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TECHNICAL REPORT

Essential requirements for setting up a stem cell processing laboratory

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The Graft Processing subcommittee of the Worldwide Network for Blood and Marrow Transplantation wrote this guideline to assist physicians and laboratory technologists with the setting up of a cell processing laboratory (CPL) to support a hematopoietic stem cell transplant program, thereby facilitating the start-up of a transplant program in a new location and improving patient access to transplantation worldwide. This guideline describes the minimal essential features of designing such a laboratory and provides a list of equipment and supply needs and staffing recommendations. It describes the typical scope of services that a CPL is expected to perform, including product testing services, and discusses the basic principles behind the most frequent procedures. Quality management (QM) principles specific to a CPL are also discussed. References to additional guidance documents that are available worldwide to assist with QM and regulatory compliance are also provided.

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INTRODUCTION

This report is intended as a guide for laboratory professionals tasked with setting up a cell processing laboratory (CPL) to support a new hematopoietic stem cell transplant program; especially in a region or country with limited resources. It describes the essential features of a laboratory's design and setup, provides basic processing principles and quality management (QM) guidelines and references additional guidance documents available worldwide. The Graft Processing subcommittee of the Worldwide Network for Blood and Marrow Transplantation[1] wrote this guideline to help physicians and laboratory technologists establish and run a CPL that will support either an autologous or allogeneic hematopoietic stem cell transplant program, thereby improving patient access to transplantation as a potentially curative modality of treatment.

We started with the assumptions that these guidelines will be used for starting a laboratory that will only produce minimally manipulated products, as defined by the US Food and Drug Administration[2] and the European Union.[3] We also assumed: (1) the availability of a clean, temperature-controlled and securable environment, with reliable electricity; (2) the laboratory is located in reasonably close proximity to the clinical site where patients would be treated and where the donor cells would be collected; (3) that the lab would be able to purchase supplies suitable for clinical use; (4) that staff would have at least a basic understanding of medical laboratory practices, as various laboratories will already be part of the hospital infrastructure; and (5) an understanding of the general principles of maintaining a clean and safe medical laboratory.

The primary objectives for laboratories supporting an autologous-only transplant program are to provide secure storage for the stem cell graft and other cellular therapy products and to adequately characterize the content of the cellular therapy product for the physicians overseeing patient care. Support for an allogeneic transplant program would additionally require the lab to provide RBC and plasma-depletion services and be prepared to thaw and infuse cord blood products. The need for labeling and product tracking and transportation to and from the hospital is required for all product types.

This guidance document is intended to assist with setting up a CPL that provides safe and effective cellular therapy product processing, storage and graft characterization services for a hematopoietic stem cell transplant program or programs. Many additional guidance documents, published literature and other useful on-line resources available internationally from relevant professional societies and governmental agencies are referenced throughout.

We emphasize that financial planning is a crucial aspect in setting up a laboratory and should be a core consideration from the outset. It continues to be a critical component once the laboratory is established in terms of revenue generation as well as maintenance and processing costs. These will all impact upon the running costs of the hospital and ultimately on the cost of SCT both for the country and to the patient. The variability of healthcare systems, modes of funding and payment as well as infrastructure support makes it difficult to offer any single model of financial planning other than to emphasize its critical importance.

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Laboratory design and physical plant requirements

When first establishing a transplant program, a relatively small dedicated space or even a clearly defined shared space is typically sufficient for a CPL, provided product safety risks such as cross contamination or product mix-ups are taken into consideration with the design and location.

The laboratory should be located as far from potential contaminants as possible and in as clean a space as possible. Sharing space or equipment with microbiology laboratories, or laboratories that utilize radioactive isotopes should be avoided, as that arrangement may increase the risk of product contamination. When sharing space with a hospital or clinic lab, locating the CPL adjacent to the Blood Bank has a number of advantages. As the equipment needed, procedures used, product safety focus and staff training needs are similar, there is an opportunity to share more than just the physical space. Standard hospital electrical supply is sufficient; however, a backup power generator and/or access to an uninterruptible power source is highly desirable in case of power outages, especially for equipment used for cellular therapy product storage. A lockable file cabinet or another system for storing facility documents and product records securely is required. A sink and water supply for hand washing and cleaning within the laboratory or nearby is important. Dedicated workstation(s) in a clean environment with restricted controlled access and away from potential contaminants are essential. Each workstation should contain a biosafety cabinet (1.2–1.8 m), approximately 2 m of counter space, a plasma extractor and a centrifuge, with access to a refrigerator, −70 °C (or colder) freezer and a microscope nearby. The number of workstations needed will depend on the number of products anticipated to be processed in a day and on the number of staff available for processing. It is important to limit each workstation and each staff member to the processing of one product at a time. Because access to liquid nitrogen (LN_2) may pose a challenge in certain regions, this document focuses on −70 °C or colder product storage in mechanical freezers. Storing products in a mechanical freezer requires a consistent, reliable electrical power source; so if the likelihood of power interruptions is high, it is safer to store the products in an LN_2 freezer. If products are to be stored in tanks requiring LN_2, the facility must be designed to facilitate regular delivery of LN_2 supply dewars. The space containing the LN_2 storage tanks and supply dewars should be separate from the processing laboratory and needs to have sufficient air handling capacity to maintain safe levels of oxygen during the times when the LN_2 tanks are filling. An oxygen sensor that alarms when levels are dangerously low is highly recommended.

It is crucial that the need for facility cleaning and waste disposal is not overlooked during the design phase. Biohazardous waste can usually be disposed of according to hospital practices. Uncluttered, readily cleanable and preferably, impervious work surfaces, availability of adequate space for raw material storage and separation of waste materials and sharps should be part of the design plan. All cleaning protocols and schedules should be clearly outlined and validated. Introduction of potential airborne contaminants into the laboratory should be minimized by process controls such as limiting access to laboratory personnel only and a design that avoids the need to deliver supplies and medical gas cylinders directly to the processing space. Temperature and humidity of the laboratory space should be controlled to the extent possible to maintain proper storage conditions for reagents and supplies, to ensure optimal performance of sensitive electronic equipment and for employee comfort. In addition, warm, humid conditions for extended periods favor the growth of contaminants, such as fungi and moulds.

Table 1. Equipment needed to start a cell processing lab

Required equipment:		
Biosafety cabinet (or equivalent)	Refrigerator	Balance (Scale)
Water bath	Centrifuge (with carriers to hold 600 mL blood bags)	Freezer (⩽ −70 °C)
Plasma extractor	Tubing sealer	Tubing stripper
Cryo-transporter (−80 °C) or liquid nitrogen dry shipper	Micropipettes (100 μL and 1000 μL)	Reference thermometer
Pipette aid	Hemostats	
Desired equipment:		
Sterile connecting device	Controlled rate freezer	LN_2 storage freezer
Label printer	CO_2 incubator	Hemocytometer
Microscope	Personal computer	
Shared equipment:		
Flow cytometer	Automated instrument for cell processing	Microbiology lab for bacterial and fungal culture
Hematology analyzer		

Abbreviation: LN_2 = liquid nitrogen.

Equipment

The equipment requirements for a CPL are fairly minimal. Table 1 lists the equipment needed in three categories: Required, Desirable, (nice to have), and Shared (equipment that because of expense, maintenance considerations and low volume use could be shared with another laboratory within reasonable proximity). Critical equipment should be maintained and calibrated on a regular basis. Backup equipment should be identified when only one device is in use by the laboratory. Each piece of equipment, including that designated as backup equipment, should undergo qualification and validation before use.[4] If an uninterruptible emergency power supply is available, the critical pieces of equipment should be connected to that supply. Product storage and supply storage refrigerators and freezers are particularly critical and must have a reliable power source. In addition, refrigerators and freezers that store patient products or critical reagents and supplies should be in a secure location, with only authorized personnel having access, and if at all possible, have full-time temperature monitoring with alarm systems that notify key personnel when temperatures are out of range. Storage devices need to be monitored by lab staff at least once or twice each day. Mechanical freezers ultimately will fail, therefore access to a backup freezer or another contingency plan in the event of a freezer outage is highly recommended. Adequate backup liquid (or vapor) nitrogen storage capacity should also be considered, as well as planning for separate quarantined product storage capacity.

Supplies and reagents

A partial list of supplies and reagents that will be needed is provided in Table 2. All product contact reagents need to be sterile and of infusion-grade. All supplies need to be sterile and disposable; used once and discarded. Reagents can be dispensed into single-use containers before use in order to minimize waste. All reagents and supplies need to be inspected before use and stored in controlled (and monitored) environments, separate from non-clinical, potentially harmful research reagents, and it is

Table 2. Minimal supplies needed to start a cell processing lab

Miscellaneous laboratory supplies		
Cryobags (for example: 50; 250; 500 mL)	Transfer packs (300; 600 mL)	Syringes (1, 3, 10, 30, 60 mL)
Safety needles; couplers	Spike to needle, spike to spike adapters; stopcocks	Alcohol swabs, iodine swabs, syringe caps, sterile swabs
Labels, laminating tags; zip ties	15, 50, 175 mL conical tubes	Pipettes (1–50 mL)
Biohazard sample bags	Tube racks	Pipette tips
Cryovials, microtubes	Biohazard bags; sharp containers; garbage bags; trash can	Dry ice
Sterile overwrap bags		
Sample reagent list (will vary depending on products and services offered)		
DMSO	Plasmalyte (or equivalent)	ACD-A
Human serum albumin	Hetastarch	Heparin
70% IPA; bleach; bactericidal and fungicidal detergent	Flow cytometry reagents	Trypan blue

Abbreviations: ACD-A = acid citrate dextrose solution A; DMSO = dimethyl sulfoxide; IPA = isopropyl alcohol.

important to document the specific lot numbers used during processing. Such careful materials management requires significant effort but contributes greatly to error prevention and overall product quality.

Personnel

Education and training of staff is critical for the establishment and operation of a CPL. Processing products for cryopreservation is not a highly technical operation; however, attention to detail, strict aseptic technique, a quality focus and consideration of the importance of each product for the patient are key factors for success. Staff with formal education in a laboratory-based discipline, and preferably with some experience in clinical hematology and/or blood banking, are most suited for the work that is required.

Although most processing operations for a start-up program can be accomplished by a single staff member, a minimum of two trained laboratory technicians is required, one of whom could be cross-trained from another laboratory. This is not only to cover absences due to illness or leave but also for planned or unplanned long working hours and the variability of workload in transplant programs. A second individual is also essential to maintain quality through verification of procedure steps and double checks of product and patient identity. Mistakes can be fatal for the patient; having two people reviewing records before product release minimizes the likelihood that a mistake will occur. It is also essential that one person works with only one stem cell product at any one time to prevent mix up of samples. If the laboratory is within a hospital, a staff sharing arrangement with the blood bank or other clinical laboratory can be a cost-effective strategy for processing staff backup.

Quality control testing of the product can be performed by the cell processing staff if necessary, but it may be more cost effective to contract with the hospital's Microbiology, Flow Cytometry and/or Hematology laboratories that perform these tests routinely. Either way, the staff performing quality control testing must be trained in the unique aspects of testing hematopoietic progenitor cell (HPC) products. The written agreements need to specify regulatory requirements for the contracted testing lab as well as procedures for timely result reporting.

We also recommend hiring or sharing part-time staff that do not have processing responsibilities to focus on Quality Assurance and Regulatory tasks, such as reviewing charts, inspecting raw materials, releasing products for infusion, doing process improvement projects and performing internal regulatory compliance audits. The time required for record review, product release and other non-technical duties can easily exceed the time spent processing products. The person responsible for the quality program needs to be familiar with the principles of QM as well as the local government's regulations and must also understand the technical aspects of the laboratory in order to design and maintain an effective quality plan that improves outcomes and reduces errors. This person needs to report quality parameters to the clinical program and to the hospital's overall QM personnel regularly and should be supervised either by the clinical program director or by someone in the hospital's compliance office.

It is important for the processing laboratory to have a close relationship with the clinical transplant program and be viewed as an integral part of the transplant team. The laboratory should be represented at clinical transplant planning meetings so that expected collection dates and transplant dates are clearly communicated, and the laboratory staff has access to engraftment data that can and must be correlated with the stem cell collection data (CD34+ cell count, total nucleated dose and viability). This will give an assurance that the clinical outcomes match the reliability of processing and can also serve as an indicator if processing standards are sub-optimal. Often, one of the transplant physicians serves as the Medical Director for the CPL, providing oversight and guidance for laboratory staff and serving as a consultant for the less experienced physicians treating transplant patients. We recommend that this person should not be in charge of daily laboratory operations however; rather there should be a designated supervisor or manager in charge of staff scheduling and ensuring standard operating procedures are followed. It is also imperative that the clinical transplant program confirms with the CPL that the products are available before beginning the patient's conditioning regimen. This is especially important in newly established facilities where document control systems may be less well tested and cryopreservation potentially less reliable.

Processing principles

The CPL's primary role in supporting an autologous transplant program is to preserve cellular therapy product viability during storage and to prevent the introduction of microbial contamination at all stages of processing. The autograft is collected before the patient receives high-dose therapy,[5] and depending on the disease and treatment plan, cellular therapy products may need to be stored from several days to a few weeks, months or even years.

Storage at 2–8 °C maintains acceptable HPC viability only for relatively short periods of time (48–72 h),[6] therefore cryopreservation is required for longer storage periods. Ideally products should be cryopreserved as soon as possible after collection. However, overnight storage is also acceptable if the product is kept refrigerated (2–8 °C). If product cellularity exceeds 2×10^8 cells per mL or contains a high proportion of mature granulocytes,

dilution with concurrent plasma may better preserve viability. HPC and Apheresis products may be more vulnerable to temperature-related cell loss during liquid storage than BM products.[7] An adequate amount of anticoagulant should be added to avoid cell aggregation if storing or transporting the product for longer periods of time (24–72 h). The use of acid citrate dextrose is recommended; heparin should be used with caution as it could potentially exacerbate bleeding complications in thrombocytopenic transplant patients.

Most cryopreservation protocols involve (stepwise) volume reduction, addition of a cryoprotectant solution, controlled rate (slow) freezing and storage at vapor-phase LN_2 temperatures ($\leqslant -160$ °C). Although the procedures for performing these steps are far from standardized, strict adherence to aseptic technique is universally required. In a start-up situation, with limited resources, it is possible to maintain cellular therapy product viability using a $\leqslant -70$ °C mechanical chest freezer instead of a programmable controlled rate freezer and LN_2 storage freezer.[8–10] Cellular therapy product stability can be maintained for several months at the warmer temperature by using a cryoprotectant consisting of 6% hydroxyethylstarch and 5% dimethyl sulfoxide (DMSO) and human albumin.[10] For longer-term storage (years) cryopreservation with a final concentration of 10% DMSO and storage in liquid or vapor phase nitrogen is still the preferred and most widely used methodology, as there has been some indication of a fall in GM-CFU (granulocyte macrophage colony-forming unit) colonies after 1 year of storage at −80 °C.[11] Protection from temperature fluctuations is an important consideration. Products stored in liquid-phase LN_2 (−196 °C) are better protected from temperature fluctuations but are at increased risk for bag breakage at thaw and cross-contamination from other products. It is recommended that they should be wrapped in an overwrap bag before or just after cryopreservation and stored in metal cassettes to protect them from damage. Also, it may be prudent to cryopreserve products in at least two bags to reduce the risk of catastrophic product loss in the event of a bag breaking. Cryopreserving small volume retain vials along with the product allows for testing the product integrity (viability; sterility) either before or after infusion in the event of an adverse reaction or processing deviation.

Cell viability during freezing and storage can be maintained using either 10% DMSO or a combination of 5% DMSO and hydroxyethylstarch as cryoprotectants.[10,12–14] Recent reports in the literature describe fewer adverse reactions to infusion with lower DMSO concentrations.[15] Detailed cryopreservation protocols are available from published manuscripts and book chapters[16–18] and from the International Society of Cellular Therapy (ISCT) and AABB professional association websites[19,20] and from their members. In general, it is beneficial if the cell suspension and cryoprotectant solution are chilled before mixing, the cryoprotectant solution is added slowly and with gentle agitation only and the exposure time to DMSO before freezing is minimized. Advanced preparation is therefore essential. The products should be cryopreserved in bags, not tubes, so that the cryopreservation media can be added and the cells distributed in a closed system, with much less risk of contamination. A start-up CPL should demonstrate their chosen cryopreservation protocol results in an acceptable postthaw viability ($\geqslant \approx 70\%$) before performing their first transplant, as part of a process validation protocol. Once established, the laboratory should routinely monitor postthaw viability and watch for adverse trending as part of their quality plan. Written processing instructions (standard operating procedures), data capture worksheets, labels, result reporting and clinical site communication documents and equipment quality control forms all need to be in place before processing the first product, so that thorough documentation of each processing step can be filed indefinitely as part of the patient's record.

In contrast to the freezing process, the products should be thawed rapidly, in a 37 °C water bath if possible, but without letting the product warm past ambient temperature before infusion. Exposure time to DMSO after thawing should be minimized to avoid cell death.[21] Special attention should be paid to minimizing the chances of contaminating the product during the thaw process. Thawing products in a sterile overwrap bag not only protects the product from contamination but also allows for product recovery in the event a bag breaks during the thaw. Most often, products that are directly thawed are done so in close proximity to the patient, requiring the transport of the product and equipment needed for thawing by laboratory personnel and the presence of laboratory staff throughout the infusion. Inspecting bag integrity before transport and bringing supplies needed in case of bag breakage to the clinical site is highly recommended.

Some laboratories use a dilution method and removal of the supernatant along with most of the DMSO by centrifuging the product.[15,22] This method greatly reduces the incidence and severity of adverse reactions. A survey of laboratory practices conducted by EBMT indicated an effect of the amount of DMSO infused with a product and toxicity reactions at infusion, with the lowest rate of toxicity seen after infusion of products from which DMSO was removed by washing.[23] Such wash methods must be performed in the laboratory. However, with DMSO removed, postthaw viability at refrigerator temperatures is maintained for longer periods of time making this a feasible approach. The thawing method chosen must consider logistical issues such as the proximity of the laboratory to the infusion site and the need for good communication between the laboratory and the infusion team to minimize the time between thawing and infusion. For all methods, products should be thawed one at a time in case of an adverse reaction to infusion requiring treatment before subsequent infusions. If DMSO cannot be removed, the volume per kg infused at a single setting should be considered, especially if a large number of bags are infused. Infusion of a large volume of DMSO (>1 mL/kg) can cause severe hemodynamic stress and infusion-related hemolysis, therefore close monitoring of vital signs, body weight and urinalysis is required.[24] DMSO toxicity is rarer with cord blood transplants, where the infusion volume is low; however, direct infusion of thawed cord blood products is not recommended. Only appropriately trained personnel—either CPL technologists or transplant ward nurses—should be authorized to thaw and administer autologous hematopoietic stem cell products.

There are several other immunological and non-immunological infusion toxicities to be aware of, and prepared for, when infusing cell therapy products. The Circular of Information for the use of cellular therapy products, an effort from multiple organizations, including AABB, ISCT and ASBMT,[24] is an excellent resource for understanding the more common types of adverse reactions that can and do occur.

All cryopreservation and thawing protocols result in some product loss; thus it is best if allogeneic products can be infused without cryopreservation whenever possible. It can sometimes be logistically challenging to coordinate the timing of the allogeneic collection from a volunteer donor so that the product is available for infusion just as the patient is ready to receive the cells. It may occasionally be necessary to cryopreserve an allogeneic donation, but this is not recommended as standard practice, unless the physician wishes to limit the cell dose infused fresh and requests that 'extra' HPC or donor T cells be cryopreserved for future use. Many allogeneic products can be infused directly, without processing other than sampling for sterility and performing cell counts and determination of CD34+ cell content. However, the laboratory needs to be prepared to perform either RBC depletion or plasma depletion on ABO-incompatible BM products and plasma depletion of mobilized peripheral blood products. There is

generally not a need for depleting additional RBC from a mobilized apheresis product as the RBC content is already quite low; however, BM products with a major ABO incompatibility to the recipient's blood type cannot be infused safely without significantly reducing the RBC content. There are a few ways to accomplish the RBC depletion; the most common and the simplest way being a gravity sedimentation procedure after adding hetastarch to the BM to agglutinate the RBC and cause them to settle more rapidly than the WBC.[25,26]

Patients receiving minor ABO incompatible transplants face a risk of hemolysis from the infusion of incompatible plasma contained in the product, particularly with marrow products. Depending on the amount of donor plasma present, the donor's Isoagglutination titers and donor/recipient blood types, plasma depletion may or may not be required for minor ABO-mismatched allogeneic products to prevent anti-ABO antibodies in the product from lysing the recipient's erythrocytes.[26] This can be accomplished with a single high-speed centrifugation and removing the majority of the plasma before infusion. A second centrifugation (wash procedure) can be helpful if the donor has a particularly high titer anti-ABO antibody ($\geqslant$1:256).[27]

If supporting an allogeneic program that utilizes unrelated donor products from around the world, the processing lab is often responsible (in most regulatory frameworks) for making sure that the donor screening and donor testing results confirm donor eligibility. Donor eligibility rules vary internationally, but most involve testing the donor within a few weeks (typically 4) of donation for the presence of infectious agents, such as HIV 1 and 2, HTLV 1 and 2, Hepatitis B, Hepatitis C, West Nile Virus and Syphilis, in a concerted effort to prevent the spread of infectious disease from donor to recipient. Additional testing for other infectious disease agents may be required depending on individual countries, range of infectious diseases and regulatory frameworks. The processing laboratory is frequently not involved in the testing itself; nonetheless, it should be part of standard operating procedure that the infectious disease test results are negative and up to date before releasing a product for infusion. If a clinical decision has been made to infuse the product despite a positive or out-of-date test result, the laboratory is then responsible for labeling the product as a non-conforming product and making sure all are aware of the risks involved. In addition, it is important for the CPL to be aware of the infectious disease marker results before receipt of any product into the CPL so that appropriate labeling is applied and so that products are stored in a manner to prevent release before appropriate approvals have been obtained. All products must be handled in a manner to reduce risks of cross-contamination and ensure staff safety.

Regardless of whether the laboratory supports an autologous or an allogeneic transplant program, strict product labeling, transportation and product-tracking systems and procedures need to be in place from the beginning through to infusion to prevent product mix-up errors and to maintain product integrity while delivering the product to the patient. Complete traceability is essential. The laboratory may be storing products from many patients in the same freezer; it is vital that labeling is clear and that product and patient identity double checks are in place at the time the product is received into the laboratory, placed into storage and removed from storage for infusion. Insulated coolers and gel packs are required to transport fresh products at cool temperatures. Transport of cryopreserved products from the lab to be thawed at the bedside should occur minimally on dry ice if the clinical site and hospital are not immediately adjacent in order to minimize the time between thaw and infusion. Products could be thawed (one at a time) in the laboratory and transported to the hospital in a cooler if products can still be infused within 15–20 min of thaw, or if the cryoprotectant is removed, products may be stored at refrigerator temperatures for up to 3 h.

If sufficient resources are available, the implementation of the ISBT 128 labeling standard is highly desirable from the start.[28] The financial investment is likely to be rapidly offset by the decrease in the workload that is associated with the design of custom and locally made labels that are compliant with national and international requirements. Whenever possible, barcodes and barcode readers should be used instead of handwritten labels.

Even though most donors are tested for the presence of several common infectious diseases before donating, cellular therapy products are potentially infectious. It is essential that staff are provided with personal protective equipment such as lab coats, gloves and safety glasses and properly trained to use universal precautions to protect themselves from possible infection and to protect the products from cross-contamination. Lab design, engineering controls and staff training should focus on reducing the risk of product contamination to prevent the introduction or spread of infectious disease in severely immunocompromised transplant patients.

Cellular Therapy Product Characterization/Quality Control Testing

BM contains a wide range of cell numbers and cell types and responses to agents used to mobilize HPCs to the peripheral blood vary, especially for the autologous donor. Therefore, it is imperative that the laboratory provide the patient's physician with cell count data and CD34+ cell count data so they can assess the potential of the cellular therapy product to engraft before initiating conditioning therapy. The total leukocyte count is important as is a calculation of the mononuclear cell count (manual differential or flow differential) and a blast cell count, as the number of immature myeloid cells correlates with engraftment better than the total cell count.[29,30] However, the total CD34 cell count is currently the best available predictor of engraftment, especially for PBSC grafts while the total nucleated cell count is often still used for marrow grafts.[31] Although the minimum number of CD34+ cells required for engraftment has not been firmly established, most investigators accept a minimum of 2×10^6 CD34+ cells/kg for optimal engraftment. When possible, higher doses of $4–5\times10^6$ CD34+ cells/kg are preferred, as they are associated with faster engraftment, reduced incidence of infection and reduced need for transfusions. Enumeration of peripheral blood CD34 counts during mobilization is useful for planning the apheresis collections necessary to obtain these grafts. Hematology analyzers can provide total cell count information, or these counts can be obtained with reasonable accuracy by making slides to perform a differential and manually counting cells in a hemocytometer using a microscope. The lab could purchase a small analyzer; however, given the low number of samples and requirements for calibration and maintenance of this piece of equipment, it may be more cost effective for a start-up lab to send the sample to the hospital's hematology lab for analysis. A more specialized flow cytometry analyzer is required for CD34+ cell detection. Flow cytometry can also be used to determine mononuclear cell content and viability. CD34+ cell enumeration is most accurately performed using a multiparameter definition of progenitor cells based on their light scatter characteristics, dim expression of CD45 and CD34 expression. References as to how to perform these tests are readily available through the professional networking opportunities offered by ISCT, Foundation for the Accreditation of Cellular Therapy (FACT) and AABB. Adhering to published guidelines for the CD34 analysis is very important as the stem cells represent a small percentage of the total number of cells and must be carefully enumerated.[32–35] The use of a 'single platform' method for enumeration of viable CD34+ cells is recommended for many low-volume start-up laboratories.[33,34] For both tests, the lab would need to purchase a dilution buffer, sample tubes, syringes and needles, a viability dye and fluorescently labeled monoclonal antibodies to CD34 and to CD45 (and

to CD14 if assessing mononuclear cells), although there are currently kits available that contain all the necessary reagents for the 'single platform' method. A small centrifuge would also be required for the CD34 testing.

A sample for cellular therapy product sterility testing for aerobic and anaerobic bacteria as well as fungus is required by most regulatory agencies to be taken before product infusion. This testing is best done in collaboration with a hospital microbiology laboratory that can also provide antibiotic sensitivity testing on any organisms found. Line infections or bacteremia at the time of collection are the most common sources of contamination for autologous peripheral blood products. Likewise, during BM harvest, skin contaminants are not infrequently introduced. Therefore, we recommend testing HPC products before and after processing, just before either infusion or cryopreservation to facilitate investigation of the cause of any identified contamination. This is especially important in a start-up situation, as the laboratory has to know that they are not introducing contaminants.

Many institutions perform a postthaw viability assessment on a retain vial, using either trypan blue or 7-aminoactinomycin D, as part of their product release procedure for cryopreserved products. Others monitor the postthaw viability of the product at the time of transplant and then look for adverse trends or outliers. Both are worthwhile product-testing objectives.

A typical HPC product testing plan is provided in Table 3. The testing plan should be designed to prove the product's identity, purity, potency and (most importantly) safety. Additional, more complex testing may be needed to ensure the safety and potency of more specialized cell therapy products, and it may be necessary to test the product before and after processing to be sure that the processing is working correctly. Note that all product release testing for fresh infusion products needs to be performed immediately so that the product can be infused in a timely manner. Products can still be infused if they do not meet predetermined acceptance criteria, but only if there is an urgent medical need and only with documentation of the transplant physician's and/or Medical Director's approval.

Table 3. Quality control testing for HPC products

Attribute	*Test method*	*Specification*
Donor screening	Summary of records; donor eligibility form	Donor eligible
Infectious disease testing	Certified laboratory	Negative (exclusive of CMV)[a]
Infusion volume	Measurement	$\leqslant$20 mL/kg/infusion
DMSO volume	Calculation	$\leqslant$1 mL/kg/day
Total nucleated cell (TNC) count	Automated cell counter; or hemacytometer	As measured
RBC content (if ABO incompatible)	Automated cell counter	$\leqslant$20–30 mL/adult infusion
CD34+ cell count	Flow cytometry	$\geqslant 2\times10^6$/kg
CD3+ cell count (if allogeneic)	Flow cytometry	As measured
Viability (pre-freeze)	Flow cytometry	$\geqslant$80%
Sterility	Bacterial culture	No growth
Sterility	Fungal Culture	No growth
Final product Labeling	Observation	Labeled correctly

Abbreviation: HPC = hematopoietic progenitor cell. [a]Infectious disease testing of autologous products is not universally required worldwide. Consult national regulations.

Product release

Standard operating procedures or policies must be in place defining the criteria that must be met for a product to be released from the laboratory for infusion. Staff releasing the product must confirm that all criteria are met; or if they are not met, that the required approvals and notifications have been obtained from the transplant physician or Medical Director. Ideally, final review of processing records and approval for product release should come from laboratory management and/or from 'independent' QM staff not directly involved in the processing operations, as is done in pharmaceutical production. A final review of processing records by laboratory management is always required.

Quality plan essentials

Finally, each of us wishes to emphasize the importance of starting the planning for building a processing laboratory by writing a Quality Plan specific to the laboratory. A quality plan summarizes and references the organization's policies, procedures and practices related to the day-to-day operation of the facility and in the case of emergency. There are many resources available for guidance through the FACT and AABB, and others,[36,37] but minimally the Quality Plan needs to describe the policies and procedures that will be used to ensure product integrity, prevent contamination from the environment, prevent cross-contamination from other products and prevent products going to the wrong patient. Starting with a description of staff safety and disaster planning procedures, a useful quality plan also describes the organization's minimal staff qualifications, staff training and competency assessment procedures, document control, record retention, materials management, process validation, process control and event reporting procedures, including a system for corrective and preventive actions. The Quality Plan needs to summarize and reference policies and procedures for handling products with positive microbial culture results and describe procedures for how to quarantine such products, document urgent medical need and release for infusion only with physician's

Table 4. Cell processing laboratory quality management plans

Quality system element	*Description*
Organization	Organizational charts; reporting structures, inter-institutional relationships
Personnel	Human resources policies; job descriptions; personnel qualifications, training and competency
Communications	Processing prescriptions; result reporting
Facilities, work environment and safety	Floor plans; cleaning schedules; mechanical systems; environmental monitoring; disaster plans
Suppliers and materials management	Materials management; supplier qualification
Equipment	Qualification; calibration; maintenance; cleaning schedules
Process controls	Change control; methods to prevent mix-ups and cross-contamination; process validation; product release; quarantine storage; product tracking; label control
Documents and records	Standard operating procedures; document and version controls; record review; record retention
Management of non-conforming events	Deviation and adverse event reporting; documentation of urgent medical need; regulatory agency reporting
Monitoring and assessment	Donor eligibility; product testing; outcome analysis; audits

approval. The Quality Plan should also describe procedures for data collection and outcome analysis (especially time to engraftment);[38] so that problems can be detected quickly and resolved. Most regulatory agencies now require that a quality plan include regular analysis of its performance, including correlation with clinical parameters like engraftment. Planning for these activities in advance enables good decisions on what supplies and equipment to purchase, what space to use or to build, staff to hire and so on.

Table 4 describes the subject headings present in a typical quality plan for a CPL. In the early stages of starting a laboratory, the quality plan could be made up of mostly policies or policy statements as guiding principles, and as the program matures, the scope and detail of the quality plan can grow as well. Having a simple QM plan from the start allows the laboratory to obtain authorizations from governmental agencies and competent authorities and pave the way for accreditation by FACT–Joint Accreditation Committee ISCT (Europe)-EBMT (JACIE), AABB[39,40] or a similar organization. Although outside the scope of this guidance document, the International Netcord Foundation[41] and FACT have collaborated to provide accreditation standards and guidance for cord blood banking operations.

Regulatory compliance

Regulatory compliance requirements will vary greatly between countries. In general, a solid quality plan that addresses all local regulations, adherence to the principles of that plan and documentation to support that adherence will likely allow the laboratory to comply with the regulatory frameworks.

FACT, AABB, JACIE, Netcord, The Alliance for Harmonization of Cellular Therapy Accreditation and others all have many resources available to assist facilities with regulatory and accreditation issues. We wish to recommend, however, that it is best to focus on product quality and patient safety first and foremost. Regulatory compliance will follow quality work.

CONCLUSION

This paper describes the essential elements and considerations in starting a stem CPL. There is considerable room for improvement and addition as resources become available or as the transplant program evolves. Table 1 highlights a list of equipment that is required vs equipment that is desirable or can be shared. This is certainly one area for development as the transplant program matures and expands. Additional personnel in addition to the minimum of two would also be an important consideration to handle the greater volume and complexity of work and to allow for independent checks as well as verifications for product release to minimize potential conflict of interest issues.

More specialized equipment may also be required for more complex stem cell processing, such as T-cell depletion. Another area of improvement is the introduction of robust and efficient information technologies to handle greater volumes of work and to minimize paper-based errors. Information technologies also improve traceability and greatly help to support a comprehensive QM plan as well as document-tracking systems. This will also complement the use of barcode printers and readers and aid in the adoption of international nomenclature, such as ISBT 128.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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