



## Determining the Quality and Sterility of Stem Cell Products: Pretransplant Testing of Stem Cell Products for Human Transplantation

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### **Glossary**

Haematopoietic Progenitor Cell (HPC) – (FACT) Self-renewing and/or multi-potent stem cells capable of maturation into any of the hematopoietic lineages, lineage-restricted pluripotent progenitor cells, and committed progenitor cells, regardless of tissue source (bone marrow, cord blood, peripheral blood, or other tissue)

Potency - (FACT) The therapeutic activity of a product as indicated by appropriate laboratory tests or adequately developed and controlled clinical data. (CFR) the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result.

Purity - (FACT) Relative freedom from extraneous matter in the finished product, whether or not harmful to the recipient or deleterious to the product.

Quality - (ASQ) A subjective term for which each person has his or her own definition. In technical usage, quality can have two meanings:  
a. the characteristics of a product or service that bear on its ability to satisfy stated or implied needs;  
b. a product or service free of deficiencies.

Safety - (FACT) Relative freedom from harmful effects to persons or products.

Sterility - (Wikipedia) An aseptic condition, meaning an absence of living things, as in [microbiology](#) or the [environment](#)

## **Introduction**

Ensuring a safe, pure and potent product – what should be tested, when should it be tested and how should I test it? All cellular therapy products must be demonstrated to be safe, pure, potent, stable, and effective for human use. Stem cell laboratories are not routine processing/testing labs, they are specialized laboratories assisting clinical programs, helping to ensure that an adequate dose of HPC is provided to each patient and that each HPC product has been tested and meets specific release criteria. With the increasing number of different stem cell therapy products being used to treat an increasing number of different diseases, the laboratory must demonstrate that they have the ability to produce consistently high quality products.

In consultation with the clinical site, the laboratory should have documented product specifications that are intended to ensure that cellular products manufactured by the laboratory consistently meet regulatory and industry requirements for sterility, safety, purity, identity, and potency. In addition, the laboratory must ensure testing has been completed to measure and evaluate stem cell products before releasing these products to the clinical site for infusion. This testing and measurement will form the product release criteria used by the laboratory.

## **Safety**

**Sterility Testing:** Also referred to as microbiology testing, is the process of testing the HPC graft for microbial contamination, including bacterial (aerobic and anaerobic) and fungal.

The two most common microbiology testing methods are the BacTAlert and Bactec systems. When developing and implementing a sterility testing procedure or performing sterility testing, a number of factors must be considered to address specificity and sensitivity. These factors include; the initial size of inoculum (larger inoculum volume increases sensitivity of testing), the ability of the method to detect the many different types of bacteria and fungus the product could encounter during collection, processing and storage and finally the timing of product sampling during the manufacturing process. Since microbiology testing methods are developed specifically for blood cultures and not cells, tissues and organs, the laboratory should maintain complete validation records for their microbiology testing procedures, or at a minimum should have literature to support the method being used. Currently, the majority of Canadian laboratories use the BacTAlert or Bactec testing systems with an inoculation volume of 0.5 mL or 1 mL. Some laboratories use the pediatric culture bottle

while others use the adult bottle. Sampling frequency should be defined by individual laboratories based on the type of processing being performed. At a minimum sampling and microbiology testing must be performed at the end of laboratory processing as this will be required as part of their release criteria.

The role of the laboratory is to provide complete testing information to the clinical transplant program. The decision to transplant a contaminated product should be made by the transplant program director. Testing information should include the microbiology testing results, and, if product is contaminated, information should also include type of organism, antibiotic sensitivity and possible sources of contamination. This information should be made available to all members of the transplant team including clinical, collection and laboratory groups. Documented notification of the program director should be maintained by the laboratory in the patient files.

In addition to the standard microbiology testing, additional testing may include endotoxin, gram stain and mycoplasma testing. Although not routinely performed for HPC minimally manipulated products, the laboratory should be aware of these additional testing methods for products processed by more than minimal manipulation.

## **Potency Testing**

General Information: Potency assays are quantitative measures of a product-specific biological activity that is linked to a relevant biological property and, ideally, a product's in vivo mechanism of action (e.g. HPC potential to differentiate to the various hematopoietic cell lineages). Since there is often a limited period of time between the completion of production and the release from the laboratory for administration to the patient, CD34 enumeration using flow cytometry is one of the most common potency assays performed by the cell therapy laboratory.

As new cellular therapies are developed and used to treat an increasing variety of diseases and patients, potency testing becomes a critical and required part of the production/testing of cellular therapy products.

Flow Cytometry - CD34 Analysis: CD34 antigen, expressed as a surface antigen on primitive hematopoietic progenitor cells (HPC), is the standard "marker" used for enumeration of HPC and ultimately is routinely used as an indicator for graft quality. Flow Cytometry is used to determine percentage of CD34+ cells in the HPC product. This percentage and the nucleated cell count are used to calculate the CD34 dosage expressed as CD34+ cells per kilogram of recipient body weight. Many Flow Cytometry laboratories use the "Stem-Kit" (Beckman Coulter)

for identification and enumeration of CD45+/CD34+ cells within the HPC product.

Flow cytometry techniques should be well defined in SOPs as well as validation studies for reagents, equipment and software to minimize variability and interpretation of data.

The clinical transplant program, in consultation with the laboratory, should have clearly defined targets for CD34 dosage and follow-up actions when these end points are not achieved.

**HPC Culture Assay:** The culture assay or colony-forming culture assay is an “ex-vivo” potency assay used to identify and enumerate HPC capable of proliferation in culture conditions. Briefly, a sample of HPC product is added to specific culture assay media and incubated (37 C/ 5% CO<sub>2</sub>) for approximately 2 weeks. The resulting cell proliferation is identified and enumerated using light microscope. Colony types are identified by their specific morphological features in culture.

Culture techniques and colony enumeration can vary between laboratories as well as between individual technologists performing the procedures. Therefore, well documented standard operating procedures should be maintained by the laboratory to minimize this variability.

There is no regulatory requirement in Canada for HPC testing. The routine use of this assay for evaluation of potency in HPC products is not performed by all laboratories or programs. Individual laboratories, in consultation with the clinical program and after reviewing current literature, should document HPC testing decisions and policies.

As with CD34 enumeration, the clinical transplant program, in consultation with the laboratory, should have clearly defined endpoints for HPC enumeration by culture assay and follow-up actions when these end points are not achieved.

**Engraftment Data:** Ultimately, patient engraftment of neutrophils and platelets from the infusion of a HPC product is the true determinant for product quality. Patient engraftment is an “in-vivo” potency assay and the laboratory should monitor clinical engraftment data as a measure of product quality.

## **Purity Testing**

**Viability:** The main goal of viability testing is to establish percentage of intact, living cells vs. dead cells. Viability testing is performed using a standard dye exclusion method and can be performed using a microscope or by flow

cytometry. Briefly, cells are incubated with a dye which live cells are able to exclude from uptake. Trypan blue is used with the light microscopy method while 7-aminoactinomycin D is used with flow cytometry. The proportion of non-intact cells is determined from the proportion of cells which fail to exclude (i.e. stain with) the dye. An advantage to using flow cytometry is the ability to monitor the viability of a specific subset of cells, i.e. CD34+ viability.

Cell Counts: Nucleated cell counts, performed either manually or by haematology analyzer, can be used as a measure of graft quality. The total nucleated cell counts are usually expressed as nucleated cell number per kilogram of recipient body weight. However, these measurements are not considered potency measurement because they do not measure biological activity within the HPC product.

Flow Cytometry – Immunophenotype Determination: As with CD34 analysis, the HPC product can be analyzed for cellular immunophenotype by flow cytometry. Immunophenotyping allows for cellular identification for the HPC product allowing for not only progenitor cell (CD34) determination but also T-cell (CD3) and B-cell (CD19) enumeration.

Tumor Cell Detection: Although not routinely performed by most HPC processing laboratories, tumor cell detection can be performed to identify contaminating tumor cells in HPC product and to evaluate any tumor cell purging procedures. The detection methods vary between laboratories but the majority of methods consists of tumor antigen identification by flow cytometry or immunohistochemistry methods. Also, molecular biology methods can be used to identify gene rearrangements and tumor cell contamination.

### **Conclusions:**

Pretransplant Testing of Stem Cell Products for Human Transplantation could also be referred to as product "Release Testing". The laboratory, before releasing product to the clinical facility for transplantation, must ensure the product meets or exceeds documented acceptability criteria to ensure product safety, purity and potency. These criteria should include:

Product microbiology testing (Sterility) complete / Negative

Donor screening complete / Negative

Donor Infectious Disease testing complete / Negative

Potency Testing complete / Acceptable (Flow Cytometry CD34+, CFU Assay)

Product labelling complete / accurate

If the release criteria is unacceptable, Program Director and Laboratory Director must approve the release of product from laboratory. This release is documented usually as “urgent medical need”.

This release testing helps to ensure product safety, purity, and potency.

Product Safety – free of detectable agents, bacteria, fungi, endotoxin, and mycoplasma (microbiology testing)

Product Purity – testing to confirm the product contents is what has been identified on the label (cells counts, viability, immunophenotype)

Product Potency – testing to demonstrate the functional activity of the product (CD34 analysis, CFU culture assay)

## References

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