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VoxSanguinis
The International Journal of Transfusion Medicine

ISBT International Society of Blood Transfusion

Vox Sanguinis (2011) **101**, 255–275

INTERNATIONAL FORUM

DOI: 10.1111/j.1423-0410.2011.01471.x

Quality controls of cryopreserved haematopoietic progenitor cells (peripheral blood, cord blood, bone marrow)

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The final quality control of cryopreserved progenitor cells is a successful and persistent three lineage engraftment after transplantation. Of course, the stem cell providing institution is obliged to have a program for controlling and monitoring the manufacturing of cellular therapy products before the patients' conditioning therapy is started. The FACT-JACIE Standards [1] and the Netcord/FACT Standards prescribe that the director of the institute shall define tests and procedures for measuring and assaying cellular therapy products to ensure their safety, viability and integrity and shall also ensure that products meet predetermined release specifications. This requires specifications of assays and the definition of thresholds to allow release.

The most common cell viability test is still trypan blue dye exclusion, although its predictive value is low and it does not seem to be a substitute for assays evaluating *in vitro* proliferative capacity [2]. Stem cell culture assays are time consuming and results are investigator-dependent. Furthermore, flow cytometry-based evaluation of viability or apoptosis markers of progenitor cells after freezing-thawing are not standardized. Reduced numbers of viable CD34(+) cells have been reported to be associated with a risk of delayed platelet engraftment or graft failure [3].

Further it has to be mentioned that interlaboratory discrepancies in the results of the assays exists, due to the fact that standardization is difficult and that the performance is variable. These problems can only be overcome by participating in external proficiency testing and by individual validation studies to establish specifications for release in each centre. In Germany the Societies of Transfusion Medicine, Haematology and Oncology and Paediatric Oncology and Haematology concluded that short-term culture assays are not suitable for defining the quality of the individual product but help validating the progenitor cell processing [4].

This International Forum of Vox Sanguinis is meant to obtain information concerning the above mentioned issues. The following questions were sent to experts of transfusion services, laboratories, transplant centers and cord blood banks:

Question 1
How many sample aliquots do you freeze together with the bags and for what purpose (quality control after short-term storage; quality control after long-term storage; sterility, back up sample)? Are these samples stored with the bags or are they stored separately?

Question 2
At what time point do you perform the quality control of frozen samples?

Question 3
What kind of quality controls do you perform routinely after the freezing and thawing of haematopoietic progenitor cells? (total cell viability; trypan blue dye exclusion; flow cytometry-based permeability marker and/or early apoptosis assay; stem cell culture assays; others).

Question 4
Do you perform stem cell culture assays and if yes which ones?

Question 5
Have you defined acceptable ranges/thresholds for the number of stem/progenitor cells that allow transplantation?

Question 6
How did you define these thresholds/requirements (by validation of your own clinical results or by published work of other groups or by national/international regulations)? If you test several types (sources) of stem cells: do the thresholds/requirements differ from type to type (PB, CB, BM)?

Question 7
What are the consequences for the product if the results do not meet the requirements (warning the transplantation unit; discarding the product; other)?

Table 1 Contributors

C	Country	Participants	Centre(s)	
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2	Austria	Nina Worel	Department of Blood Group Serology and Transfusion Medicine	Medical University of Vienna
3	Belgium and the Netherlands	M. Grommé, F. W. M. B. Preijers, E. Braakman, G. J. Schuurhuis and I. Van Riet	Maastricht, Nijmegen, Rotterdam, Amsterdam, Brussels	WSN, Dutch and Dutch-speaking Belgian processing facilities
4	Brazil	Silvano Wendel, Rita Fontão-Wendel, Arlete Lazar	Blood Bank Hospital Sírio Libanês	Hospital Sírio Libanês São Paulo
5	Canada	Mindy Goldman, Mike Halpenny, Anthony Giulivi, Brenda Letcher, Locksley McGann	Head Office Ottawa, Ottawa , Edmonton	Canadian Blood Services
6	Finland (CBB)	Matti Korhonen	Cell and Tissue Therapies	Finnish Red Cross Blood Service
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16	USA (CBB)	J.-A. Reems, J. Oh	Puget Sound Blood Center (PSBC)	Seattle, collects and stores autologous peripheral blood stem cell products

CBB, cord blood center. PSBC also collects and stores autologous peripheral blood stem cell products.

Question 8

Does your laboratory take part in external proficiency testing (national, international) and if so, for which assays?

We received 16 contributions from 13 countries, five of the centres deal with cord blood exclusively (Table 1).

The number of cell sample aliquots that are frozen ranges between two to five per product, but may depend on the source. Additional samples are stored for donor identification and other purposes if cord blood was collected. The samples are in most cases stored with the bags or at least under the same conditions in liquid nitrogen containers.

A first sample is assessed within 1–2 weeks after collection and cryopreservation in most centres. However, in some centres quality controls are only retrospectively evaluated in case the engraftment is delayed (C3–C5) or because this is their validated process (C8). Some of the contributors test samples that have been stored for more than one year, if scheduled for transplantation (C 1, C2, C4, C8, C15), to assess the quality after long-term storage ranging from one to five years. CBs are usually tested just before release.

The quality controls undertaken after freezing-thawing differ among the contributing centers and even among

members of a corporate organisation. A few participants do not perform viability or clonogenic assays. One of the reasons may be an automated and validated process to differentiate between products collected within the center from those obtained from outside. Partly this reflects the fact that nearly all quality control methods in this field have modest predictive value.

A commercially available stem cell culture assay (MethoCult®, Stem Cell Technologies Inc., Vancouver, Canada) is still popular and used by 14 of 16 contributors, and is used by all participating cord blood banks. Sometimes it is used only for confirmation of the functionality of the cells, rather than for quantitative purposes or as a process validation test without relevance for the release of the products.

Most of the centres have defined thresholds for products to be transplanted but quite often leave the final decision to use the stored product till after a discussion with the clinicians. A different strategy applies to cord blood banks. Please find the detailed answers from contributors C6, C10–C12 and C16.

There is a good consensus to the question how to deal with a product if results do not meet the requirements. Products that do not meet the release criteria are discarded if the clinician refuses to use the inferior product. Most of the contributors have exceptional release policies and only two stated that they do not discard any cryopreserved products (C4 and C14).

All of the centers take part in various forms of proficiency testing workshops, but not in all cases for viability or culturing tests. An international proficiency testing of clonogenic growth is organized by Stem Cell Technologies, Inc., and this opportunity is widely accepted among the contributors although critical views are mentioned (C5 and C12).

We advise the reader to read the individual contributions of the experts, since they contain many interesting details. This Forum clearly shows that standardisation for processing of cryopreserved progenitor cells is not yet adequately standardised. For the future it is important that more consensus should be reached about minimal quality requirements for processing and storage of this blood component.

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By way of background, Australian requirements for the quality control of cryopreserved HPC are determined by the regulatory status of the laboratory, which in turn define the relevant product standard with prescribed tests. Haemopoietic progenitor cells are currently regulated as a medicine by the Therapeutic Goods Administration (TGA).

A number of exemptions from the requirement to hold a Manufacturer's Licence exist within the Therapeutic Goods Act, with the result that most hospital-based HPC facilities are exempted. These 'exempt' facilities are generally accredited to a National Pathology Accreditation Advisory Council Requirements document [1], that is based on FACT-JACIE, and defines necessary quality control and product testing. TGA-licensed HPC facilities have their regulatory Product Standard defined by the Act, in this case *Ph. Eur* monograph 2323.

1) The NPAAC Requirements state that "at least two test samples of the product (cryopreserved and stored under conditions that ensure a valid representation of the clinical product) must be available for testing, as and when required".

Our facility stores five 0·5 ml vials from each cryopreservation. One vial is used within 72 h for CFU-GM assays to

validate the cryopreservation process and is stored in liquid phase LN_2 for that period. Two vials are placed in each inventory cassette together with a cryobag and are used for pre-release quality control testing after long-term storage (which is defined as > 5 years at our institution).

Any remaining vials are stored in a separate vial rack within the same LN_2 storage tank and are used as a back-up sample should the initial CFU-GM assay require repeating.

2) The NPAAC Requirements state that, before the issue of a cryopreserved product, the viability and enumeration of a relevant target cell population must be evaluated from a sample of the product using a relevant and validated test.

Our practice, as an exempt facility, is to perform initial quality control testing of the frozen product on a reference vial within 72 h of the cryopreservation by CFU-GM assay.

As a previous study [2] provided some (weak) evidence of deterioration during long-term cryostorage, we repeat quality control testing using (CFU-GM assay and viable CD34+ HPC enumeration) on a reference vial stored with a cryobag prior to release of product that has been stored for five years or longer.

3) We perform CFU-GM assays on a thawed reference vial generally within 72 h of the cryopreservation as initial quality control of the frozen sample. A trypan blue exclusion is performed as part of that assay but is 'for information only' and is not validated against engraftment times. As stated above, we also repeat CFU-GM and perform viable CD34+ HPC enumeration prior to the issue of product has been in cryostorage for longer than five years.

4) Yes. CFU-GM assays using Methocult GFH 4534.

5) Yes. We have determined threshold dosages that are associated with engraftment within an acceptable timeframe.

6) We validated institutional threshold numbers for CD34+ HPC and CFU-GM content of autografts by monitoring engraftment times against the infused dosage of these cells. The engraftment criteria used were (1) Days to ANC > 0·5/nl and (2) Days to platelet count > 20/nl (unsupported) with a maximum acceptable engraftment time of 14 and 20 days, respectively. We set the threshold content for CD34+ HPC and CFU-GM just above the respective infused cell dosages where platelet engraftment first exceeded the maximum acceptable time. Interestingly, delayed platelet recovery times were used as the threshold trigger as delayed neutrophil recovery was only observed at comparatively lower CD34+ HPC and CFU-GM content, and delayed neutrophil recovery was always associated with delayed platelet engraftment. Engraftment times and infused CD34+ HPC and CFU-GM dosages are formally reviewed, and the validation confirmed, on an annual basis.

We have different threshold dosages for the two HPC sources we utilise for our autografts – mobilised PBSC and GCSF-primed bone marrow. Our internal validation showed that delayed platelet engraftment occurred at a lower CD34+ HPC and CFU-GM dosage when GCSF-primed BM was used as the autograft source.

7) If the initial frozen product testing reveals insufficient CFU-GM content, then our first response is to repeat the CFU-GM assay using a second cryopreserved reference vial and concurrently perform a viable CD34+ HPC enumeration by flow cytometry. This allows a determination of whether an event has arisen during the cryopreservation process that has resulted in the loss of viable CD34+ HPC with a concomitant reduction in CFU-GM potential. Initial CD34+ HPC enumeration is performed on a fresh (immediately prior to cryopreservation) PBSC sample in order to provide a rapid result to aid clinical decision making as to whether a subsequent apheresis collection is required.

Our institutional policy is that both CD34+ HPC and CFU-GM thresholds must be exceeded in order for the product to meet acceptance criteria. CD34+ HPC and CFU-GM content of cryopreserved autografts are reported to the requesting physician and the transplant unit, generally within 2–3 weeks from collection. If a product does not meet these requirements, then the report contains a recommendation that a second mobilisation and collection be performed in order to 'top up' the autograft.

Issuing/release of cryopreserved units that contain CD34+ HPC and/or CFU-GM numbers below our institutional threshold constitutes an Exceptional Release. The Exceptional Release process informs the Transplant Unit of the suboptimal CD34+ HPC and/or CFU-GM content (and as such informs their clinical decision making) and also requires the informed consent of the recipient for the release to occur.

8) The Royal College of Pathologists of Australasia operates a national external proficiency testing program for CD34+ HPC enumeration. Participation is mandatory for all laboratories who are accredited to perform this test.

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1. In our institution, one aliquot of the whole product (quality control after freezing and one week of storage) and one aliquot for every single bag (quality control before transplantation) are frozen and stored with the stem cell product under equal conditions. A serum aliquot (back up sample) of the patient/donor is frozen and stored for 30 years according to the guidelines of the tissue bank regulations.
2. Samples from the whole product are analysed after one week of storage to assess cell loss during the freezing procedure. If one bag is ascertained for transplantation the appropriate sample is thawed and analysed for CD34 viability before start of the conditioning therapy.
3. Routinely, after freezing and thawing flow cytometry based total cell viability is assed by a single platform method (TruCount; CD45/34/7-AAD).
4. CFU assays are performed on 1% of the products. Moreover they are assessed for validation of new harvest or processing methods.
5. A minimal number of 2×10^6/kg CD34 positive cells is defined as the threshold for successful transplantation [1].
6. The thresholds of CD34 positive cells for successful transplantation are well defined in the literature and differ between bone marrow, peripheral blood stem cells ($> 2 \times 10^6$/kg CD34+ cells) and cord blood ($> 3.7 \times 10^7$/kg nucleated cells; $> 2 \times 10^5$/kg CD34+ cells) [2,3]. Our requirements are defined according to the published data and no graft failure in patients undergoing transplantation with cryopreserved products have been seen in the last 10 years.
7. In case of an insufficient collection or cell loss during processing leading to $< 2 \times 10^6$/kg CD34+ cells the respective physicians are informed. Products are discarded if no additional cells can be collected in consecutive collections or the transplant physician refuse to use an insufficient product.
8. Our stem cell laboratory takes part in external national proficiency testing on a three monthly basis for the following assays: flow cytometry based single and dual platform analysis (CD34/45) and complete blood count on a hematology counter.

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Question 1

Three samples (approximately 0.5 mL each) are collected directly from the bags, immediately before freezing, and are kept in the same freezer (−80 °C) together with the bags. Our freezing protocol is a non-controlled-rate freezing using dimethyl sulfoxide (DMSO) and hydroxyethyl starch (HES) combination [1]. Their main purpose is to assure back-up samples. They are kept for a maximum period of two years after the transplant. If for any reason the transplant is not performed, the samples and the bags are kept until a final medical decision is made according to the patient's needs.

Question 2

As mentioned above three samples are frozen together with the bags. If there is a problem with the microbiological tests performed in the freezing day (i.e. positive results), one sample is also used as a back-up for microbiological studies.

If the transplantation occurs after two years of storage, one of these samples is used to check for viability (by Trypan Blue dye exclusion). Based on this viability test, there is an estimation of remaining CD34+ cells. If there is more than 2×10^6 CD34+ viable cells/kg, the material may be used for transplantation.

Question 3

During the freezing procedure, two samples are collected for microbiological tests and mononuclear cell count, before and after addition of DMSO. CD34+ cell count including molecular exclusion with 7-amino actinomycin D is performed only in the sample collected before DMSO addition [2]. This dosage is the one used to calculate our threshold for peripheral blood collections ($2{\cdot}5 \times 10^6$ CD34+ viable cells/kg).

After thawing, a sample is collected from each bag to be transplanted and microbiological tests (anaerobic and aerobic bacteria and fungal cultures) are performed.

Question 4

No.

Question 5

There are thresholds for our patients, but if the minimum numbers are not achieved, the final decision is based on the clinical analyses of the patient with the transplantation team. The minimum requirement for autologous bone marrow transplantation is $2{\cdot}0 \times 10^8$ nucleated cells and $1{\cdot}0 \times 10^6$ CD34 cells/kg [1]; for peripheral blood is $2{\cdot}5 \times 10^6$ CD34 cells/kg. There is a slight difference for allogeneic transplants; the minimal requirement for bone marrow transplantation is $3{\cdot}0 \times 10^8$ nucleated cells/kg [1] and although CD34 is measured, it is not considered as a threshold. For peripheral blood we recommend a dose between $3 - 5 \times 10^6$ CD34/kg.

Question 6

Our threshold is based on the current medical literature [3]. Although Brazil has a national regulation about cryopreserved haematopoietic progenitor cells [4], there is not a defined threshold. There are variations on the threshold according to the origin of the progenitor cells (see answer to question 5). We do not perform Umbilical Cord Blood Transplantation.

Question 7

If the product does not meet the target cell dose, we advise the transplantation team to try another mobilization strategy to accomplish the required cell dose. We do not discard the product and the transplantation team decides what to do on individual basis, considering the patient's condition and published literature concerning bone marrow and peripheral progenitor cells transplants using lower cell doses than recommended [5–7].

Question 8

Yes. We participate in the UK-external Quality Assessment Service (UK-NEQAS) for determination of absolute and relative numbers of CD34+ stem cell enumeration. We also participate in a national proficient test for leukocytes enumeration.

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Question 1

Canadian Blood Services operates two haematopoietic progenitor cell programs, in partnership with clinical transplantation programs. Our Ottawa stem cell program, run jointly with the Ottawa Hospital, provides collection and laboratory processing services of predominantly autologous progenitor stem cells to three external sites, Ottawa, Kingston and Sudbury performing approximately 140 transplants per year. Our Edmonton program, run in conjunction with the University of Alberta and the Cross Cancer Institute, provides autologous progenitor stem cells to the Cross Cancer Institute, performing approximately 60

transplants per year. There are some differences between the programs, in order to meet customer needs and expectations of the clinical transplant programs.

In the Ottawa program, two 1 ml sample aliquots are cryopreserved together with the bags. At the present time, they are stored separately but under the same conditions as the bags. We are reviewing this policy and will likely change to storing the samples together with the bags. In addition, two plasma samples of 2 ml each are frozen.

In our Edmonton program, three sample aliquots of 1·5 ml are cryopreserved in parallel with the bags and are stored with the bags.

In both programs, the purpose of the samples is for both short-term and long-term QC as outlined in Questions 2 and 3 below. This may include viable CD34+ enumeration, CFU-GM enumeration, and repeat sterility testing. Unused samples may be utilized for research or developmental projects if donor consent has been obtained at the time of donation.

Question 2

In our Ottawa program, QC of frozen samples is not routinely performed. Decisions regarding QC testing are made based on patient outcome and discussions from the Clinical and Laboratory Directors and Supervisors. The majority of sample QC testing will be done for patients with delayed engraftment or clinical complications, or products with positive microbial testing.

In our Edmonton program, viable CD34+ and CFU-GM enumeration is performed on thawed samples when the total fresh product CD34+ cell cut-off falls below $3{\cdot}64 \times 10^6$/kg and/or the fresh product CFU-GM falls below $3{\cdot}23 \times 10^5$/kg. This is in part based on our own data [1, 2]. Other testing is performed as needed, for example in cases of processing or storage deviations, positive sterility testing on the component, delayed engraftment or adverse reactions.

Question 3

As mentioned above we do not perform routine quality controls in our Ottawa program. When required, post-thaw analysis testing consists of CFU-GM culture assay and viability by trypan blue dye exclusion.

In our Edmonton program, Viable CD34+ counting and culture assays using CFU-GM enumeration are performed as outlined in Question 2.

Question 4

In both programs, stem cell culture assays are performed using methylcellulose based commercial media from Stem Cell Technologies (MethoCult GF H4434) without density-based cell separation.

Question 5

In both programs, we do not have a strictly defined acceptable range or threshold for transplantation. However, we have a recommended dosage that physicians hope to achieve for transplant.

In our Edmonton program, the laboratory provides guidance to the transplant facility stating that infusion of products with cell counts below a recommended level may be associated with neutrophil engraftment of > 14 days and/or platelet engraftment of > 21 days.

Question 6

Acceptable ranges were defined by clinical sites based on both previous clinical results and current literature. Some studies were done in our Edmonton program to establish local clinical data [1, 2]. In both programs the same value is used for bone marrow and peripheral blood, although bone marrow processing is rarely performed. Ranges do differ for autologous vs. allogeneic products and may differ for individual patient diagnosis.

Question 7

If a product does not meet the usual requirements, the clinical site will be notified and subsequent patient transplant and collection decisions will be made by the transplant physician. In our Edmonton program, we have an exceptional release process which requires specific acknowledgement by the laboratory medical director and transplant physician that the product cell count is below accepted levels.

Question 8

Both our programs are FACT accredited and participate in external proficiency testing for CD34 enumeration. The Ottawa program participates in a provincial proficiency program for flow cytometry, Quality Management Program Laboratory Services, while our Edmonton program participates in College of American Pathologists proficiency program. Difficulties with proficiency programs for CFU assays have been related to expiration of samples prior to arrival in our laboratories.

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1. We store two attached segments. When a cord blood unit is ordered, we will carry out confirmatory HLA typing, trypan blue exclusion assay and colony forming unit (CFU) assay. In addition to the contiguous segments, we freeze the following separate samples for possible additional tests: (1) four test tubes of plasma (e.g. for infectious agent testing); (2) two tubes of red blood cell waste (with enough leukocytes for DNA analyses); (3) two 0.5 ml samples of the final cryopreservate and (4) a segment of the cord.
2. Quality controls of the frozen unit are performed when the unit is ordered by a transplant centre.
3. TNC count, cell viability by trypan blue exclusion, CD34 + cell enumeration, CD34 + cell viability by 7-AAD exclusion, CFU cultures, and sterility testing.
4. Stem cell technologies, Methocult assay.
5. No thresholds have been set. The CFU counts are used for quality control of the processing of units.
6. Not applicable (see Question 5).
7. Not applicable (see Question 5).
8. We participate in international quality assessment for CD34 + cell enumeration, as well as for CFU enumeration.

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1. For each bag frozen, five satellite tubes are cryopreserved in parallel. One of the frozen satellite tubes, for anaerobic and for aerobic analysis, are for sterility testing. One tube is used for evaluation of viability and one serves as a back up sample for a potential repetition of viability testing together with analysis of clonogenic potential. The fourth tube is for possible testing after storage for longer time periods and the last tube is stored as a retain sample after transplantation for legal purposes according to the national guidelines.

In case that more than one transplantation dose was harvested and split in several bags, these bags are stored in separate liquid N_2 tanks for safety reasons. It is always aimed to store bags and the corresponding satellite tubes in the same tank although this can not always be achieved.

2. Sterility testing is assessed on the next working day after storage in the vapour phase of liquid N_2. Viability analysis is performed after a minimum storage time of 24 h at $\leq$ -140 °C. Repetitions of this analysis together with analysis of clonogenic growth are performed in between 1–2 weeks after cryopreservation.

3. After freezing and thawing of HPC analysis of membrane integrity by staining with the viability dye 7-AAD (7-Aminoactinomycin A) is performed by flow cytometry according to ISHAGE guidelines [1] for CD45+ cells as well as for CD34+ cells as published recently [2]. In case of a viability below 70% for CD34+ cells or between 50–70% for CD45+ cells, the analysis is generally repeated and complemented by analysis of clonogenic growth.

4. In case of a decreased viability of CD45+ cells or CD34+ cells or in case of any abnormality or deviation during processing analyses of clonogenic growth are performed. Such analysis consists of the evaluation of colony forming unit-granulocyte macrophage (CFU-GM) by a commercially available kit (MethoCult, Stemcell Technologies, Vancouver, Canada).

5. A minimum number of 2×10^6 CD34+ cells/kg of body weight before cryopreservation in the autologous and 4×10^6 CD34+ cells/kg of body weight in the allogeneic setting are the aimed dose for transplantation. After cryopreservation, the threshold viability of CD45+ cells is 50% and for CD34+ cells is 70%. In case of undershooting the latter value it is aimed that at least 1×10^5 CFU-GM/kg of body weight can be detected in the sample.

6. The values before cryopreservation for HPC derived from peripheral blood (PB) of a mobilised healthy donor or from PB of a mobilised patient are derived from literature [3, 4] and from guidelines of the German national authorities (Paul-Ehrlich-Institute (PEI), http://www.pei.de). The values after cryopreservation for these grafts are deduced from own data and are in agreement with the published specifications of the PEI.

Additionally, HPC from bone marrow (BM) are processed and tested in our laboratory. Although freezing of HPC derived from BM occurs only rarely in our laboratory the

specifications regarding viability of CD34+ cells or CD45+ cells after cryopreservation should supposedly be the same as for cryopreserved HPC derived from mobilised PB. Furthermore, the same assumptions should be correct for CFU-GM analysis of HPC derived from BM.

HPC from CB are not processed or tested in our laboratory, yet.

7. In case of a low viability of CD34+ cells and no clonogenic growth the responsible transplant physician is informed and the product is not released. In such very rare cases, patients or allogeneic donors are mobilized and harvested a second time.

8. Our laboratory participates in the German (INSTAND e.V.) and the Austrian (OEQUASTA) external proficiency testing for the enumeration of CD34+ cells. In addition, the laboratory participates in the international external proficiency testing for the analysis of clonogenic growth offered by Stemcell Technologies.

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Question 1

Two different procedures are followed:

(I) When harvesting and freezing are both performed within our centre, we have validated the collection and freezing process as a standardized procedure. Frozen sample aliquots are retained by controlled freezing alongside the product bag, but not routinely used for release-determining quality controls. Instead, they serve for ongoing quality control purposes and/or look-back. Before freezing a sterility test is performed and the number of viable CD34+/CD45+ cells/kg body weight (BW) of the harvested product is determined by flow cytometry using a single platform validated test.

(II) When freezing in cooperation with external centres, four reference samples are frozen together with the respective haematopoietic progenitor cell graft using a programmable freezer and are also stored at −140 °C in the vapour phase of nitrogen, but not in the same container.

Following protocol (II), one sample is thawed > 24 h after cryopreservation for quality controls (see Question 3) and serves as prerequisite to release the cryopreserved stem cell graft for transplantation. The second sample is stored for two years following autologous and for five years following allogeneic transplantation as a repository sample for potential re-examination. The third sample is used as quality control for long-time storage if parts of the graft are stored for a longer period of time. The fourth sample can be used for additional investigations, e.g. study protocols for tumour cell contamination of autologous grafts.

Sterility testing is only done of the ready-to-freeze product and of the additives involved for cryopreservation (prerequisite for reagent clearance).

Question 2

As applicable (see above), within one week of concluding the cryopreservation process the first sample aliquot is thawed and analysed in order to release the frozen stem cell graft for clinical use. This applies specifically to products collected elsewhere, if we are responsible for their release.

Question 3

At one of the two sites of the German Red Cross Blood Service reporting here, quality controls of the frozen sample aliquot for releasing the cryopreserved stem cell grafts are as follows:

1) Total nucleated cell (NC) count and calculation of NC-recovery after freezing and thawing.
2) Viability tested by trypan blue dye exclusion.
3) Plating of 1×10^5 viable NC for *in vitro* culture assays (BFU-E and CFU-GEMM).

Less stringent quality criteria are applied at the other site, in that no post-thaw data are generated from companion tubes. Release is solely based on pre-freezing CD34+ and NC counts, microbiology/virology and viability, and the

quality of the freezing process/freezing curve. The rationale is that the freeze–thaw process is automatized and thus produces cell products of consistent quality, as confirmed by regular re-validations (flow with viability dyes, colony assay). Recommendations for required CD34+ cell doses are based on pre-freeze numbers.

Question 4

In vitro culture assays (BFU-E and CFU-GEMM) for evaluating the proliferative capacity of the cells after freezing and thawing are performed in semi-solid, cytokine-replete methylcellulose-based media using a commercial kit according to the manufacturer instructions (StemCell Technologies, Vancouver, Canada). Clearance is based on qualitative, not quantitative data.

Question 5

Our target dose for autologous peripheral blood stem cell (PBSC) grafts is $\geq 2\text{–}4 \times 10^6$ viable CD34+ cells/kg BW and for allogeneic PBSC grafts $\geq 4\text{–}5 \times 10^6$ viable CD34+ cells/kg BW of the recipient. Depending on the site, after cryopreservation the cells have to prove their multilineage proliferative capacity by *in vitro* colony assay prior to release.

Question 6

The thresholds and requirements for the several sources of haematopoietic progenitor cell grafts are defined by our own clinical experience, requests from our clinical colleagues, published data and by national/international recommendations/regulations [1–4].

For allogeneic bone marrow grafts our target dose is $\geq 2\text{–}4 \times 10^8$ NC/kg BW and for cord blood grafts $\geq 3 \times 10^7$ NC/kg BW, respectively. For autologous and allogeneic PBSC see Question 5.

Question 7

If a stem cell product does not meet release criteria, the individual risk and situation of the patient/donor are discussed between the responsible physicians of the stem cell processing unit and the transplantation unit and if possible, a further collection of stem cells is performed. Grafts proven positive for bacterial contamination would only be released for transplantation if absolutely necessary and using antibiotic prophylaxis of the recipient adapted to the antibiotic sensitivity of the respective bacterial strain.

Question 8

Our laboratory takes part in external national and international proficiency testings at regular time intervals for:

1) Automated cell counting (NC, differential blood count, Hb, Hct, platelet count).
2) Sterility test (aerobic and anaerobic).
3) Viability testing using trypan blue dye exclusion.
4) Flow cytometry using a single-platform technique including viable CD34+/CD45+ cells.
5) *In vitro* culture assay (BFU-E, CFU-GEMM, colony identification).

All described techniques are performed in accordance with manufacturers' instructions and in agreement with the recommendations of the European Pharmacopeia [5].

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P. Accorsi

Question 1

In regards to HPC collection and storage our policy is the following:

We cryopreserve minimum two bags for each HPC-A/HPC-M collection and we aliquote one sample for each bag of HPC-A/HPC-M (total minimum two samples for each collection).

The samples are stored with the bags.

We perform the following quality control (QC) on one sample [1,2]:

1) Cell count with hemocytometer
2) Cell count CD34+ with flow cytometry method
3) Viability 7AAD and trypan blue
4) Clonogenic tests for:
   - CFU-GM
   - BFU-E
   - -CFU-GEMM
   - -CFU-C.

The second sample is stored for back-up:

1) In case of adverse events during transplantation course
2) Or for long term studies.

The sterility is performed immediately before cryopreservation.

Question 2

We perform QC before transplantation, at the moment that Clinic Unit asks us for the availability of the HPC (generally from three to two weeks before thawing).

We verify:

1) bags integrity
2) temperature during the storage
3) QC on one sample (as above mentioned).

Question 3

We perform the same QC that is performed on the sample:

1) Cell count with hemocytometer
2) Cell count CD34+ with flow cytometry method
3) Viability 7AAD and trypan blue
4) Clonogenic tests for:
   - CFU-GM
   - BFU-E
   - -CFU-GEMM
   - -CFU-C.

Question 4

We perform the following clonogenic testing [3,4]:

- CFU-GM
- BFU-E
- CFU-GEMM
- CFU-C.

Question 5

Our HPC-A target dose for transplantation is CD34+ = 5 $\times 10^6$/kg $\pm$ 10%.

At times we perform the transplantation procedure with lower CD34+ values in accordance with the Director of Clinic Unit. The minimum CD34+ value for transplantation is 2·5 $\times 10^6$/kg.

Our HPC-M target dose for the transplantation is TNC 1–3 $\times 10^8$/kg and MNC = 0·5–1 $\times 10^8$/kg.

Question 6

Our policy derives from our clinical experience dating back since 1978 and as according to published works.

Actually, the HPC-M source is used routinely fresh for allogeneic transplantation; very rarely the cryopreserved HPC-M source is applied for autologous setting.

Question 7

If the products do not meet our target dose standard or the products do not meet the required standards (e.g. sample with viability lower than 50%, sterility alteration before cryopreservation, etc.) we inform the Director of Clinic Unit and together we reach a decision on the use of the HPC cryopreserved.

In our experience, patients sign an informed consent form stating that if the HPC are not utilized within 5 years, they will be discarded.

Question 8

Our laboratories take part of national and international proficiency testing (Valutazione Esterna di Qualitá) for all of the abovementioned tests [5,6].

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T. Bonfini & E. Liberatore

Our cord blood bank (CBB) policies are constantly updated according to the issues of the national laws and the improvement of international guidelines. At the present, we are applying for FACT accreditation and validating new procedures and policies to meet the standard requirements [1, 2]. The answers to the questions below mentioned describe our current practices.

Question 1

For each CB unit (CBU) we collect the following reference samples:

- Four aliquots (0·5 ml) of CBU final product (buffy coat including DMSO) are stored in the same conditions of the CBU in order to perform the following quality controls (QCs) before the release of the CBU: two samples for identity and potency assay as described in the answer to question 3, the third is retained as backup sample to repeat the test in case the results do not meet the established criteria or a look back sample in case of failure or delayed engraftment and the fourth sample is sent to the Transplant Centre together with the CBU;
- Three plasma cord (1·8 ml) are stored at −80°C, for infectious disease markers (IDMs);
- Three mother plasma/serum (1·8 ml) are stored at −80°C, for IDMs;
- A DNA cord blood sample (≥50 μg) is stored at −80°C;
- A DNA mother sample (≥50 μg) is stored at −80°C.

Sterility is performed on fresh product immediately after addition of cryopreservation solution.

Question 2

When we receive a CBU request from a Transplant Centre through our National Registry, we perform QCs on frozen reference samples to give the availability of the CBU. The cryopreservation process was validated when we optimized the method. Furthermore, we perform QCs on selected thawed CBUs and reference samples on a monthly basis. This QC is also considered as 'stability testing' because thawed CBUs are chosen according to the length of their storage ranging from one to ten years, going back to when we started to bank CBUs for clinical use.

Question 3

After freezing and thawing a CBU sample, we routinely perform the following identity e potency QC:

- HLA confirmatory testing;
- ABO blood group by molecular testing;
- Nucleated Cell and NRBC count by automatic analyzer;
- Nucleated cell / MNC viability by flow cytometry-based permeability marker (7-AAD);
- CD34+ cell count and viability by single platform flow cytometry method (CD34/CD45/7-AAD, ISHAGE protocol, EWGCCA modified);
- Stem cell culture assays by metilcellulose-based clonogenic test (STEM CELL Technologies).

Question 4

We perform stem cell culture assay by metilcellulose-based clonogenic test (MethoCult GFH8444, Stem Cell Technologies) enumerating CFU-GM, BFU-E, CFU-GEMM.

Question 5

Published works and clinical experiences generally recommend to ensure a nucleated cell dose/kg recipient weight $\geq 3/3{\cdot}5 \times 10^7$ with an accepted threshold dose of 2×10^7 NC [3]. These values are established on fresh products, before freezing, but recoveries at thawing can change widely from one CBU to another. Based on these criteria, each CBB establishes a banking threshold. Our policy changed during time. In the past we have banked CBUs with a content of 8×10^8 TNC but, since 2009, we bank only CBUs with the same TNC value and a content of CD34+ cells $\geq 2 \times 10^6$. Considering the fact that the cell content of CBUs issued is higher, we are starting to bank only the CBUs with at least 10×10^8 TNC, according to Italian national requirements as well as current literature [4].

Question 6

We defined acceptable ranges for stem/progenitor cell recovery of the thawed CBUs according to our own process validation and published works [5]. Our acceptable thresholds of cell recovery rate and viability to allow release are the following:

- TNC/MNC/CD34/CFC recovery ≥ 50%;
- NC viability ≥ 50%;
- MNC viability ≥ 70%;
- CD 34+ viability ≥ 80%;
- Clonogenic efficiency ≥ 10%.

Question 7

If results of the QC do not meet the requirements, and if it is still available, we thaw a second sample to repeat the

tests; the type of discrepancy and the grade of deviation are evaluated and the Transplant Centre (TC) and our National Registry are informed. If viability is satisfactory, but other criteria are not met, we check if a change in the laboratory methods has been adopted for fresh samples as compared to the current method (i.e. single/double platform for CD34 count, number of seeded cells in the CFU assay and a different clonogenic efficiency). In this case, we explain to the TC the reasons of the inconsistency and, if they agree, we negotiate the release of the CB considering also if, in any case, the content of the CBU ensures a minimum cell/progenitor dose required from the transplant program. Instead, if the results confirm low viability and low potency assay, we inform the Transplant Centre and our National Registry and withdraw the CBU from the inventory; the CBU is discarded from clinical use and QCs are repeated on the thawed CBU to have the confirmation of the deviation.

Question 8

Our laboratories participate to the following external proficiency testing programs:

- Cell count every two months (Proficiency Testing for Haematology – Hemocytometer cell counter, DICS – DASIT)
- CD 34 count every two months (Stem Cell Quantitation Program, UK NEQAS)
- Stem Cell Culture assay every six months (Proficiency Testing Program for frozen cord blood colony assay – STEMCELL Technologies).

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M. Takanashi

The following answers refer to The Metro Tokyo Red Cross Cord Blood Bank

Question 1

We freeze 14 sample aliquots, 11 of the cord blood and three blood samples from the mother. The 14 aliquots are stored in the ways and for the purposes listed below:

(1–3) The final product, three tubes of 0·2 ml each, including DMSO, at −196 °C. One is for quality control before release of the cord blood unit, one is for HLA confirmation before release, and the third is a backup.

(4) The final product, 0·15 ml in the 3 cm segment of the tube attached to the cord blood unit, including DMSO, at −196 °C, as a backup for quality control. This is the only aliquot stored with the bag.

(5) Cord blood (whole blood) with anticoagulant, one tube of 0·5 ml, at −80 °C. A source of DNA.

(6,7) Cord blood plasma, two tubes of 0·5 ml each, at −80 °C, for a CMV IgM test when the mother's CMV IgG/IgM is positive. The leftover is stored for a look back test.

(8) Cord blood buffy coat, one tube of 1·0 ml, at −80 °C. A source of DNA.

(9,10) Cord blood plasma including HES, two tubes of 1·5 ml each, at −80 °C. One for a HIV NAT, the other as a backup for a NAT.

(11) Cord blood RBCs separated by HES sedimentation, one tube of 1·5 ml, at −80 °C. A source of DNA.

(12,13) Mother's serum, two tubes of 0·5 ml each, at −80 °C. Backups for infectious marker screening.

(14) Mother's blood clot, one tube of about 2·5 ml, at −80 °C. A source of DNA.

Question 2

Before releasing the unit to the transplant centre.

Question 3

Before releasing the unit we perform the following tests:

1) Cell count, with a Sysmex auto-cell analyzer.
2) Total cell viability, with EB/AO fluorescence counting.
3) CD34+ cell counting, with CD34/CD45 double staining and 7AAD.
4) Stem cell culture assay.

For regular quality control and validation of our procedures, we perform the same tests on one unit of every 100 units that are cryopreserved, testing each of the sample tube, the segment tube, the 5 ml compartment and the 20 ml compartment of the cryobag.

Question 4

The stem cell culture assay we perform is the counting of CFU-GM, BFU-E and CFU-mix after two weeks of culture in a medium from STEMCELL Technologies.

Question 5

The viability of the total nucleated cells should be 70% or more. The recovery rate of CD34 positive cells compared with before freezing should be 60% or more. The recovery rate of the total CFU compared with the total CFU before freezing should be 50% or more, though the CFU assay result is used only for validation, and not for the release decision.

In Japan, the preferred minimum cell dose is 2×10^7/kg patient weight, calculated based on the cell count before freezing.

Question 6

Our cord blood bank established the pre-release test requirements by ourselves. From our test result experience, the average of the viability is 80% with a full recovery of the total nucleated cell count. The average recovery rate for CD34 positive cells is 80% and the recovery rate of CFU is 70%, though each test result shows a wide range.

The cell dose, calculated before freezing, was originally established based on early international reports on CBT [1, 2].

Question 7

When the viability or the recovery rate of CD34 or CFU does not meet the criteria, we do the test again with the segment of the tube stored with the cryobag.

When the test result does not meet the criteria again, we call the transplantation centre and explain the result. In the cases when the cell viability is satisfactory but one of the other criteria is not met, and the prefreezing test result looks to have a possible overcounting, we suggest to use the cord blood unit. Otherwise, we search for other units and suggest to the transplantation centre to change the donor cord blood unit.

Question 8

Yes. For national proficiency testing, taking turns, one of the 11 public cord blood banks in Japan prepares cord blood aliquots, and sends frozen samples to other banks, to test cell counts, viability, CD34+ cells and CFU.

For international proficiency testing, our laboratory joins the Proficiency Testing Programs of STEMCELL Technologies.

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M. Grommé, F. W. M. B. Preijers, G. J. Schuurhuis, E. Braakman & I. Van Riet on behalf of the WSN

Question 1

In the Netherlands, the Ministry of Health has assigned hospitals that are allowed to perform allogeneic hematopoietic stem cell transplantations with family and/or unrelated donors and/or autologous transplantations in adults and/or children. In Belgium, only EBMT-accredited transplantation centres can receive reimbursement of costs from health care authorities. JACIE accreditation of the collection facility, the cell processing facility and the clinical unit of these hematopoietic transplantation centres is mandatory by law in the Netherlands, but not in Belgium. The majority of the transplantation centres in the Netherlands and Belgium have obtained JACIE accreditation, the others have applied. Hematopoietic progenitor (HPC) bone marrow cells and peripheral blood stem cells are cryopreserved for transplantation purposes. The Dutch and Dutch-speaking Belgian processing facilities are united in a working group of the HOVON called the WSN.

JACIE requires the availability of sample aliquots of the cell product that is to be cryopreserved. These aliquots must be stored under such conditions that they are a valid representation of the product [1]. WSN processing facilities cryopreserve 2–5 aliquots simultaneously with the transplant in the same cryopreservation device. These frozen aliquots are stored either in the same or another cryostorage tank as the clinical product. The aliquots are used for quality control testing of the clinical product and as back up sample for instance if the sterility testing results are positive.

Question 2

The JACIE standards describe the tests that need to be performed on cellular therapy products (D6.1.3), but do not indicate at which time point. Between WSN laboratories there is no uniformity concerning the time points at which the frozen samples are analysed. Some processing facilities have included the quality control test results of the frozen aliquots in their release criteria; other laboratories evaluate quality control test results retrospectively.

Question 3

There is also no uniformity in the quality control tests that are performed on these frozen aliquots. Quality controls tests include viability testing and CD34-positive cell analysis and may include HPC colony assays. Viability testing and CD34-positive cell analysis are usually combined in flow cytometry analyses with a fluorescent viability dye. The WSN provides in a proficiency testing program which includes CD34-positive cell analysis and HPC colony assay of cryopreserved samples.

Question 4

Most stem cell laboratories of the WSN perform HPC colony assays to detect the proliferation and differentiation capacity of the cell product. To evaluate BFU-E, CFU-GM and CFU-GEMM colonies, the culture media of StemCell Technologies Inc. and Miltenyi Biotec are used. In addition to the WSN proficiency testing program, the majority of the laboratories that perform this assay also participate in the proficiency testing program of HPC cultures provided by StemCell Technologies.

Question 5

There are defined thresholds for the number of peripheral blood CD34-positive cells/kg of bodyweight of the patient for specific transplantation indications depending on the stem cell source (HPC, apheresis, HPC, marrow, HPC, cord blood). In general, these numbers are all based on CD34-positive cell numbers in the graft before cryopreservation, although the quantity of viable CD34-positive cells infused may vary significantly due to cell death as a result of the cryopreservation and thawing process [2]. For bone marrow stem cell transplantations the number of (mono)nucleated cells, rather than the number of CD34-positive cells, is frequently used to define thresholds. This is based on historical studies in which CD34-positive cell analysis was not common practice yet [3].

Question 6

The thresholds for the number of CD34-positive cells/kg in the graft are derived from national (HOVON) and international (e.g. HOVON/SAKK, EORTC) clinical studies. Results of these studies are discussed in working parties and at the EBMT annual meetings and published in peer-reviewed international journals. However, each transplantation centre can (and in practice does) define their own thresholds and there are differences in doses of stem cells given for specific indications between centres.

Question 7

When a peripheral blood stem cell harvest does not contain the requested target cell number, then multiple harvests can be performed by continuing the mobilisation by G-CSF or other mobilising agents, e.g. Plerixafor. Also a bone marrow harvest can be performed to ultimately transplant with the HPC-A and HPC-M cell products to infuse sufficient cells. When in the end a product does not meet the cell number requirement, then the transplantation physician is informed. The decision to infuse or discard the product is at the discretion of this physician.

Question 8

The stem cell laboratories participating in the WSN take part in the WSN proficiency testing for CD34-positive cell analysis and HPC colony assays on frozen samples, as well as in the HPC colony assay proficiency testing program of StemCell Technologies and the UK NEQAS CD34-positive stem cell proficiency testing programme for CD34-positive HPC cell enumeration in stabilised samples. Also workshops are provided to train laboratory technicians in the state of the art flow cytometric analysis protocols and to improve performance reflected by the reduction of intra- and inter-laboratory variation [4].

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J. M. Van Beckhoven & A. Brand

Question 1

Cryopreserved bone marrow and peripheral blood stem cells are in practice only stored for autologous use. This is in the Netherlands performed by hospitals with an autologous stem cell transplantation program. Umbilical Cord Blood (UCB) is – except a patient-named minority intended for related transplantation – banked as cryopreserved product available for treatment of unrelated recipients in transplant centres worldwide.

In the Netherlands, a Cord Blood Bank (CBB) was founded in 1994 by one of the regional Red Cross Blood Banks. Since the merge of the Dutch Blood Banks in 1998, one national CBB operates under responsibility of the national blood supply foundation, Sanquin. Currently the CBB has ca. 3000 units available for request by a transplant centre.

It is obvious that during the 15 years of her existence, knowledge in CB banking advanced. Cord blood processing prior to freezing changed from whole blood to various methods of red cell and plasma depletion. For quality control procedures gradually guidelines, laws and international accreditation requirements and audits became available.

From the start of the CBB the policy was to store additional reference samples from the mother and the UCB, also suitable for preparation of genomic DNA. In the period 1994–2002, this material was processed and cryopreserved in ampoules separated from the UCB product.

In 2002, besides above mentioned maternal and UCB separated reference ampoules for post cryopreservation viability and progenitor cell growth assays, we introduced the segments which are sealed and integrally attached to the UCB freezing bag, intended for confirmatory HLA typing.

Since 2007, the Dutch CBB operates in accordance to the NetCord/FACT guidelines [1], which require a minimum of reference UCB samples and maternal samples.

Todays practice is volume reduction using an AXP-device (Thermogenesis, USA), cryopreservation in 10% DMSO (v/v) and three segments, integrally attached to the UCB freezing bag.

One of these segments is used for confirmatory HLA typing by the CBB (Identity or verifying HLA typing) and another segment is used for viability and CFU assays, both to be used prior to release for distribution of the UCB unit to a Clinical Program. The third segment is intended for the Transplant Center for investigations of their choice.

In addition, we cryopreserve four reference vials (0·9 ml), stored separated from the UCB bag which can be used for viability and CFU assays prior to release for listing of the UCB unit in international search files (or prior to release for distribution when results of the segment needs to be confirmed; see Question 5). This material is only used in selected cases, when routinely applied quality control reveals uncertainty about the process (e.g. freezing curve, pre-cryopreservation cell recovery).

Question 2

The UCB quality control is performed on (1) a once-monthly randomly selected UCB and related samples for process control, (2) product control of every product prior to release for administrative listing and (3) product control of every product prior to release for distribution to a Clinical Transplant Program.

1) For process control every month one randomly selected cord blood donation, including segments and reference ampoules, is subjected to the complete procedure of pre and post-volume reduction and post-cryopreservation and thawing. The counts of different cell populations (NC, MNC, CD45, CD34) are determined by flowcytometry and a HPC culture assay (CFU-GEMM) is performed on reference samples and the thawed UCB unit. This allows to evaluate the two

critical phases of UCB processing compared to the whole blood CB unit. Since 2000 the freezing bag consist of two compartments, both are separately tested after thawing. This process control is intended to verify stability of the production process including cryopreservation and the thawing procedure. Process control requires > 80% recovery of all cell subsets and > 90% viability post-processing, prior to cryopreservation. Post cryopreservation recovery of all cell subsets must be higher than 50% and the viability of CD34+ cells higher than 50%.

2) Prior to release for listing in the international search administration, quality control is performed on every product. Routinely, product control for each UCB includes a pre- and post-volume reduction (pre-cryopreservation) recovery measurement of TNC, which must be higher than 70%. In a sample of the volume reduced UCB the TNC, MNC, CD45+ and viability of CD34+ cells are enumerated.
3) Prior to release for a Clinical Program the post cryopreservation quality control is performed using a segment (see Question 1) as product control. Viability and CFU-GEMM are determined.

Questions 3 and 4

CD34+ cell viability after thawing is estimated by single platform (ISHAGE protocol) flowcytometry (permeability of 7-AAD) and HPC (CFU GEMM) culture assay (StemCell Technology, Grenoble, France).

Question 5

We have no specified ranges for the number of stem/progenitor cells in a CB unit in relation to transplantation (e.g. based on kg body weight of recipient); this is at the judgement of the Transplant centre. We distinguish growth or no growth of CFU post cryopreservation. When no growth is associated with a $CD34^+$ cell viability below 50% we consider the CBU not suitable for release to a transplant center. When no growth is associated with > 50% viability we perform a progenitor test on a second sample of one of the cryopreserved reference vials.

Question 6

Our own Quality Control Program results form the basis to define the thresholds for transplantation.

This *process control* requires a mean (mean of at least five sequential CB units) recovery post processing for different cell populations (NC, MNC, CD45 and CD34) each higher than 80% of whole blood cell population. The mean post processing viability must be higher than 90% and the mean recovery post cryopreservation higher than 50% for all the different cell populations compared to the whole blood prior to volume reduction.

The mean viability of CD34+ cells post cryopreservation in the freezing bag and vial or segment must be higher than 50%.

Consequently, if *product control*, performed on every unit prior to cryopreservation, shows a recovery post processing for the TNC higher than 70%, the CB unit is cryopreserved. Product control post-thawing prior to release for transplantation (segment or vial) requires > 50% viability and growth of HPCs.

In collaboration with nine CBB from the three continents within [2] the BEST consortium (Biomedical Excellence for Safer Transfusion) we observed that only TNC counts in thawed UCB samples show good intra- and inter-laboratory agreement. All post-thaw viability assays overestimate the proportion of viable cells. Non-viable cells, not producing any CFU in progenitor assays, were in eight out of nine experiments associated with < 50% viability estimated with the 7-AAD methodology. Using other methods (e.g. trypan blue in particular, but also acridine-orange- ethidium bromide or acridine orange-propidium bromide) could yield values above 50% in non-viable samples [3]. Therefore we have chosen for process as well as for product control for a post-thaw viability value > 50% using the 7-AAD method, associated with growth in the CFU-GEMM. We use no quantitative thresholds for CFUs because our intra-laboratory variation with post-thaw samples of UCB is huge.

Recently, within the setting of double cord transplantation it has been suggested that a CD34 cell viability below 75% is associated with non-engraftment and discussions are ongoing to adjust the quality requirements [3].

Question 7

The consequences for the CB unit when this product does not meet the requirements is not to release the product for a clinical program and discarding the product from the Bank.

Question 8

Our quality control laboratory takes part in two different proficiency testings.

For flowcytometry this a national proficiency test program of fresh blood (not on frozen samples) and for the HPC culture this is the international proficiency test program of StemCellTechnology. Unfortunately these external proficiency tests are not suited to control for the critical steps in cord blood processing, cryopreservation and thawing protocols. The lack of proficiency tests suited for cord blood hampers the speed of development of optimal processing and thawing protocols.

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D. Gounder, A. Wong & R. Doocey

Question 1
We routinely freeze three sample aliquots with the bags and these are stored separately. One vial is used for post cryopreservation stem cell culture and viability assays. The other two aliquots are back up samples with the majority used for repeat stem cell culture and an occasional repeat test for bacterial contamination. Post cryopreservation CD34 assays are done upon request.

Question 2
Majority of the routine quality control on frozen samples is performed within a week after freezing.

Question 3
In every case, we would perform trypan blue dye exclusion viability and stem cell culture assays.

Question 4
We perform CFU-GM culture assays using Methocult media.

Question 5
There is a historical threshold for a fresh HPC harvest which is a CFU-GM growth of $> 5 \times 10^4$/kg. There are no defined acceptable ranges/thresholds for the number of progenitor cells required for transplantation determined from a cryopreserved sample. The assay is used as a qualitative potency test i.e. growth or no growth [1].

Question 6
We have no validation data of our own and will be guided by published literature. CFU-GM culture assays are performed on peripheral blood harvests, cord blood and bone marrow and there are no specified threshold requirements for any of these.

Question 7
The transplant team is made aware of the CFU-GM culture results. Where the growth is considered to be poor, again not well defined, there will usually be a request to repeat assays using one of the stored aliquots. Where there is no growth at all including the stored aliquots the transplant team will make the decision on discard of the product [2].

Question 8
Our laboratory participates in two external proficiency testing for CD34 assays on fresh samples.

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E. Forrest & G. Galea

Question 1
4 sample aliquots of final product are frozen together with the stored bags. These are taken from the final product prior to aliquotting.

Samples are used for the following:

1. Quality control after short time storage:
   A) Post cryopreservation WBC $\times 10^9$/l.
   B) Post cryopreservation total nucleated cell (TNC) viability.
2. Historic samples for virology re-testing if required. The original donor blood samples for virology testing are archived at the testing site.

Question 2

The quality control of frozen samples is normally performed within 7 days of freezing and is always completed prior to release of the product for clinical use.

Question 3

The following quality control tests are performed after freezing:

1. Post cryopreservation WBC $\times 10^9$/l. Target is 50% of the pre freeze WBC.
2. Post cryopreservation TNC viability via a flow based cytometric assay using the permeability marker 7-Amino Actinomycin D (7-AAD).

Question 4

No stem cell culture assays are performed.

Question 5

The following thresholds/ranges are defined for transplantation:

1. *Autologous HPC, Apheresis transplant*
   Minimum CD34 Dose: $2{\cdot}5 \times 10^6$/kg of recipients body weight.
   Maximum CD34 Dose: $10{\cdot}0 \times 10^6$/kg of recipients body weight.
2. *Autologous HPC, Marrow transplant*
   Minimum CD34 Dose: $1{\cdot}0 \times 10^6$/kg of recipients body weight.
   Maximum CD34 Dose: $10{\cdot}0 \times 10^6$/kg of recipients body weight.
3. *Allogeneic HPC, Apheresis transplant*
   Minimum CD34 Dose: $3{\cdot}0 \times 10^6$/kg of recipients body weight.
   Maximum CD34 Dose: $10{\cdot}0 \times 10^6$/kg of recipients body weight.
4. *Allogeneic HPC, Marrow transplant*
   Minimum CD34 Dose: $2{\cdot}0 \times 10^6$/kg of recipients body weight.
   Maximum CD34 Dose: $10{\cdot}0 \times 10^6$/kg of recipients body weight.

Question 6

Thresholds for apheresis products have been defined by validation of our historical clinical results over the past 15 years.

Thresholds for marrow products have been defined by adopting data from other transplant centres. We have done this in view of the small number of marrow procedures that are performed at our centre.

Question 7

We work in very close contact with the apheresis team and the transplant clinicians. The transplant clinician is informed of the product dose obtained at collection and whether this meets the required dose for transplantation. Further collections may take place as necessary.

The product would remain in storage unless otherwise directed by the patient's transplant clinician in which case it will be released on a concessionary basis.

The product is never discarded.

Question 8

Assays are performed by the local hospital haematology laboratory on our behalf. This laboratory participates in the UK NEQAS Scheme for CD 34 positive stem cell enumeration and full blood counts.

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J. Smythe & R. Pawson

Question 1

In the UK, National Health Service (NHS) Blood and Transplant run seven Stem Cell and Immunotherapy laboratories and the NHS Cord Blood Bank. These facilities process human progenitor cells (HPCs) from bone marrow, peripheral blood and altruistic and directed cord blood collections. Practice including quality control is standardised across these facilities where possible.

Four 1 ml sample aliquots are frozen in tubes together with the stem cell storage bags. These are used for quality control after short-term storage, after long-term storage and two for back-up. The samples are stored in the same cryotank as the stem cell bags.

Question 2

The quality control of frozen samples is performed routinely 2–10 days after cryopreservation. Viability testing is also repeated on cellular products that have been in long-term storage for more than one year and are under consideration for transplantation. Testing will also be performed on a frozen vial where there was delayed engraftment after use for a transplant.

Question 3

After cryopreservation we perform a routine single platform, lyse no-wash, flow cytometry assay that incorporates

