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PERSPECTIVE

POTENTIAL IMPACT OF THE FORTHCOMING EUROPEAN UNION SUBSTANCES OF HUMAN ORIGIN REGULATION ON THE PROGRAMME OF ALLOTRANSPLANTATION OF CRYOPRESERVED VASCULAR TISSUE GRAFTS

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Abstract

The Czech vascular tissue transplantation programme has operated under the conditions set by European Union (EU) directives since 2013. The aim of this analysis was to assess the impact of the new EU regulation on it and to analyse potential non-conformance. The impact of the new regulation was assessed based on the texts of the regulation and the recommendations of the European Directorate for Quality of Medicines and Healthcare (EDQM) and the European Centre for Disease Control (ECDC). Areas requiring improvements in the future were identified. The non-compliance analysis was performed on a group of 30 vascular tissue donations. We found that the areas requiring

attention are: 1) inclusion of West Nile Fever testing of donors; 2) more frequent particle counting and aeroscopy during processing; 3) determination of shelf life on the basis of a cryostability study; 4) implementation of the ISBT 128 standards for product labelling; 5) use of European Good Tissue Practice II (EURO GTP II) and Microbiologcal Risk Critical Assessment (MiRCA) tools for risk assessment; 6) implementation of the clinical outcome monitoring plan; and 7) staff participation in EDQM e-learning courses. The analysis showed a high level of compliance with the EDQM microbiological criteria for tissue procurement and processing. The temperature during processing complied with the EDQM limits, while relatively high non-compliance with the relative humidity limits occurred. In conclusion, the new regulation, which should be implemented by 2027, represents an important step towards improving vascular tissue transplantation safety. Our plans aim to meet its requirements within this period.

Keywords: allotransplantation; cryopreservation; European Union directive; European Union regulation; substances of human origin; tissue establishment; vascular grafts.

INTRODUCTION

The European Union (EU)'s regulation of cell and tissue procurement, processing, storage and distribution was based for a long time on the Directive of the European Parliament and Council No. 2004/23/EC (1) and two European Commission (EC) directives, 2006/17/EC and 2006/86/EC (2, 3). The former EC directive dealt with the selection of suitable donors, their screening and serological testing, while the latter dealt with the processing, labelling, storage and distribution of cells and tissues of human origin for clinical application. EU member states were obliged to harmonize their national legislation with the requirements of these directives within two years. This was completed in the Czech Republic in 2008 with Act No. 296/2008 Coll. and the Decree of the Department of Health No. 422/2008 Coll. All existing or newly established Cell or Tissue Banks (called Tissue Establishments [TEs] (4), together with their collection centres (called Procurement Establishments [PEs]) and laboratories performing serological testing of donors (called diagnostic laboratories [DL]), were obliged to complete the process of being licensed or accredited by the national competent authority.

To prevent unauthorized distribution of cells and tissue for transplantation, TEs have been registered in the European Compendium since 2017 and receive TE identification codes from the EC (5, 6). Since then, each product must be labelled using the single European code consisting of a unique donation identification sequence and the cell or tissue product identification sequence (5, 6). The list of registered EU TEs with their codes, the extent of their authorization and the cell and tissue product list including product codes was made available on the EU TE Cell and Tissue Coding Platform (6). In the USA, similarly strict rules were established under federal law (7), and Tissue Banks were supervised by the Food and Drug Administration (FDA).

The EC evaluated the regulatory situation in the EU member states 15 years after Directive 2004/23/EC came into force (8). The conclusion was that considerable diversity existed in the approaches of individual member states towards harmonization of national norms with the directives. Moreover, the directives did not cover all clinically used substances of human origin (SoHO). Breast milk stored in Human Milk Banks and used widely for feeding premature babies (9, 10, 11, 12), regulated until then as food (13), and intestinal microbiota recently introduced as a treatment for infection caused by *Clostridium difficile* (14) and for treating graft versus host disease after allogeneic bone marrow transplantation (15) were presented as examples. It was also noted that these directives were based on scientific knowledge from the 1990s and that they urgently needed to be updated. The situation became even more urgent during the COVID-19 pandemic, as it became clear that a mechanism for quick reaction in response to changing epidemiological situations was also lacking.

The original idea for improving the EU regulatory system was to replace the existing three directives with a single one that would cover blood, cells and tissues of human origin as well as SoHO (substances of human origin) not yet covered by the directives. It was also planned to make this norm more general and to

have expert groups formed at the level of the European Directorate for Quality of Medicine and Healthcare (EDQM) and the European Committee for Disease Prevention and Control (ECDC) to review the technical issues. These groups would prepare specific guides that would be updated at two-year intervals. The proposals for the new EU norm considered two options for changing the current regulatory situation:

1. To adopt a single directive to replace the three existing ones to be implemented into the national legislation of the member states within two years.
2. To make a change to uniform regulation at the EU level to prevent the possibility of changes at the national level.

The final version of the new norm from June 2024 (16) was in favour of the second option. Full implementation of this regulation by future SoHO Establishments is expected to be completed before the end of 2027.

The aim of this study was to assess the potential impact of the forthcoming EU regulation on the system of procurement, processing, distribution, and clinical transplantation of allogeneic cryopreserved vascular tissue in the Czech Republic. Before the start of the programme of allotransplantation of cryopreserved vascular tissue, only fresh allografts collected from deceased donors and stored in organ preservation solution or simple saline in refrigerators at +4 °C were available in the Czech Republic (17, 18). The great disadvantage of simple cold storage is its limited shelf life (19, 20), resulting in a lower chance of finding an AB0-matched graft of suitable type and size, especially in urgent situations. To prevent graft rejection, a clinically usable immunosuppression protocol was elaborated by a group of Prague authors (21, 22), and the idea of establishing a bank of cryopreserved vascular tissue grafts emerged with the aim of increasing the chance of transplanting AB0-compatible grafts. After an experimental stage of allotransplantation of cryopreserved vascular tissue in rats (23, 24) and validation of the cryopreservation methods planned to be used in humans within the Czech national competent authority's process of licensing TEs and PEs, the cryopreserved vascular tissue transplantation programme began in 2013 (25).

METHODS DEVELOPMENT

Procurement and cryoprocessing of vascular grafts

Since the beginning of the cryopreserved vascular graft allotransplantation programme aseptic procurement of arterial and venous grafts has been performed at licensed PEs by vascular surgeons within the framework of multi-organ harvesting from deceased donors (25). The collected vessels were put into a sterile plastic jar containing organ preservation solution (Custodiol CE, Dr. Kohler Chemie GmbH, Bensheim, Germany) supplemented with gentamicin and immediately transported in a polystyrene thermo-box filled with melting ice by a licensed courier company (Ambulance Meditrans Ltd., Prague, Czech Republic) to the TE (i.e., the Tissue Bank of University Hospital Hradec Králové), where the vessels were processed and cryopreserved within 24 hours. This TE was granted a TE licence by the State Institute for Drug Control (SÚKL, Prague) in 2011 and received the EU Tissue Establishment Code CZ000427 from the EC in 2017.

Processing in the clean rooms of the TE began with decontamination in an antibiotic solution according to the van Kats method (26). Freezing in a programmable freezer (Planer Biomed, Sunbury-on-Thames, England) in cryobags (Maco Biotech freezing EVA bags, Maco-Pharma, Mouvaux, France) in the presence of 10 % CE-certified dimethyl sulfoxide (DMSO) (Cryosure WAK Chemie GmbH, Steinbach, Germany) followed. Storage of the cryobags housed in metal cassettes (Fig. 1) was performed in the vapour phase of liquid nitrogen in a Kryo CE liquid nitrogen container (Taylor Wharton, Milstedt, Germany) with automatic filling and continuous temperature registration. The high-temperature alarm was adjusted to −160 °C. The data on the graft positions inside the container were uploaded into SW Freezeworks software (SIAD Healthcare, Assago, Italy). The declared shelf life of the cryopreserved vascular allografts was set to 5 years (27).

Figure 1. The cryostorage frame with inserted metal cassettes containing cryopreserved vascular grafts in the liquid nitrogen storage vessel.

Control of environmental parameters during processing and storage of vascular grafts

In addition to continuous monitoring of the pressure differences between the clean room (grade B) with a critical processing area (grade A), the adjacent clean rooms (grade C) and surrounding corridors, the temperature and relative humidity inside the clean rooms were continuously monitored at 5-min intervals and recorded.

The liquid nitrogen container used for storage was placed inside the cryobank of the TE (28) equipped with forced ventilation. This facility is also used to store haematopoietic progenitor cells (29) and reproductive cells and to store both registered and investigational CAR-T therapy products (30) used to treat haematological malignancies (31). The cryobank area, which is under continuous video supervision, can be accessed only by staff using personal electronic cards. The environmental parameters, temperature, and relative humidity inside the cryobank are monitored and recorded in the same way as during processing. To control the risk of oxygen deficiency, wall-mounted oxygen monitors (Polytron Draeger, Lubeck, Germany) are placed at a height of approximately 1 m above the floor of the cryobank, and the atmospheric oxygen content is continuously monitored and recorded at 5-min intervals (32). The transport of cryopreserved grafts released for clinical application by the

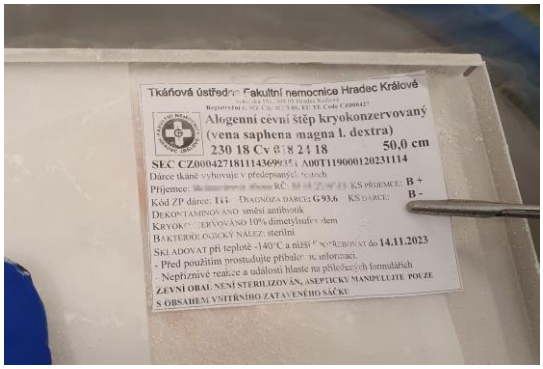

Figure 2. Current vascular graft labelling method.

responsible person of the TE and finally labelled (Fig. 2) is performed in dry shippers owned by the courier company. Thawing and transplantation are performed exclusively at authorized specialized centres of organ and/or vascular transplantation surgery. Common slow thawing (33) and immunosuppression protocols (Table 1) are used in all centres.

Table 1. Protocol for immunosuppressive treatment.

1.	For grafts implanted for critical limb ischaemia, immunosuppression is given from the first post-operative day. In patients on antibiotic treatment for infections, it is given on the seventh post-operative day.
2.	Tacrolimus monotherapy (Prograf, Advagraf) is used with target levels of 4–7 ng/mL.
3.	The levels of immunosuppression and renal functions are examined at 3-month intervals in the first year, then at 6-month intervals afterwards.
4.	If there are no clinical or computer tomography signs of graft rejection, the dose is reduced to 0.5 mg a day after 24 months (at minimum plasma concentrations).
5.	For patients who are not able to control the levels of tacrolimus, cyclosporin A (Sandimmun 2 x 25 mg) is an alternative.
6.	Patients with advanced renal disease are immunosuppressed individually.

Outcomes

The main indications for clinical use of cryopreserved grafts are infections of prosthetic vascular grafts and critical limb ischaemia in

patients lacking autologous vein grafts, mostly with poor outflow tracts. All potential graft recipients are referred by individual centres to the central waiting list, and the grafts are distributed according to compatibility in the AB0 system, and the urgency criteria listed in Table 2.

Table 2. Urgency criteria: waiting list for vascular allograft transplantation.

I. Urgent – life-saving procedure.
• Bleeding from vascular anastomosis (infection caused by virulent pathogen)
• Soft tissue defect above vascular anastomosis/visible vascular graft
• Vascular graft infection – patient with elevated infection markers on antibiotic therapy
II. Urgent – limb-saving procedure.
• Critical limb ischaemia – rest pain
• Critical limb ischaemia – soft tissue defect
III. Normal
• Vascular graft infection caused by pathogens with low virulence.
• Chronic ischaemic defect of soft tissues (peripheral artery disease)
• Vascular access implantation

All data describing the harvest processing distribution and clinical application of the grafts are uploaded and referred to the monitoring TISSISS SW operated by the Transplant Coordination Centre in Prague, Czech Republic.

The cryopreserved vascular graft allotransplantation programme, which is repeatedly verified by inspections carried out by the State Institute of Drug Control (SÚKL Prague) and by audits of the PEs performed by the TE, is fully compliant with the requirements of the current EU legislation.

The data on the number of cryopreserved and transplanted vascular tissue grafts presented in this study were taken from the TE annual reports publicly available on the University Hospital Hradec Králové website. The data on the number of transplanted fresh grafts were provided by the Transplant Coordination Centre.

The impact of the forthcoming regulation was assessed on the basis of the regulation itself and the accompanying recommendations of the European Directorate for Quality of Medicines and Healthcare (EDQM) version 5 (34) and the ECDC and on information received by the authors at various international meetings: i) the European Association of Cell and Tissue Banks [EATCB] congresses in Leiden, The Netherlands in 2019; in Warsaw, Poland in 2022; and in Zagreb, Croatia in 2023; ii) the European Group for Blood and Bone Marrow Transplantation [EBMT] congress in Paris, France in 2023, and iii) the conference on the new regulation on SoHO held in Brussels, Belgium in 2024. Subsequently, areas that would probably require organizational or technical improvements were identified.

NON-COMPLIANCE ANALYSIS

As many future criteria were already included in version 5 of the EDQM safety and quality guide (34), a preliminary analysis of non-compliance with the microbiological criteria after procurement and after processing of the vascular tissue and of non-compliance with the temperature and relative humidity limits during processing in the group of 30 vascular tissue donations was performed in the post-COVID period from November 2022 until August 2024.

Tissue bank and/or tissue establishment authorization and registration codes

An overview of the authorization obtained and of national and the European registration codes is presented in Table 3.

Number of cryopreserved and transplanted vascular grafts

From 2014 until 2023, a total of 290 grafts were cryopreserved by the TE (Fig. 3), and 296 fresh grafts and 185 cryopreserved grafts were used clinically (Fig. 4**)**. The mean percentage of transplanted cryopreserved grafts from the total transplanted ones during the whole observed period was 38.46% and ranged between 49.2% in 2015 and 13.0% in 2017 (Table 4).

The level of vascular grafts in stock over this period was maintained at between 18 and 36 items (Fig. 5).

Expected organizational changes

In the current system, the Czech TEs, PEs and DLs receive authorization from the national competent authority (SÚKL), and since 2017, the TEs have also been registered with the EC (Table 3). Specific authorization for clinical units using cell and tissue grafts is not needed unless it is required by national competent

authorities, which is the case for vascular tissue transplantation. The data on cell and tissue harvest processing and clinical application are collected by the Transplant Coordination Centre and referred to the EC (System Eurocet) once a year.

In the new system, all medical care units involved in harvesting, processing, testing and clinical application of vascular tissue will be registered with the EC as SoHO entities. The Tissue Bank of University Hospital Hradec Králové will be obliged to apply for the SoHO preparation authorization that will be issued by the national competent authority (SÚKL, Prague) under the condition of proof of compliance of the applied technologies with the requirements of the new regulation. Clinical units, specialized centres of organ and/or vascular transplantation surgery will become new SoHo entities and will be obliged to produce a clinical outcome monitoring plan documenting established continuous follow-up of clinical results. All the abovementioned SoHO entities will need to possess appropriate tools for direct electronic communication with the future EU SoHO platform (now the EU TE plattform), which is planned to be used for direct transfer of data on vascular tissue harvest processing and clinical applications.

The expected changes in techniques and the results of the non-compliance analysis are presented below.

Table 3. Overview of tissue bank and tissue establishment authorization and registration obtained by the tissue bank of University Hospital Hradec Králové.

Year	Type of authorization	Granted by	Registered at	Registration number
2004	National multi-tissue bank licence (provisional)	Department of Health, Czech Republic	Department of Health, Czech Republic	MTB 06
2011	National Tissue Establishment licence	State Institute for Drug Control (SÚKL)	Department of Health, Czech Republic	MTB 06
2017	National Tissue Establishment licence	State Institute for Drug Control (SÚKL)	European Commission	CZ000425* CZ000426** CZ000427

*Reproductive tissue; ** haematopoietic tissue.

Table 4. Number of transplanted fresh and cryopreserved vascular grafts in the period 2014–2023.

Year	Fresh grafts (n)	Cryo-preserved grafts (n)	Total (n)	Ratio cryo-preserved/ total grafts (%)
2014	36	26	62	41.94
2015	33	32	65	49.23
2016	32	27	59	45.76
2017	40	6	46	13.04
2018	25	13	38	34.21
2019	24	17	41	41.46
2020	24	10	34	29.41
2021	28	19	47	40.43
2022	28	12	40	30.00
2023	26	23	49	46.94
Total	296	185	481	38.46

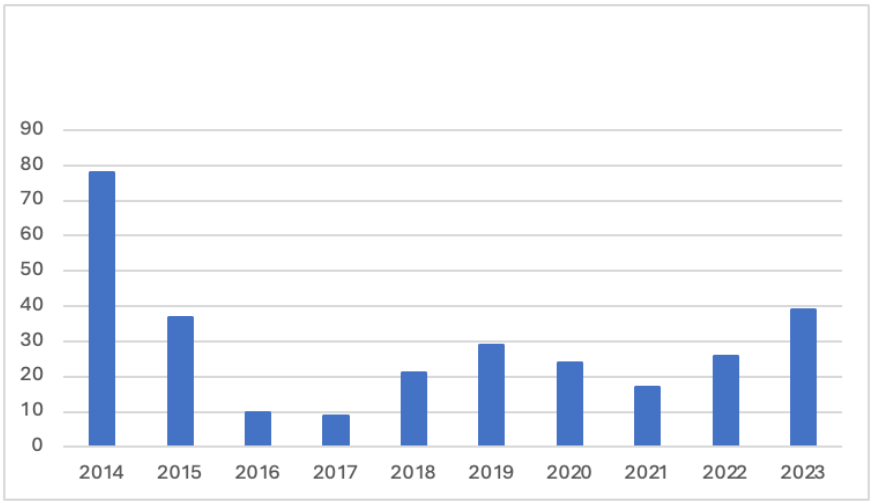

Figure 3. Annual number of cryopreserved vascular grafts, 2014–2023.

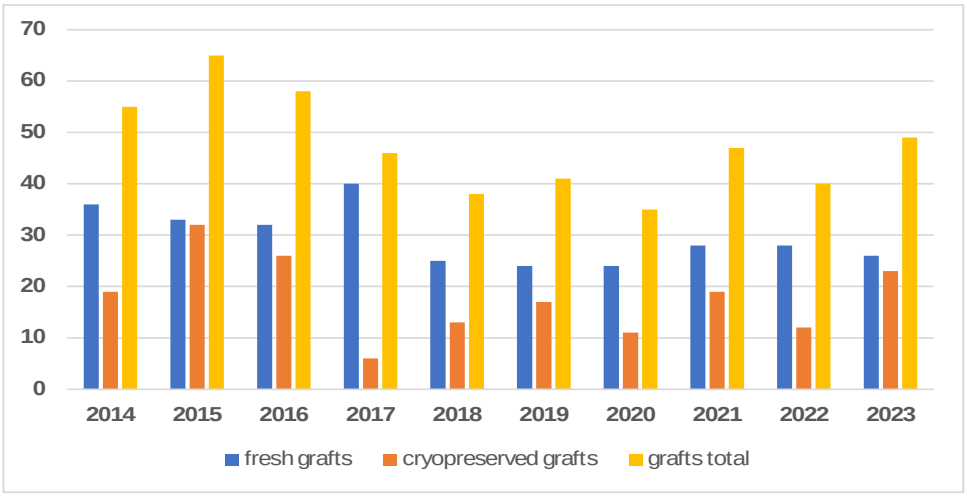

Figure 4. Annual number of transplantations, 2014–2023.

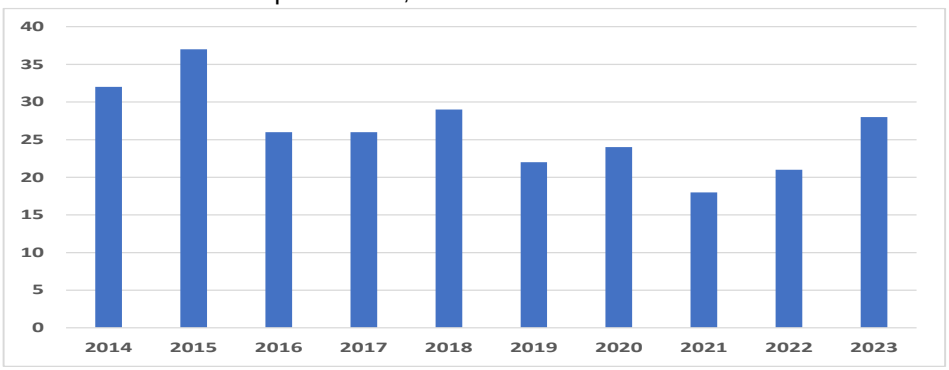

Figure 5. Number of cryopreserved grafts in stock at the end of the year, 2014–2023.

Vascular tissue procurement

The current protocol for donor eligibility assessment and harvesting vascular tissue in operating rooms during multiple organ harvesting in deceased donors (Fig. 6) is fully compliant with the EDQM guide. The current spectrum of obligatory serology tests includes anti-HIV 1 and 2, and p24-Ag, HBsAg, anti-HBc, anti-HCV and two tests for the detection of syphilis. The new ECDC recommendation is also expected to include West Nile fever testing.

The results of the analysis of non-compliance with the EDQM microbiological criteria for release of collected tissues for

processing performed in the 18 arterial and 38 venous graft harvests are shown in Tables 5 and 6. In the arterial graft harvests, non-compliance

Table 5. Non-compliance with microbiological European Directorate for Quality of Medicine and Healthcare criteria after procurement – arterial grafts

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Type of graft	Compliant grafts (n)	Non-compliant grafts (n)	Microbiological finding
Aortic bifurcation	8	1	*Klebsiella pneumoniae*
A. iliaca + arteria femoralis supeficialis	0	1	*Candida albicans*
Aorta thoracica	3	0	Not applicable
Arteria femoralis superficialis	5	0	Not applicable
Total	16	2	

Table 6. Non-compliance with microbiological European Directorate for Quality of Medicine and Healthcare criteria after procurement – venous grafts.

Type of graft	Compliant grafts (n)	Non-compliant grafts (n)	Microbiological finding
Vena cava inferior	1	0	Not applicable
Vena saphena magna	33	4	1 x *Enterococcus faecalis* 1 x *Proteus mirabilis* 2 x *Escherichia coli*
Total	34	4	

Table 7. Non-compliance with the sterility criterion at the output control in arterial grafts.

Type of graft	No. of compliant grafts	No. of non-compliant grafts	Microbiological finding at input control	Microbiological finding at output control
Aortic bifurcation	8	0	Not applicable	Not applicable
Aorta thoracica	2	1	*Streptococcus mitis** 20 CFU/mL	*Streptococcus mitis** < 1 CFU/mL
Arteria femoralis superficialis	5	0	Not applicable	Not applicable
Total	15	1		

*Resistant to antibiotics of the decontamination solution.

Table 8. Non-compliance with the sterility criterion at the output control in venous grafts.

Type of graft	No. of compliant grafts	No. of non-compliant grafts	Microbiological finding at input control	Microbiological finding at output control
Vena cava inferior	1	0	Not applicable	Not applicable
Vena saphena magna	33	1	*Staphylococcus epidermidis* < 1 CFU/mL	*Staphylococcus epidermidis* < 1 CFU/mL
Total	34	1		

with the indication for graft discarding occurred in two cases (11.11 % non-compliance rate) (Table 5) and in venous grafts in four cases (10.53 % non-compliance rate) (Table 6).

In future, the risk of microbiological contamination during vascular tissue harvesting is planned to be assessed by the Microbiological Risk of Contamination Assessment tool (MiRCA) considering the number of staff present and the quality of the aseptic work and of the critical material and equipment used.

Vascular tissue processing

The current method of vascular tissue processing in the clean rooms designed and built according to the International Society for Pharmaceutical Engineering standard (28, 35) with the critical area of grade A with a background of grade B and the use of 10 % DMSO as a cryoprotectant is fully compliant with the EDQM guide.

The results of the analysis of non-compliance with the current criterion of sterility at the output control of processed grafts performed in 16 arterial grafts released for processing showed only one discarded case on the basis of non-sterility caused by *Streptococcus mitis* (6.25% non-compliance rate). The same analysis performed on 36 venous grafts released for processing showed one case of non-sterility caused by *Staphylococcus epidermidis* (2.78% non-compliance rate) (Tables 7 and 8). Similarly, for harvesting, the MiRCA tool will be used for microbiology risk assessment in the future. Full compliance with the limits for optimal temperature (15–25°C) in the 30 cases of processing of vascular tissue donations performed from November 2022 until August 2024 is shown in Figure 7. Deviations from the optimal range for relative humidity (30 %–65 %) occurred in five cases (20% non-compliance rate): in three cases, the values were above the limit (in summer 2023), and in two cases, the values were slightly below the limit (in autumn 2022) (Fig. 7).

A considerable change will be necessary in vascular tissue labelling. The current label written in the Czech language (Fig. 2) contains obligatory data identifying the producer and the donor using the donor identification sequence. Basic identification of the intended recipient and identification of the vascular graft type using the Cellular Therapy ISBT 128 product code is included. The full single European code makes it possible for the clinician to verify after it is loaded into the EU TE coding platform that the product originated from the TE with valid authorization for vascular tissue procurement, processing and distribution. This labelling system is planned to be replaced by labels using a format and content compliant with the ISBT 128 standard (36, 37) and the English language.

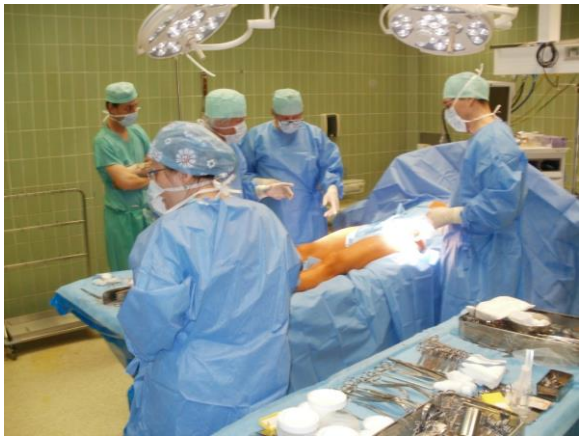

Figure 6. Aseptic tissue procurement within multi-organ donation procedure.

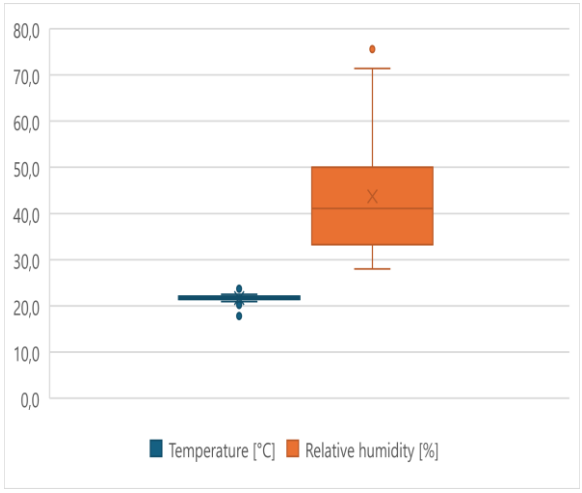

Figure 7. Enviromental parameters during processing.

Vascular tissue storage

Exclusive storage in the vapour phase of liquid nitrogen and adjusting the high-temperature alarm to −160 °C is fully compliant with the EDQM guide, which prescribes storage temperatures below −140 °C. Any temperature rise above the EDQM limit is to be evaluated during the process of the release of the graft for clinical application. The 5-year shelf life was determined based on a cryostability study (27), as required by the EDQM guide.

The existing forced ventilation can maintain the oxygen level inside the cryobank above 19 %, as prescribed by the EDQM guide. The TE is also obliged to settle the temperature and relative humidity limits inside the cryostorage facility. As the recommended limit is not specified by the EDQM guide, we selected limits prescribed by the manufacturer of the liquid nitrogen containers (18–27°C for the temperature and below 50% for the relative humidity).

The EDQM guide also has no limits for the purity of the environment of the cryostorage facility. To prevent the pollution of liquid nitrogen inside the biological containers by condensed water particles containing airborne microbes, regular particle counting and aeroscopy control of the cryobank premises is highly advisable. Our preliminary study (38) showed the purity grade to be at least class D.

Clinical outcome monitoring

The existing clinical monitoring system is based on the return of early clinical outcome report forms to the TE and on the reporting of severe adverse reactions and events to the national competent authority (SÚKL, Prague). Long-term outcomes are assessed by the Task Force on Vascular Substitutes and Allotransplantations of the Czech Society for Cardiovascular Surgery. Secondary assisted patency rates with median of almost 2 years were achieved in a group of patients with critical limb ischemia using cryopreserved venous grafts. It is of course less than in a group with autologous saphenous vein graft, but sufficient for limb salvage in most cases. In individual cases of transplantation of arterial grafts, an even longer patency was described. In the future, it will be necessary to upgrade this system by reporting long-term outcomes to the TE according to the clinical outcome monitoring plan, which should be uniform for all clinical centres participating in the national vascular tissue transplantation programme.

Staff education and training

Education of all staff members has been organized by the TE itself, by cooperating PEs and by the Task Force on Vascular Substitutes and Allotransplantations of the Czech Society for Cardiovascular Surgery. The key persons (the TE and/or PE responsible persons and quality managers) regularly take part in the congresses of the Czech Transplantation Society, the Czech Society for Organ Transplantation, and the Czech Society for Cardiovascular Surgery. They also participate in international meetings, namely in the EATCB congresses and the Society for Low Temperature Biology and the Society for Cryobiology annual meetings. However, the frequency of participation and the number of participating staff members have been limited by available funding. The dissemination of information on the forthcoming regulation has recently been strengthened by participating in the free certified e-learning EDQM courses. In recent years, three TE staff members participated in the course on the EDQM quality and safety guide version 5, and one staff member took the Euro GTP II guide course.

Novelty assessment

The existing Czech national system requires submission of the results of three validation lots to the national competent authority (SÚKL, Prague) in the case of major changes to the existing vascular tissue procurement or processing standard operating procedures (SOPs) or if a new product is introduced to clinical practice. This system is to be replaced by an approval process at the EC level. The TE will be obliged to submit the data on the new product together with the risk assessment performed according to the Euro GTP II tool.

DISCUSSION

TEs became a standard part of EU member states' healthcare systems after the EU directives of 2004 and 2006 came into force (4). However, this required investments of one million EUR per TE, according to a 2015 EU economic study (39). At University Hospital Hradec Králové, it was necessary to re-engineer the Tissue Bank processing and storage procedures of the technology using a combination of cryogenic and clean room technology (28). This change, which required reconstruction of the TE premises, facilitated the immediate granting of a provisional multi-tissue licence by the Department of Health in 2004 (Table 3). Nevertheless, the process of fully meeting the requirements of these directives necessary for obtaining the standard TE licence was relatively slow. One obstacle to faster progress was the fact

that the detailed instructions for submitting applications for authorization (40) were not available before August 2008. As higher levels of safety and quality assurance were required than expected by the TEs, additional investments and changes to internal rules, such as specifications of cryopreserved vascular tissue and upgrading existing SOPs for harvesting and processing vascular tissue, were necessary. Specialized vascular surgery departments were required to be licensed as PEs and to sign cooperation agreements with the TE. All these issues were solved in close cooperation with the Task Force on Vascular Substitutes and Allotransplantations of the Czech Society for Cardiovascular Surgery, the Czech Transplantation Society and the Transplant Coordination Centre, and the process was completed in 2013 (41). The process also included validation of SOPs and calculation of harvesting and processing costs on a non-profit principle. Additional changes were required because of the implementation of the EU coding system in 2017 (5, 6).

Regardless of the difficulties to be overcome at the beginning of the programme, the data from the years 2014 until 2023, when the TE operated under the directives (Figure 3), showed that after the period of frequent harvesting necessary for establishing a sufficient stock of grafts of different types and blood groups in 2014, the annual number of cryopreserved grafts was relatively stable. The exception was for the years 2016 and 2017, when a scarcity caused by unavailability of CE-marked organ preservation solution occurred. On the other hand, the COVID-19 pandemic in the years 2020–2022 did not substantially lower the number of cryopreserved grafts, This was because there were no restrictions on the organ transplantation programme, and COVID-19 testing of all organ donors was ordered by the Transplant Coordination Centre at the beginning of the pandemic based on the ECDC recommendation (42). This was not the case for the low number of delivered cryopreserved grafts and the low total number of transplanted grafts in 2020 and 2022, because the vascular transplantation centres were partly converted to COVID-19 treatment units. Table 4 documents that fresh grafts remained the most frequently used ones. Nevertheless, the delivered cryopreserved grafts represented a valuable contribution to the quality of the vascular transplantation programme.

Since several requirements of the forthcoming legislation have already been introduced into the TE internal rules, we could assess compliance with the microbiological EDQM criteria during procurement and processing and with the environmental physical parameters during processing. The non-compliance rate during procurement was lower than reported by other authors (43, 44) and did not differ substantially from our data from the beginning of the vascular tissue transplantation programme (45). Incidental cases of non-sterility at output control are presented in Tables 7 and 8. In one case, the decontamination process failed because the non-pathogenic microbe was multi-resistant (Table 7). In the second case, the microbe was present at a low concentration, below 1 CFU/mL. Nevertheless, it caused non-sterility of the product (Table 8). These results reflect the advantage of tissue harvesting in operating rooms by vascular surgeons and of processing in a clean room facility at the Good Manufacturing Practice level as well as the high level of skill of the TE staff. Other beneficial factors leading to improved safety and quality were improving laboratory diagnostics with matrix-assisted laser absorption/ionization (MALDI) time of flight mass spectrometry from 2014 and performing sterility tests in the clean room facility of the Department of Microbiology since 2016, as well as maintaining a stable temperature (mean 21.7 °C, SD 1.0 °C) (Fig. 7) in the clean rooms of the TE, thus preventing growth of microbes during processing.

The rate of non-compliance with the recommended relative humidity limits was relatively high (20 %). These deviations are unlikely to have had an adverse effect on graft quality because grafts are submerged into different solutions during processing. However, high relative humidity (Fig. 7) is inconvenient for staff working in anti-emission garments covering the whole body.

Our previous study (38) identified high relative humidity in the cryostorage facility as a technical issue to be solved in the future. In this case, high humidity causes formation of ice accretion on the lids and formation of an ice layer at the bottom of the cryo-container, which increases the necessity of frequent defrosting of the container.

Vascular tissue cryopreservation protocols based on the use of DMSO, slow controlled freezing, storage in the vapour phase of liquid nitrogen and slow thawing assured high viability of cells of the vessel wall in the 5-year cryostability study (27). It also avoided the presence of micro-fractures (46) and lowered graft immunogenicity in experiments in rats (23, 24). The potential DMSO toxicity described in cases of infusion of the product (29, 47) did not represent any risk in our case, because the DMSO residuals were washed out by immersion of the thawed grafts into organ preservation solution before implantation. The use of DMSO-free cryoprotective media can be considered in the future if an efficient CE-marked or FDA-approved product becomes available on the market. Such a technological change would be an example of a novelty that would require careful assessment of risk based on an experimental study before introduction to clinical practice.

The new SoHO regulation contains many common features with the JACIE EBMT accreditation process, which is based on a combination of proof of compliance with the JACIE EBMT standard (48) and a risk assessment approach. In the SoHO regulation, the risk assessment approach is strengthened by the obligation of the TE to use specific tools, such as the MiRCA or Euro GTPII tools.

Similar to the process of implementing the EU directives, technical improvements will be necessary to achieve compliance with the new regulation. In addition to implementation of the full ISTB 128 standard requiring the purchasing of adequate printers and licences and upgrading relative humidity regulation in the TE's processing and storage facilities, the tools for direct communication with the EU SoHO platform must be available for all SoHO entities. In general, however, we expect to need a much lower investment than during the process of implementing the original directives (39). Similarly, faster implementation of the new regulations than in the case of the implementation of the directives is expected. However, this is not possible without timely action by the TEs if completion of the process is to be achieved within 2 years.

Our analysis of non-compliance with the forthcoming SoHO regulation was based especially on comparison of our current practice with the standards of the 5th version of the EDQM guide and is to be supplemented by the results of application of risk assessment tools. Further corrections will be necessary as more information becomes available from upgrades of the existing EDQM guide and the new ECDC recommendations that are expected to be issued soon, as well as from new manuals and future training at e-learning and face-to-face events.

CONCLUSIONS

The new regulation, which should be fully implemented by 2027, represents an important step for the improvement of vascular tissue transplantation safety. To achieve this deadline, timely action by TEs is necessary. The presented results of our preliminary analysis of compliance of our technology for vascular tissue procurement processing, storage, and distribution with the forthcoming SoHO regulation are based especially on comparison with the standards of the EDQM guide version 5. Actions to avoid the identified areas of non-compliance should include enlarging the spectrum of serological testing of donors, fully implementing the ISBT 128 standard of graft labelling, introducing the use of risk assessment tools into the TE's practice, implementing technical improvements for better regulation of relative humidity during processing and storage of vascular grafts, implementing the clinical outcome monitoring plan at cooperating clinical centres and educating the TE and PE staff. Corrective actions will become necessary as more detailed information becomes available.

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REFERENCES

1. European Union (2004) *The Official Journal of the European Union* **102**, 48-58.
2. European Commission (2006) *The Official Journal of the European Union* **38**, 40-44.
3. European Commission (2006) *The Official*

Journal of the European Union **294**, 32-50.
4. Morales Pedraza J (2016) *Cell and Tissue Banking* **17**, 179-188. DOI: 10.1007/s10561-015-9534-5
5. Caramia V, Ghirardini A, Di Ciaccio P, Vespasiano F, Mareri M & Nanni Costa A (2017) *Transfusion Medicine and Hemotherapy* **44**, 391-394. DOI: 10.1159/000481144
6. European Commission Health and Food Directorate – General Directorate B – Health systems, medical products, and innovation B4 – Medical products: quality, safety, innovation (2021) *EU Coding Platform User Manual*, Brussels.
7. Food and Drug Administration (2004) *Federal Register* **69**, 68611-68688.
8. European Commission (2019) *Evaluation of the Union Legislation on Blood, Tissues and Cells*, Brussels.
9. Moro GE & Arslanoglu S (2012) *Journal of Pediatric Gastroenterology and Nutrition* **54**, 165-166. DOI: 10.1097/MPG.0b013e318235d629
10. Weaver G, Bertino E, Gebauer C, Grovslien A, Mileusnic-Milenovic R, Arslanoglu S, Barnett D, Boquien CY, Buffin R, Gaya A, Moro GE, Wesolowska A & Picaud JC (2019) *Frontiers in Pediatrics* **7**, 53-62. DOI: 10.3389/fped.2019.00053
11. Cormontagne D, Rigourd V, Vidic J, Rizzotto F, Bille E & Ramarao N (2021) *Toxins* **13**, 123-136. DOI: 10.3390/toxins13020123
12. Jandová M, Měřička P, Fišerová M, Landfeld A, Paterová P, Hobzová L, Jarkovská E, Kacerovský M & Houška M (2021) *Foods* **10**, 10-25. DOI: 10.3390/foods10122955
13. European Parliament and Council (2002) *Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 Laying Down the General Principles and Requirements of Food Law, Establishing the European Food Safety Authority*, Brussels.
14. Chopra T, Hecht G & Tillotson G (2023) *Frontiers in Medicine* **9**, 32-38. DOI: 10.3389/fmed.2022.1093329
15. Weber D, Meedt E, Poeck H, Thiele-Orberg E, Hiergeist A, Gessner A & Holler E (2024) *Visceral Medicine* **40**, 1-6. DOI: 10.1159/000538303
16. European Parliament and Council (2024) *Regulation (EU) 2024/1938 of the European Parliament and of the Council of 13 June 2024 on Standards of Quality and Safety for Substances of Human Origin Intended for Human Application and Repealing Directives 2002/98/ and 2004/23/EC*, Brussels.
17. Matia I, Janoušek L, Marada T & Adamec M (2007) *European Journal of Vascular and Endovascular Surgery* **34**, 424-431. DOI: 10.1016/j.ejvs.2007.04.005
18. Matia I, Adamec M, Janoušek L, Lipár K, Marada T, Klein D, Baláz P, Varga M, Chlupáč J & Rokosný S (2010) *Rozhledy v Chirurgii* **89**, 45-54.
19. Páral J, Ferko A, Měřička P, Slízová D, Nozicka J, Chovanec V & Raupach J (2000) *Rozhledy v Chirurgii* **79**, 244-249.
20. Kieffer E, Gomes D, Chiche L, Fléron MH, Koskas F & Bahnini A (2004) *Journal of Vascular Surgery* **39**, 1009-1017. DOI: 10.1016/j.jvs.2003.12.040
21. Adamec M, Matia I & Janoušek L (2011) *Rozhledy v Chirurgii* **90**, 130-133.
22. Matia I, Fellmer P, Splith K, Varga M, Adamec M, Kämmerer I, Feldbrügge L, Krenzien F, Hau HM, Atanasov G, Schmelzle M & Jonas S (2014) *Annals of Transplantation* **19**, 225-232. DOI: 10.12659/AOT.889870
23. Špunda R, Hrubý J, Mericka P, Mlček M, Pecha O, Splith K, Schmelzle M, Krenzien F, Lindner J, Matia I & Špaček M (2018) *PLoS One* **13**, 124-138. DOI: 10.1371/journal.pone.0201984
24. Hrubý J, Špunda R, Měřička P, Mlček M, Pecha O, Splith K, Schmelzle M, Krenzien F, Lindner J, Špaček M & Matia I (2020) *PLoS One* **15**, 167-178. DOI: 10.1371/journal.pone.0230234
25. Špaček M, Měřička P, Janoušek L, Štádler P, Adamec M, Vlachovský R, Guňka I, Navrátil P, Thieme F, Špunda R, Burkert J, Staffa R, Němec P & Lindner J (2019) *Advances in Clinical and Experimental Medicine* **28**, 529-534. DOI: 10.17219/acem/90037
26. van Kats JP, van Tricht C, van Dijk A, van der Schans M, van den Bogaerdt A, Petit PLC & Bogers JJC (2010) *European Journal of Cardio-Thoracic Surgery* **37**, 163-169. DOI: 10.1016/j.ejcts.2009.07.011
27. Měřička P, Janoušek L, Benda A, Lainková R, Sabó J, Dalecká M, Prokšová P, Salmay

M, Špunda R, Pecha O, Jandová M, Gregor J, Štěrba L, Špaček M & Lindner J (2021) *International Journal of Molecular Sciences* **22**, 135-148. DOI: 10.3390/ijms221910653
28. Měřička P, Straková H, Štěrba L, Schustr P & Vinš M (2014) *Cryogenics: Proceedings of the 13th IIR International Conference*.
29. Acker JP, Bondarovych M, Brunotte R, Buriak IA, Fuller BJ, Glasmacher B, Goltsev AM, Gregor J, Gryshkov O, Herrity K, Honegrová B, Hunt CJ, Jandová M, Johnstone BH, Kilbride P, Lánská M, Mann J, Měřička P, Musall KG, Mutsenko V, Mykhailova O, Petrenko Y, Radocha J, Sherry AM, Stacey GN, Štěrba L, Vokurková D, William N & Woods EJ (2022) *Cell Engineering and Regeneration*, **12**, 1-69.
30. Jandová M, Stacey GN, Lánská M, Gregor J, Rozsívalová P, Beková L, Woidigová Ducháová Z, Belada D, Radocha J, Měřička P & Fuller B (2023) *CryoLetters* **44**, 123-133. DOI: 10.54680/fr23310110112
31. Costa LJ, Lin Y, Cornell RF, Martin T, Chhabra S, Usmani SZ, Jagannath S, Callander NS, Berdeja JG, Kang Y, Vij R, Godby KN, Malek E, Neppalli A, Liedtke M, Fiala M, Tian H, Valluri S, Marino J, Jackson CC, Banerjee A, Kansagra A Schecter JM, Kumar S & Hari P (2022) *Clinical Lymphoma, Myeloma and Leukemia* **22**, 326-335. DOI: 10.1016/j.clml.2021.10.013
32. Měřička P, Vávra L, Vinš M & (2004) in *The Eighth Cryogenics 2004 IIR International Conference*, (ed) V Chrz, Icaris Ltd, Prague, pp 242-247.
33. Špaček M, Měřička P, Janoušek L, Dalecká M, Benda A, Krs O, Slížová D, Špunda R, Hrubý J, Matia I, Honegrová B & Lindner J (2020) *Annals of Vascular Surgery* **64**, 347-354. DOI: 10.1016/j.avsg.2019.11.026
34. European Directorate for the Quality of Medicines (2024) *Conseil d'Europa Guide to the Quality and Safety of Tissues and Cells for Human Application* (5th ed.), Brussels. Available online: https://www edqm.eu/en/news/new-guidequality-and-safety-tissues-and-cells-human application (accessed 29 May 2022).
35. Society for Pharmaceutical and Medical Device Professionals (1999) *ISPE: Baseline Pharmaceutical Engineering Guide for New and Renovated Facilities, Volume 3, Sterile Manufacturing Facilities*, ISPE Headquarters, ISPE European Branch Office, North Bethesda .
36. Distler P, (ed) (2015) *United States Consensus Guidance for the Uniform Labelling of Cellular Therapy Products using ISBT 128. Version 1.3.1*. International Council for Commonality in Blood Banking Automation, San Bernardino, CA. Available online: http://www.iccbba.org/tech-library/iccbba-documen (accessed 25 May 2018).
37. International Council for Commonality in Blood Banking Automation (2022) *ISBT 128 Standard. Standard Terminology for Medical Products of Human Origin* Available online: https://www.isbt128.org/_files/ugd/1a7593 74dd535431f1457b9c5a3174dd535431f1457 b9c5a31adb6145ec0.pdf (accessed 27 December 2022).
38. Měřička, P., Jandová, M., Fuller, B., Gregor, J., Studená., Lánská, M., Vokurková, D., Jankovičová, K., Fátorová, I., Brandejs, D., Guňka, I. Benda, A., Sabo, J, Janoušek, L., Špaček, M. (2024) *Problems of Cryobiology and Cryomedicine* **34 (2),** 71-112. https://doi.org/10.15407/cryo34.02,71
39 . European Commission (2015) *Final Report EAHC/2012/Health/19 Contract No 201236301: Economic Landscapes of Human Tissues and Cells for Clinical Application in the EU*, European Commission, Brussels.
40. SÚKL Pokyn č. VYR-39 (2009) *Povolení činností souvisejících se zajištěním lidských tkání a buněk určených k použití u člověka*, 1-5.
41. Špaček M, Měřička P, Janoušek L, Štádler P, Adamec M, Vlachovský R, Guňka I, Navrátil P, Thieme F, Mitáš P, Špunda R, Špatenka J, Staffa R, Němec P & Lindner J (2018) *Cell and Tissue Banking* **19**, 437-445. DOI: 10.1007/s10561-018-9691-4
42. European Centre for Disease Prevention and Control (2020) *ECDC Report: Coronavirus Disease (COVID-2019) and Supply of Substances of Human Origin in the EU/EEA – First Update*, ECDC, Stockholm.
43. Jashari R, Van Hoeck B, Ngakam R, Goffin Y & Fan Y (2013) *Cell and Tissue Banking* **14**, 589-599. DOI: 10.1007/s10561-012-9359-4
44. Jashari R, Bouzet V, Alcaraz Blanco MJ,

Oleffe A, Lecocq E & Mastrobuoni S (2023) *Cell and Tissue Banking* **24**, 613-625. DOI: 10.1007/s10561-022-10063-z
45. Měřička P, Špaček M, Janoušek L, Dvořáček L, Štádler P, Vlachovský R, Guňka I, Honegrová B, Brandejs D, Štěrba L, Voxová B, Štěpánová V, Navrátil P, Staffa R, Němec P & Lindner J (2015) *Cryobiology* **71**, 546-547. DOI: 10.1016/j.cryobiol.2015.10.041
46. Novotný R, Měřička P, Chlupáč J, Matějka R, Křístek J, Marada T, Konaříik M, Ivak P, Štěrba L, Hlubocký J, Pirk J, Janoušek L & Froněk J (2020) *BioMed Research International* **20**, 1-7. DOI: 10.1155/2020/6545190
47. Awan M, Buriak I, Fleck R, Fuller B, Goltsev A, Kerby J, Lowdell M, Měřička P, Petrenko A, Petrenko Y, Rogulska O, Stolzing A & Stacey GN (2020) *Regenerative Medicine* **15**, 1463-1491. DOI: 10.2217/rme-2019-0145
48. Foundation for the Accreditation of Cellular Therapy and the Joint Accreditation Committee ISCT and EBMT (JACIE) (2018) *International Standards for Hematopoietic Cellular Therapy Product Collection, Processing, and Administration*, 7th ed, FACT–JACIE, Omaha, NE.