Strengthening capabilities and capacities of the interdisciplinary laboratory at the National Centre of Tissue and Cell Banking for the safety and quality control of human tissues used for transplantation

1. Basic Information

1.1 CRIS Number: 2006/018-180.03-04
1.2 Title: Strengthening capabilities and capacities of the interdisciplinary laboratory at the National Centre of Tissue and Cell Banking for the safety and quality control of human tissues used for transplantation
1.3 Sector: Free movement of goods
1.4 Location: Poland, the Ministry of Health, National Centre of Tissue and Cell Banking ul. Chalubinskiego 5, 02-004 Warsaw

2. Objectives:

2.1. Overall Objective(s):

2.2. Project purpose(s):

1. Development of interdisciplinary laboratory at the National Centre of Tissue and Cell Banking;
2. Increase the safety and quality of human tissue grafts.

2.3. Justification

Tissue and cell transplantation is a commonly used method in human therapy. The Community should promote the highest possible level of protection to safeguard public health regarding safety and quality of tissues and cells used for transplantation.

Directive 2004/23/EC of the European Parliament and Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells, in order to ensure a high level of health protection in the Community, and will come into force on April 7, 2006 in all Member States.

The Act of the Polish Parliament of 1 July 2005 on procurement, storage and distribution of cells, tissues and organs has come into force on January 1st 2006.

Regulations concerning this Act are already in force or at the stage of preparation by the Minister of Health. The recommendations derived from Directive 2004/23/EC and Technical Directives will be subsequently implemented in Poland. Tissue banks have to implement national regulations based on the European regulations by the end of 2008.
3. **Description**

3.1 **Background and justification:**

Preserved human connective tissue grafts such as bone, cartilage, tendons, skin and amnion that are prepared and distributed by tissue banks, are widely used nowadays in various clinical disciplines. The highest demand is for bone allografts which are commonly used for reconstructive surgery in orthopaedics and traumatology. The general purpose of tissue banks is to provide safe and effective allografts for transplantation.

The risk of infectious disease transmission with human tissue grafts is a major concern in tissue banking practice. Non-sterilised, fresh or frozen bone allografts collected under aseptic conditions have been associated with transmission of viral infections such as HIV, hepatitis C and B viruses (HCV, HBV) and bacterial infections including tuberculosis. Recently, *Clostridium sordelli* sepsis, of 23-year-old male, who received a contaminated bone allograft was the cause of his death. A year ago, three patients have died because of *rabies* transmitted during organ transplantation.

To minimize the hazard of infectious disease transmission, several steps should be undertaken: careful donor screening and selection, proper tissue procurement, processing preservation and storage. Even if all these procedures are done under aseptic conditions, the possibility of bacterial and viral disease transmission of donor origin can not be excluded. Therefore, sterilisation of tissue allografts is strongly recommended. Several methods have been applied for sterilisation of human tissue grafts including chemicals (e.g. ethylene oxide, glutaraldehyde, formaldehyde, paraacetic acid, glycerol), heat (boiling, autoclaving, pasteurizing), UV and ionizing radiation.

Radiation sterilisation is a low temperature method, which can be applied when the more commonly used heat sterilisation would cause unacceptable damage to products. The sterilisation efficiency of ionising radiation lies in its good penetrability inside matter (especially gamma rays) and its high effectiveness in the inactivation of pathogens. It allows the sterilisation of materials in previously closed wrapping and in this way, avoids recontamination during packing.

This method of sterilization has been recommended by the IAEA and implemented for sterilization of tissue allografts and is routinely applied in many tissue banks including National Centre of Tissue and Cell Banking (the Central Tissue Bank in Warsaw) and other multi-tissue banks in Poland where the dose of 35 kGy is used.

Two types of irradiation are used: gamma rays (\(^{60}\)Co source) and/or beam of accelerated electrons (10 MeV). Due to the limited activity of \(^{60}\)Co sources in Poland, electron beam accelerators are mainly used. It should be kept in mind, however, that the penetration of accelerated electrons in respect to dense compact bone is limited and this type of irradiation might be insufficient for sterilization of massive bone grafts. Therefore the installation of the high dose rate gamma source is strongly needed for sterilization of human tissue grafts to increase their safety.
It should be kept in mind however, that high doses of ionising radiation used for sterilization purposes can evoke numerous chemical and physical changes which may affect biological quality of tissue allografts such as osteoinductive capacity of bone, mechanical properties of bone and other connective tissue allografts as well as the rate of their resorption in vivo. The safety and quality of polymers used for graft packing may also be affected.

To safeguard public health and prevent the transmission of infectious diseases by human tissue grafts, the National Centre of Tissue and Cell Banking is responsible for implementing a tissue banking surveillance system at the national level by creation of a quality control & management system for tissue banking practice according to Directive 2004/23/EC, GLP and ISO family standards and to optimise preservation and sterilization procedures for various types of tissue allografts.

In the scope of previous project 2004/016-829.01.05 it is envisaged to create National Centre of Tissue and Cell Banking in order to harmonize national regulations with the UE Directive 2004/23/EC and preparation of standards concerning proper classification of donors, tissue procurement, testing, processing, preservation, storage and distribution of human allografts. The Centre will elaborate training programme for various groups of health professionals engaged in tissue banking practice. Finally, there will be assistance in building a database allowing for tissue traceability at each stage of procedures and upgrading of tissue bank processing laboratories.

The aim of the present project is to build the new capabilities and capacities of the existing interdisciplinary laboratory for control the safety and quality of human tissue grafts at the National Centre of Tissue and Cell Banking. Realization of this project does not depend on the implementation of the previous Transition Facility project (see Annex 5).

Although existing interdisciplinary laboratory at the National Centre of Tissue and Cell Banking possess highly qualified personnel, it is poorly equipped to fulfil the requirements of safety and quality control of human tissue grafts.

The main purposes are to extend competence of the laboratory by completing necessary equipment to enhance safety of human tissue grafts by implementing radiation sterilization and to control their quality. These tasks will be achieved by installation of $^{60}$Co source used for graft end-sterilization and by evaluation of the effect of various preservation procedures (e.g. lyophilisation, deep-freezing) and radiation-sterilization conditions (doses, temperatures of irradiation) on physical, chemical and biological properties of different types of human connective tissue grafts, evaluation of toxicity of irradiated tissue components (e.g. bone medullary lipid degradation products) and of toxicity of irradiated polymers used for graft packing.

High technology equipment listed in the project fiche is needed for the interdisciplinary laboratory of the National Centre of Tissue and Cell Banking for evaluation the quality, as well as to increase safety of human tissue grafts prepared and distributed in Poland. Obviously, such equipment is not a standard for tissue bank, but the National Centre of Tissue and Cell Banking is a leading, reference institution responsible for coordination of tissue banking activities in Poland, namely
safety and quality control of human tissue grafts, and the interdisciplinary laboratory is an important part of the surveillance system.

Although in the Directive 2004/23/EC is not mentioned directly, that the end sterilization of human tissue grafts is always required, but this problem has been described in the Draft Commission Directive implementing Directive 2004/23/EC of the European Parliament and of the Council as regards traceability requirements, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells.

In the part D of Annex I of Article 6 of the above-mentioned document it is stated, that tissues or cells exposed to the environment during processing without subsequent microbial inactivation air class A is required. Less stringent environment may be acceptable where validated microbial inactivation or terminal sterilization is applied. National Centre is going to upgrade cell culture facilities to air class A environment but in the other laboratories used for tissue processing air class C is planned. For that reason and due to the high risk of infectious disease transmission (see Annex I of that project fiche) end sterilization procedure (i.e. radiation-sterilization) must be implemented.

Implementation of new types of tissue grafts, modification of preparation, preservation or sterilization methods require the evaluation of their effects on human tissue safety and quality before grafts distribution. The evaluation of graft quality will be done by measuring physical, chemical and biological properties of such prepared human tissue grafts.

In case of adverse reaction of the recipient to transplanted tissue graft several procedures have to be performed. In such a situation a corrective action is not only stopping the distribution of these grafts, but also the evaluation of physical, chemical and biological properties of that particular tissue graft (if possible) and other grafts prepared from the same donor by the same method, etc. This in turn, may help to find and understand the reason of the adverse reaction and will allow avoiding it in a future.

Such a well-equipped interdisciplinary laboratory localised at the National Centre of Tissue and Cell Banking and supervised by the Polish Ministry of Health will provide services to control the safety and quality of human tissue grafts prepared by tissue banks in Poland and in other Member States, if requested.

3.2 Linked activities:

Transition Facility Programme of 2004 - Establishment of institutional control on the safety and quality of human tissues and cells used for transplantation - Development of a National Centre of Tissue and Cell Banking will be implemented in 2006. The project will be implemented through two technical assistance components and small scale investment component.
3.3 Results:

1. The capability and capacity of newly equipped interdisciplinary laboratory to control the safety and quality of human tissue grafts enhanced.
2. Sterile human tissue grafts after their exposure to radiation sterilization as an end-sterilization process obtained.
3. The effect of various preservation procedures and radiation-sterilization conditions on physical, chemical and biological properties of different types of human connective tissue grafts evaluated.
4. The toxicity of irradiated tissue components and toxicity of irradiated polymers used for graft packing evaluated.

3.4 Activities:

**Contract 1 - Technical Assistance** focuses on:

Assistance of radiation chemists in the application of ionising radiation to sterilise various types of human tissue grafts and to validate sterilisation procedures by measuring absorbed dose using various types of dosimeters is needed.

It is expected from the experts (2 persons):
- radiation chemistry background,
- over 10 years of experience in radiation sterilization,
- knowledge of the technical aspects of radiation sterilization,
- knowledge of estimation of the absorbed dose of the ionizing radiation,
- fluent English or Polish speaking.

Tissue allografts will be prepared and preserved by different methods (e.g. fresh, lyophilised or deep-frozen) at the National Centre of Tissue and Cell Banking according to its Standard Operating Procedures (SOPs). The radiation chemists will be responsible for irradiation of such prepared human tissue grafts with:

1. gamma ray irradiator (gamma ray source should be purchased in this project)
2. electron beam irradiator (linear 10 MeV electron accelerator is available at the Institute of Nuclear Chemistry and Technology at Warsaw).

Irradiation will be carried out by applying various doses at different temperatures.

Validation of radiation sterilization procedures will be performed using various types of dosimeters, including:

1. bone powder dosimeter
2. L-alanine dosimeter

based on Electron Paramagnetic Resonance (EPR) Spectrometry.

Reports concerning irradiation procedures and their validation will be prepared.

The project cost is approx. 0.12 MEUR (Transition Facility – 0.12 MEUR)
Contracts 2 and 3 - Investment focuses on:

Building of new capabilities of the interdisciplinary laboratory to control the safety and quality of human tissue grafts by:

1. Application of two types of ionising radiation, i.e. gamma rays and a beam of accelerated electrons for sterilisation of tissue allografts, validation of radiation sterilisation procedures
2. Enhancement of the knowledge regarding the safety and quality of tissues used for transplantation by:
   (a) evaluation of chemical, physical and biological properties of preserved and radiation-sterilised tissue allografts
   (b) evaluation of the toxicity of irradiated tissue components (e.g. bone medullary lipids) and polymers used for graft packing

1. Investment – works: adaptation of facilities for new equipment – project cost approx. 0.05 MEUR (0.05 MEUR of Polish co-financing)

Adaptation of some existing facilities for interdisciplinary laboratory according to GLP (Good Laboratory Practise).

2. Investment – equipment: project cost approx. 0.99 MEUR (Transition facility – 0.7425 MEUR and 0.2475 MEUR of Polish co-financing)

The task of the laboratory will be to control of the safety and quality of human tissue grafts before and after various types of sterilization.

The interdisciplinary laboratory at the National Centre of Tissue and Cell Banking is poorly equipped and the most of the graft examinations is carried out of the laboratory eg. bone mechanical properties tests are performed at the Polytechnic School in Warsaw, the other like EPR dosimetry, amino acid analysis are not available for these kinds of studies.

Purchase of equipment listed below will allow for evaluation of effects of various preservation and sterilization methods on chemical, physical and biological properties of human tissue grafts, evaluation of cytotoxicity of human tissue grafts and packing materials. This will allow fulfilling the safety and quality control requirements of the Directive EC/23/2004.

National Centre of Tissue and Cell Banking is the only beneficiary of the project. Personnel of the Centre include radiation chemist, biochemist and biotechnologist, who are able to provide measurements using equipment present in the laboratory and that, which may be purchased within this project. There is no necessity to involve any other institutions in order to achieve the objectives of the project.

Equipment needed for these activities:
  a) gamma rays source unit with activity approx. 20 000 Ci and a dose rate of approx. 20 kGy/hour – for radiation sterilization of human tissue grafts, including those preserved by deep freezing, with the time of radiation sterilization not exceeding 2 hours, to keep tissues frozen on dry ice during irradiation. The National Centre of Tissue and Cell Banking possesses adequate facilities for this unit and preliminary acceptance from the Radiation
b) Amino Acid Analyser – will be used for quantitative analysis of amino acids released from various human tissue grafts preserved and irradiated under different conditions (cost approx. 155 000 EUR)

c) High Performance Liquid Chromatography (HPLC) System with Fluorescence Detector – will be used for the quantitative analysis of collagen cross-linking molecules present in situ and extracted from irradiated human tissue grafts after application of various preservation procedures and sterilization conditions (cost approx. 70 000 EUR)

d) Confocal microscope – will be used for 3D evaluation of osteoinductive potential of preserved bone grafts and rate of their resorption in vivo (on animal models); and for estimation of in vitro (on cell cultures) cytotoxicity of radiation sterilized tissue components and polymers used for graft packing (cost approx. 350 000 EUR)

e) Gas Chromatography with Mass Spectrometry Detection (GC/MS) – will be used for analysis of lipid decomposition and products of peroxidation of medullary lipids in bone tissue allografts radiation sterilized with gamma rays (cost approx. 100 000 EUR)

f) Vertical Laminar Flow Cabinets (3 pcs) – to be used for tissue allograft preparation under aseptic conditions (Grade A) (cost approx. 35 000 EUR)

The total investment project cost is approx. 0.99 MEUR (Transition Facility – 0.7425 MEUR and 0.2475 MEUR of Polish co-financing).

3.5 Lessons learned:

Following the Commission’s remarks on the coverage pf the Evaluation Plan 2005 and 2006 communicated during the JMC meeting in December 2005, the NAC Evaluation Unit (OCEI) commissioned two thematic evaluation of the Transition Facility projects 2004-2005 within the framework of the TA contract under Phare 2003/005-710.01.07.01.

The scopes of these two evaluations have been envisaged for twinning and non-twinning projects. The evaluators commenced their work at the beginning of 2006 and the both evaluation reports are expected to be ready by May 2006.

The general lesson to be drawn from the both evaluations for this project is to learn whether it is relevant to its objectives as well as the administrative structure within key institutions involved operate efficiently and effectively and whether the EDIS system has been adopted effectively for the benefit of the referenced TF project.

4. Institutional Framework

The Ministry of Health, as the main beneficiary of the National Centre of Tissue and Cell Banking will coordinate the project as activities are planned to be on a national level.

SPO: Anna Dziedzic-Goclawksa, Director, National Centre for Tissue and Cell Banking.
Strengthening capabilities and capacities of the interdisciplinary laboratory at the National Centre of Tissue and Cell Banking for the safety and quality control of human tissues used for transplantation

The Unit responsible for the realization and coordination of the project:
National Centre for Tissue and Cell Banking, ul. Chalubinskiego 5, 02-004 Warsaw,
Contact person: Anna Dziedzic-Goclawska, Director, National Centre for Tissue and Cell Banking, Phone/Fax (+4822) 621 75 43, Phone: (+4822) 696 13 36
email: agoclaw@ib.amwaw.edu.pl

Implementation of the project does not change the above institutional framework.
The equipment purchased is to belong to the National Centre of Tissue and Cell Banking supervised by the Polish Ministry of Health.

5. Detailed Budget

<table>
<thead>
<tr>
<th>€M</th>
<th>Transition Facility support</th>
<th>Co-financing</th>
<th>Total cost (TF plus cofinancing)</th>
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<td>Investment Support</td>
<td>Institution Building</td>
<td>Total Transition Facility (=I+IB)</td>
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<td>Contract 1 Technical Assistance</td>
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<td>0,12</td>
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<tr>
<td>Contract 2 Investment – equipment contract</td>
<td>0,7425</td>
<td>0,7425</td>
<td>0,2475</td>
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<tr>
<td>Contract 3 Investment – works contract</td>
<td>0,05</td>
<td>0,05</td>
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<tr>
<td><strong>Total</strong></td>
<td>0,7425</td>
<td>0,12</td>
<td>0,8625</td>
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</table>

(*) contributions from National, Regional, Local, Municipal authorities, FIs loans to public entities, funds from public enterprises
(/**) private funds, FIs loans to private entities

The amounts for national co-financing indicated in the table correspond to cash co-financing, unless otherwise stated. Contributions from the Polish administration for effective implementation of the TA may be further detailed in the Terms of references. Unless otherwise indicated joint cofinancing is provided.

VAT does not constitute eligible expenditure except where it is genuinely and definitely borne by the final beneficiary. VAT which is considered recoverable, by whatever means, cannot be considered eligible, even if it is not actually recovered by the final beneficiary or individual recipient.

According to the joint financing procedure, the total cost of the investment - equipment contract (0,99MEUR) will be financed with both Transition Facility funds and Polish funds, in proportions corresponding with contributions declared in the budget table.
In the case of Joint Co-financing, where the final overall cost is lower than foreseen in the project fiche, the National Public and Transition Facility Co-financing are reduced proportionally so as to maintain the agreed rate of co-financing. In the case of Parallel Co-financing, where the final cost is lower than foreseen in the project fiche, it must be shown that the overall objectives of the project have been fully achieved.

6. Implementation Arrangements
There will be a Project Steering Committee (PSC) established in order to speed up the implementation process of the given project components in the first months after Financial Decision for Transition Facility 2006 is taken. The structure of the Committee will be working as an advisory and monitoring body until particular components are contracted and thus where appropriate may be replaced by the Steering Committees for TA or investment components independently.

The participants of the Project Steering Committee will be representatives of the following institutions: PAO, NAC, CFCU and beneficiary (SPO, contact person as indicated in the fiche and representative from Office for Foreign Aid Programmes in Health Care). It is also recommended to invite representatives of NAO services while the issues of financial management flow are to be comprehensibly discussed. The Project Steering Committee will meet every quarter starting from the date of signing the Financial Decision and will concentrate on discussing the problem occurred at the beginning phase of project implementation as well as on defining possible solutions and corrective measures. The PAO representative will organise and chair the PSC meetings.

6.1 Implementing Agency
PAO: Tadeusz Kozek, Under-secretary of State at the Office of the Committee for European Integration, Aleje Ujazdowskie 9, 00-918 Warsaw, Phone: (+4822) 455 52 41

Finance and Contacts Unit, Foundation “Co-operation Fund”, CFCU Director, ul. Góроnośląska 4a, 00-444 Warsaw, Phone: (+48 22) 622 88 20
The CFCU is responsible for handling tendering, contracting and payments of contracts on behalf of the beneficiary.

6.2 Twinning
N/A

6.3 Non-standards aspects
N/A

6.4 Contracts

- Contract 1 - Technical Assistance
  Transition Facility - 0.12 MEUR – gross value

- Contract 2 - Investment – equipment contract
  Transition facility – 0.99 MEUR – gross value (0.7425 MEUR and 0.2475 MEUR of Polish co-financing) – joint co-financing.

- Contract 3 - Investment - works contract
  Polish co-financing – 0.05 MEUR – gross value
7. Implementation Schedule-

- Contract 1 - Technical Assistance
  7.1 Commencement of contracting process: - 4th quarter of year 2006 – 2nd quarter of year 2007
  7.2. Start of project implementation (signature of contract): 1st and 2nd quarter of year 2008
  7.3. Project completion: 2nd quarter of year 2009

- Contract 2 - Investment – equipment contract
  7.1 Commencement of contracting process: - 4th quarter of year 2006 – 2nd quarter of year 2007
  7.2. Start of project implementation (signature of contract): 1st and 2nd quarter of year 2008
  7.3. Project completion: 4th quarter of year 2008

- Contract 3 - Investment - works contract
  7.1 Commencement of contracting process: - 4th quarter of year 2006 – 2nd quarter of year 2007
  7.2. Start of project implementation (signature of contract): 1st and 2nd quarter of year 2008
  7.3. Project completion: 4th quarter of year 2008

8. Sustainability

The National Centre of Tissue and Cell Banking was established by the Polish Minister of Health. The annual budget of this centre, including activities of the interdisciplinary laboratory, is a part of the budget of the Polish Ministry of Health.

The beneficiary has foreseen an adequate staff and financial resources to maintain the administrative function. The National Centre of Tissue and Cell Banking will cover the costs for maintenance and up-date equipment. Personnel trained by TA activity will provide the knowledge to other staff of the Centre.

9. Conditionality and sequencing

9.1 Conditionality:

The results of the previous project: creation and upgrading of national standards concerning tissue banking, creation of training programmes for professionals engaged in tissue banking practice, upgrading of tissue bank facilities and creation of a software for tissue banking are to be met before implementing this project.
9.2. Sequencing:

Preparation of TORs - 3rd and 4th quarter of year 2007
Expert selection for technical assistance – 2nd quarter of 2008
    1) Tender procedures for equipment contract - 1st and 2nd quarter of 2008
    2) Completion of works contract - 3rd quarter of year 2008
    3) Purchasing the equipment for interdisciplinary laboratory – 3rd and 4th quarter of 2008
    4) Radiation sterilization of human tissue grafts and validation of the process – 4th quarter of 2008 and 1st quarter of 2009
    5) Performing of measurements – 1st and 2nd quarter of 2009
    6) Fulfilment the requirements of a quality control & management system in tissue banking practice according to Directive 2004/23/EC, GMP and ISO family standards - 1st and 2nd quarter of 2009
ANNEXES TO PROJECT FICHE

1. Logical framework matrix in standard format (compulsory)
2. Detailed implementation chart (compulsory)
3. Contracting and disbursement schedule by quarter for full duration of programme (including disbursement period) (compulsory)
4. Annex 4 - Supplementary justification (1)
5. Annex 5 - Supplementary justification (2)
6. Annex 6 - Technical specification of equipment
### Annex 1: Logframe matrix

<table>
<thead>
<tr>
<th>LOGFRAME PLANNING MATRIX FOR THE PROJECT</th>
<th>Programme name and number</th>
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<tr>
<td>Project</td>
<td>Contracting period expires 4Q2008</td>
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<tr>
<td>Strengthening capabilities and capacities of the interdisciplinary laboratory at the National Centre of Tissue and Cell Banking for the safety and quality control of human tissues used for transplantation</td>
<td>Disbursement period expires 4Q2009</td>
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<tr>
<td>Total budget</td>
<td>1.16 MEUR</td>
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<tr>
<td>Transition Facility Budget</td>
<td>0.8625 MEUR</td>
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<table>
<thead>
<tr>
<th>Overall objective</th>
<th>Objectively Verifiable Indicators</th>
<th>Sources of Verification</th>
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<tr>
<td>Project purpose (Immediate Objectives)</td>
<td>Objectively Verifiable Indicators</td>
<td>Sources of Verification</td>
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| Development of interdisciplinary laboratory at the National Centre of Tissue and Cell Banking       | Interdisciplinary laboratory of the National Centre of Tissue and Cell Banking equipped to serve as a safety and quality control laboratory.  
Implementation of gamma irradiation for sterilization of human tissue grafts.  
Evaluation of biological (osteoinductive), physical (yield and stability of free radicals) and biochemical (collagen degradation) properties of human tissue grafts prepared for distribution to check its quality.  
Estimation of toxic effect of irradiated tissue components and polymers used for packing to increase the safety of distributed tissue grafts. | Reports from the National Centre for Tissue and Cell Banking.  
Experimental and statistical data.                                                                 | Payment for the project from Transition Facility budget and Ministry’s budget.  
Professional staff involved in the project.                                                                 |

Strengthening capabilities and capacities of the interdisciplinary laboratory at the National Centre of Tissue and Cell Banking for the safety and quality control of human tissues used for transplantation
<table>
<thead>
<tr>
<th>Results</th>
<th>Objectively Verifiable Indicators</th>
<th>Sources of Verification</th>
<th>Assumptions</th>
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<tbody>
<tr>
<td>1. The capability and capacity of newly equipped interdisciplinary laboratory to control the safety and quality of human tissue grafts enhanced.</td>
<td>1. Laboratory equipped and personnel trained (about 4 persons) to perform measurements on quality and safety of human tissue grafts by the end of the project.</td>
<td>Reports from the National Centre for Tissue and Cell Banking. Experimental and statistical data. Project realisation reports. Adequate equipment.</td>
<td>Payment for the project from the Transition Facility budget and Ministry’s budget. Professional staff involved in the project.</td>
</tr>
<tr>
<td>2. Sterile human tissue grafts after their exposure to radiation sterilization as an end-sterilization process obtained.</td>
<td>2. Evaluation of the absorbed dose of ionising radiation form gamma rays source using various types of dosimeters including bone dosimeter – validation of radiation sterilisation procedures.</td>
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<tr>
<td>3. The effect of various preservation procedures and radiation-sterilization conditions on physical, chemical and biological properties of different types of human connective tissue grafts evaluated.</td>
<td>3a. Physical properties estimated by the yield and stability of free radicals and other paramagnetic entities induced by ionising radiation of gamma rays source in bone grafts.</td>
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<td></td>
<td>3b. Chemical properties estimated by degradation of collagen - a major constituent of various human connective tissue grafts in vitro (measurements of neutral and acid soluble collagen and its susceptibility to enzymatic digestion) by High Performance Liquid Chromatography (HPLC) and by Amino Acid Analyser.</td>
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<td>3c. Biological properties estimated by the osteoinductive potential of human tissue bone allografts and the rate of their resorption in vivo by Amino Acid Analyser and on model of heterotopic bone induction.</td>
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<td>4. Application of alternative methods (in vitro toxycology) tests to evaluate any toxic effect of irradiated tissue components (e.g. lipids) and polymers used for packaging of tissue grafts by Gas Chromatography with Mass Spectrometry Detection (GC/MS), confocal microscopy and Vertical Laminar Flow Cabinets.</td>
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Strengthening capabilities and capacities of the interdisciplinary laboratory at the National Centre of Tissue and Cell Banking for the safety and quality control of human tissues used for transplantation
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<tr>
<th>Activities</th>
<th>Means</th>
<th>Assumptions</th>
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<td>Technical assistance of radiation chemists.</td>
<td>Technical assistance contract signed.</td>
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<td>Adaptation of existing facilities for the specialised equipment needed for the interdisciplinary laboratory.</td>
<td>Work contract signed.</td>
<td>Professional staff involved in the project.</td>
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<tr>
<td>Providing adequate equipment.</td>
<td>Equipment contract signed.</td>
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Preconditions
The results of the previous project are to be met before implementing this project.
Annex 2-3: Implementation, contracting and disbursement schedule

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<th>Date of Drafting</th>
<th>November 2005</th>
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<td>Planning Period</td>
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Key:
- D = design of contract and tendering
- C = signature of contract
- I = contract implementation and payment
- * show amounts in MEuro increasingly
Annex 4

Re: 2006 Project
“Strengthening the capabilities and capacities of the interdisciplinary laboratory at the National Centre of Tissue and Cell Banking for the safety and quality control of human tissues used for transplantation”.

SUPPLEMENTARY JUSTIFICATION

The general purpose of the activities carried out by a tissue establishment, according to Directive 2004/23/EC, is to provide safe and effective human tissue grafts which can be used for transplantation. Connective tissue allografts, such as bone, cartilage, tendons, pericardium, heart valves, skin or amnion are prepared by multi-tissue banks.

The risk of infectious disease transmission with human tissue grafts is a major concern in tissue banking practice. Microorganisms can be introduced into grafts during tissue procurement, processing, preservation or storage. Even if all these procedures are carried out under aseptic conditions, the possibility of bacterial, fungal or viral disease transmission of donor origin can not be excluded.

Non-sterilised, fresh or frozen bone allografts collected under aseptic conditions, even after careful donor screening and selection, have been associated with the transmission of viral infections such as HIV, hepatitis C and B viruses (HCV, HBV) and bacterial infections including tuberculosis. Not long ago, Clostridium sordelli transmission with bone allograft collected under aseptic conditions (but non-sterilised) was the cause of sepsis and death of the recipient. A few patients were also recently infected with rabies transmitted thru organ and tissue transplantation and three of them died.

Therefore, to fulfil the requirements of Directive 2004/23/EC of providing safe human tissue grafts, it is recommended that tissue grafts be sterilised.

Several methods for sterilisation tissue allografts, including chemicals (e.g. ethylene oxide, glutaraldehyde, formaldehyde, peracetic acid), heat (boiling, autoclaving, pasteurising), UV and ionising radiation have been used.

After chemical sterilization some amount of the applied agent can remain in the tissue (e.g. ethylene oxide) and be toxic to the graft recipient.

Radiation sterilisation is a low temperature method which can be applied when the more commonly used heat sterilisation would cause unacceptable damage to the heat-sensitive biological materials (e.g. human tissue grafts). The sterilisation efficiency of ionising radiation lies in its high effectiveness in the inactivation of pathogens and its good penetrability inside matter (especially gamma rays). It is efficient at room temperature and even at temperatures below zero. It allows the sterilisation of materials in previously closed wrapping and, in this way, avoids recontamination during packing. For this reason, ionising radiation, i.e. gamma rays or a beam of accelerated electrons can be used to sterilise human tissue grafts.

SAFETY OF HUMAN TISSUE GRAFTS

Why we need $^{60}$Co source with a high dose rate of gamma radiation?
We have over 40 years of experience in tissue banking. Our Tissue Bank was established in 1963 and since then, ionising radiation has been routinely applied to sterilise connective tissue grafts with a dose of 35 kGy. Over 270 000 radiation-sterilised tissue grafts have been prepared and used in hospitals throughout Poland and not one case of infectious disease transmission has been reported.

Earlier, tissue allografts were preserved by lyophilisation (freeze-drying) and subsequently radiation sterilised at room temperature in a $^{60}$Co source with a dose rate 1 kGy/h, so to achieve a dose of 35 kGy, the time of exposition was 35 hours. The irradiation was performed in the Institute of Applied Radiation Chemistry, Technical University in Lodz.

Meanwhile, the results of interdisciplinary research developed in our Tissue Bank indicate clearly, that it is possible to reduce radiation-induced damage to tissue grafts if the irradiation is carried out at low temperature. This, in turn, allows for preparation of bone grafts with better mechanical and biological properties. Thus, we decided to preserve tissue grafts by deep-freezing and to irradiate them routinely at low temperature on dry ice. Unfortunately, the very low dose rate (1 kGy/h) delivered by the only $^{60}$Co source available in Poland that meets our requirements does not allow for the irradiation of deep-frozen tissue grafts at low temperature. The exposition time in this source is approximately 35 hours, while after a few hours dry ice evaporates completely. This is the main reason why we critically need a powerful gamma ray source unit with a high dose rate of at least 20 kGy/hour to be used for radiation sterilization at low temperature of human bone and other connective tissue grafts, including those preserved by deep-freezing. With such a powerful source, it will be possible to shorten the time of radiation sterilization to under 2 hours, and this will allow the tissues to keep frozen on dry ice during the whole time of irradiation.

Since it has been proven that radiation treatment at low temperature effectively reduces radiation-induced damage to any connective tissue grafts, we now started to preserve other soft tissue grafts, such as tendons, skin, amnion, pericardium by deep-freezing and to also irradiate them on dry ice with a dose of 35 kGy at 10 MeV electron accelerator in the Institute of Nuclear Chemistry and Technology in Warsaw. The time needed to achieve 35 kGy with electron beam irradiation is only a few minutes. Unfortunately, it is impossible to sterilise bone allografts with a beam of accelerated electrons due to the less effective penetration of fast electrons in dense, mineralized bone tissue. Since bone grafts constitute over 75 % of tissue allografts prepared by tissue banks in Poland – a powerful gamma source is badly needed.

The National Centre of Tissue and Cell Banking possesses adequate facilities that meet the requirements of this unit and has obtained preliminary permission from the Radiation Protection Department of the Polish Atomic Energy Agency for its installation.

QUALITY CONTROL OF TISSUE ALLOGRAFTS PRESERVED AND STERILISED BY VARIOUS METHODS

At the National Centre of Tissue and Cell Banking, we possess tissue bank laboratories, cell and tissue culture laboratories, animal quarters, experimental animal surgery rooms and biochemical laboratories equipped with e.g. deep-freezers –150°C (2pcs), -85°C (10 pcs), lyophilisation apparatuses (3 pcs), control-rate freezer for cells and tissues, light and UV fluorescent microscopes connected to a CCD camera and PC with software for morphometric image analysis, reverse microscopes, CO₂
incubators for cell and tissue culture, vertical biohazard laminar flow cabinets (2 pcs in Tissue Bank laboratory), rotary microtomes, a diamond saw microtome for cutting undecalcified bone tissue, an UV-vis spectrometer, Elisa (microtiter) plate readers, SPEX freezer miles, etc.

All the above mentioned facilities and equipment, apart from the tissue bank laboratories, are used as an interdisciplinary laboratory to control the quality of connective tissue allografts prepared by various methods in tissue banks in Poland. We are open to co-operation with other European tissue establishments, if needed.

We have collaborated with the Polytechnic University in Warsaw where the evaluation of mechanical properties of untreated and preserved connective tissue grafts is carried out using an Instron Universal Testing Machine.

The major constituent of all connective tissue grafts is fibrillar collagen. Its structure, spatial arrangement, chemical composition and susceptibility to enzymatic digestion influences the mechanical properties of connective tissue grafts, as well as the rate of their resorption in vivo. Collagen is also a carrier of bone morphogenetic proteins (BMPs) responsible for osteoinductive potential of bone grafts, i.e. their ability to induce a new bone formation at the site of transplantation. These features are of great clinical importance, and thus studies on the effect of various preservation procedures (e.g. lyophilisation, deep-freezing) and radiation sterilization conditions (type, doses and temperature of irradiation) on chemical, physical and biological properties of connective tissue grafts, i.e. on their quality, have been preformed in the interdisciplinary laboratory at the National Centre of Tissue and Cell Banking.

The above studies must be carried out to optimise preservation and sterilization procedures to prepare safe tissue grafts. The optimisation procedures have to be constantly developed in accordance with the actual progress made in science. To fulfil these requirements, new research techniques and methodology should be implemented and that is why the modern equipment listed below is necessary to strengthen the capabilities and capacities of the interdisciplinary laboratory at the National Centre of Tissue and Cell Banking.

Amino Acid Analyser to be used for qualitative and quantitative analysis of amino acid released by different tissue grafts after application of various preservation and sterilisation procedures.

High Performance Liquid Chromatography (HPLC) System with Fluorescence Detector to be used for qualitative and quantitative analysis of collagen cross-linkages present in untreated and preserved connective tissue grafts; the type and amount of cross-linkages influence mechanical properties and the resorption rate of connective tissue grafts in vivo.

Gas Chromatography with Mass Spectrometry Detection (GC/MS) to be used for the analysis of lipid decomposition and determination of the peroxidation products of medullary lipids present in bone tissue allografts after various preservation and sterilisation procedures. It is assumed that some of these products will be toxic and thus the bone grafts will have to be deprived of fat.

Confocal microscope to be used for 3D evaluation of osteoinductive potential of preserved bone grafts and their resorption rate in vivo (using an animal model) as well as for evaluation of the morphological structure of preserved skin, tendons, amnion and pericardium. This equipment can also be used in the cytotoxicity evaluation of
tissue graft components (e.g. bone medullary lipids) and of polymers used for graft packing, carried out in vitro cell cultures.

**Vertical Laminar Flow Cabinet** (3 pcs) to be used for preparation and manipulation with samples under aseptic (sterile) conditions.

We would like to stress that the equipment mentioned above will serve for the quality control of connective tissue grafts preserved and sterilised by different methods and not the routine preparation of tissue grafts in bank laboratories.
Annex 5

RE: currently proposed 2006 Project
“Strengthening the capabilities and capacities of the interdisciplinary laboratory at the National Centre of Tissue and Cell Banking for the safety and quality control of human tissues used for transplantation”

SUPPLEMENTARY JUSTIFICATION

It has been suggested that our currently proposed 2006 Project and our previously accepted 2004 Project overlap.

It has to be stressed, however, that the accepted 2004 Project (2004/016-829.01.05) almost entirely focuses on education performed with the help of twinning experts that will allow us to create national standards and training programmes for tissue bank personnel, according to requirements Directive 2004/23/EC.

Additionally, upgrading tissue bank facilities to reach sanitary and epidemiological protection conditions according to EU standards will be performed.

Creating a special software programme allowing the traceability of tissues from their procurement up to clinical application of tissue grafts and long-term follow up after transplantation is also planned.

The purpose and results of this project are listed below.

Purpose:
2. Developing and strengthening the competence, capabilities and capacities of the National Centre for Tissue and Cell Banking.
3. Enhancement of the knowledge of the parties involved with respect to the safety and quality of tissues and cells used for transplantation.
4. Creation of a software programme to serve in tissue banking practice.

Results
1. Development of a tissue banking surveillance system at the national level.
2. Creation and updating national standards for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells according to Directive 2004/23/EC and EATB standards.
3. Creation of training programmes for various groups of health professionals engaged in tissue banking practice to increase their qualifications.
4. Upgrading tissue bank facilities to reach sanitary and epidemiological protection conditions according to EU standards.
5. Creation of a special software programme to hold several databases allowing traceability from tissue procurement via donor screening and selection, tissue processing, preservation and storage up to clinical application and long-term follow up post surgical treatment with tissue allografts.
6. Optimisation of preservation and sterilisation procedures for various types of tissue allografts.

The aim of the currently proposed 2006 Project is to build new capabilities and capacities of the existing interdisciplinary laboratory established for the control of the safety and quality of human tissue grafts. It will be achieved by supplementation of the laboratory with modern equipment and this, in turn, will allow for implementation of new techniques and methodology to control the quality of human tissue grafts according to the progress of science in this field (see Annex I).

The purpose and the results of the proposed 2006 Project are listed below.

Purpose:
1. Development of interdisciplinary laboratory at the National Centre of Tissue and Cell Banking
2. Increase the safety of human tissue grafts
3. Increase the quality of human tissue grafts

Results:
1. Building new capabilities and capacities of the interdisciplinary laboratory at the National Centre of Tissue and Cell Banking to control the safety and quality of human tissue grafts.
2. Diminishing the risk of infectious disease transmission and to increase their safety by application of ionizing radiation for end-sterilization of human tissue grafts.
3. Evaluation of the effect of ionizing radiation on chemical, physical and biological properties of human tissue grafts.
4. Evaluation of toxicity of irradiated tissue components (e.g. bone medullary lipids) and of irradiated polymers used for graft packing.

Although the results of the currently proposed 2006 Project may appear to be similar to those of the already accepted 2004 Project, it should be pointed out that different tools are used in these two projects to realize the requirements of the Directive 2004/23/EC.

We would also like to stress that we did not previously apply for equipment neither in PHARE programme.
ANNEX 6

Re: 2006 Project
“Strengthening the capabilities and capacities of the interdisciplinary laboratory at the National Centre of Tissue and Cell Banking for the safety and quality control of human tissues used for transplantation”.

TECHNICAL SPECIFICATION OF EQUIPMENT

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