

BIORESOURCE PAPER

VetBiobank, Vetmeduni Vienna: A bioresource for clinical animal biospecimens

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The VetBiobank was established in 2007 as part of the VetCore Facility for Research at the University of Veterinary Medicine, Vienna. The primary objectives of the VetBiobank are (1) to achieve comprehensive collections of high quality animal samples to support research projects and (2) to provide biological reference material as controls e.g. for method verification. Currently, collections mainly contain tumor tissues and healthy reference tissues from cats and dogs, but also a small number of biospecimens from other diseases and other species, including macaque, horse, pig, and small companion animals. A total number of around 58000 individual samples are stored. The VetBiobank is partner of BBMRI.at (the Biobanking and BioMolecular resource Research Infrastructure Austria [1]), the Austrian national node of the European research infrastructure BBMRI-ERIC [2], comprising the only member with a focus on veterinary medicine.

Keywords: clinical animal samples; high quality samples and data; tumor and normal tissue collections; project specific collections; samples from animal experiments

Funding statement: The VetBiobank is part of the central research facility of the University of Veterinary Medicine, which takes over personnel and running costs.

Additionally, the VetBiobank was and is currently supported by two grants from the federal ministry of Austria to BBMRI.at (BMWFM GZ: 10.470/0016-II/3/2013, BMBWF GZ: 10.470/0010-V/3c/2018).

(1) Bioresource Overview

Project description

The VetBiobank was established in 2007 as part of the technology platform VetCore, the central research facility of the University of Veterinary Medicine, Vienna, aimed to support research projects by state of the art technologies. The major goal of the VetBiobank is to collect high quality animal samples and associated data in order to lay the foundation for high quality research and development of new methods. Its mission is to preserve residual (left-over) biospecimens obtained from the campus and to make them available to local and external researchers.

At the beginning, the sampling was restricted to animal tissues derived from local clinics after routine diagnostic or therapeutic intervention, in particular animal tumor tissues and healthy reference tissues, mainly from cats and dogs. The intention was to save samples of spontaneously developed tumors in sufficient numbers to support oncology research projects to improve animal diagnostics and treatment and to boost comparative medicine [3]. Healthy reference tissues were selected as controls for all kinds of investigations.

In 2014 the VetBiobank became a member of the BBMRI.at consortium, which attributes great importance on the sample quality by controlling the pre-analytical sample management process. The VetBiobank implemented systematically an internal quality management system, which is periodically evaluated by BBMRI.at consortium partners [4]. Hence, tissue samples have been collected in accordance to the CEN pre-analytical standards 16826: 1-2 and 16827: 1-3 since 2015, which have recently been developed further to ISO standards [5, 6].

Currently, the collection consists of about 4000 primary tissue samples and about 55000 aliquots (FFPE, frozen pieces in optimal cutting temperature medium-O.C.T.TM, frozen pieces in RNA stabilizing reagent-RNALaterTM, native frozen pieces) processed from these samples. Clinical data about the animal, the health status, the medication, the intervention and the diagnosis are documented and available.

Recently, the scope of the VetBiobank has expanded by offering sample management of prospective samples assigned to certain study projects including tissue samples as well as body fluids (plasma, serum, urine, feces).

Classification (1)

Animal.

Species

Collected samples come from patients of the University of Veterinary Medicine, mainly cats (*Felis silvestris catus*) and dogs (*Canis lupus familiaris*), occasionally other species such as horses (*Equus caballus*), pigs (*Sus scrofa domestica*), sheep (*Ovis aries*), goats (*Capra aegagrus hircus*), or small companion animals such as guinea pigs (*Cavia porcellus*), rats (*Rattus norvegicus*), ferrets (*Mustela putorius fura*), rabbits (*Oryctolagus cuniculus*), hamsters (*Mesocricetus auratus*), mice (*Mus musculus*) are treated. Occasionally, samples from animal experiments are archived e.g. from mice, sheep, pigs and macaques (*Macaca fascicularis*).

Classification (2)

Biological samples and associated data (clinical data, sample management data).

Context**Spatial coverage**

Latitude 48.253595N

Longitude 16.428717E

Description: VetBiobank, University of Veterinary Medicine, Vienna, Veterinärplatz 1, 1210 Vienna, Austria

Temporal coverage

Start 26 September 2007 to present. No date of closure is foreseen.

Temporal coverage for accessibility

N/A.

(2) Methods**Steps**

The VetBiobank implemented an internal quality management system, which is periodically audited by BBMRI.at consortium partners under the leadership of the quality expert group.

All working steps during handling and storage of samples are described in standard operating procedures (SOPs), which are managed by an automatic document control system. One key aspect of the quality management system is the control of the sample management; tissue samples are already collected in accordance to appropriate CEN standards (16826: 1-2 and 16827: 1-3). Samples are picked up from the surgery by VetBiobank staff members and transported to the VetBiobank preparation lab under temperature and time controlled conditions. The processing time, the time of stabilisation/fixation and all steps during FFPE preparation are documented. Consequently, critical parameters during the sample management like the warm ischemia time, the cold ischemia time and the fixation time are available for most samples collected after 2014.

The high quality of tissue samples are achieved by:

- application of SOPs for the entire sample collection process

- documentation of key data regarding the sample collection process (e.g. warm ischemia time, cold ischemia time, fixation time) to enable comparability of samples
- sample evaluation of H&E stained FFPE sections
- sample storage under optimized conditions in round-the-clock monitoring storage systems

Stabilization/preservation

The following types of stabilization methods and containers are used for standard collection of tissue samples:

- FFPE tissues: standard formalin (4% neutral buffered formaldehyde) fixation of tissue samples, 2D labelled cassettes
- Fresh frozen tissues, three kinds of sample types
 - ~ 1 × 0.5 cm piece of tissue, in a cryomold embedded in OCT
 - ~ 0.3 × 0.3 cm pieces in a 1.8 ml cryovial with RNAlater
 - ~ 0.3 × 0.3 cm pieces in a 1.8 ml cryovial without any additives (native)
- Snap frozen in liquid nitrogen

Type of long-term preservation

The following long-term preservation types are used:

- FFPE tissue samples are stored at room temperature in a manual storage system, temperature and humidity are monitored.
- Fresh frozen tissue samples are stored in the vapor phase of liquid nitrogen (-130°C to -180°C) with manual tube handling; temperature of the liquid nitrogen tanks is continuously monitored and recorded. The storage facility is equipped with an alarm system and monitored 24/7 throughout the year.

Storage temperature

FFPE samples are stored at room temperature; fresh frozen tissues are stored in the vapor phase of liquid nitrogen with tube handling at room temperature.

Shipping temperature from patient/source to preservation or research use

The VetBiobank is notified by the surgical staff about suitable samples. Staff members of the VetBiobank pick up samples immediately after collection directly from the operating theatre and transfer them in a prepared cooling box to the VetBiobank lab for processing. Temperature and time stamps are recorded by a data logger.

Shipping temperature from storage to research use

Fresh frozen tissue samples are shipped on dry ice (-80°C), and FFPE samples at room temperature.

Quality assurance measures

The entire workflow of sample and data management is defined by a process description, which is based on precise and detailed SOPs.

All main working steps are recorded manually to ensure compliance to the SOPs.

Tissue samples are collected in accordance to the the CEN standards 16826: 1-2 and 16827: 1-3. A database was developed which allows the documentation of all data postulated by these standards, automatically generating the SPREC code (Version 2.0) for solid samples. The SPREC code is a general indicator for sample quality [7].

Routine procedures for ensuring sample quality:

- documentation of warm ischemia time, cold ischemia time and fixation time
- documentation of all data needed for generation of the SPREC code
- preparation and evaluation of H&E stained slides of each FFPE sample; digital images are available
- storage of samples temperature monitored; temperature profiles are available
- samples are safeguarded by a proper alarm system and by the availability of an adequate back-up storage system

Checks upon request

- location check
- check of required aliquots
- fresh frozen tissue samples: preparation of H&E stained cryosections for morphological evaluation
- analysis of RNA integrity number (RIN) values

Source of associated data

Data associated with the samples are collected both via electronic and manual methods and finally entered into the VetBiobank data storage system.

Clinical data from animal patients are recorded in the Clinical Data Registry, provided by the University on request. Data regarding the sample management are recorded on forms during single working steps e.g. sample transportation, sample preparation, FFPE processing and storage in accordance to appropriate SOPs. Based on the entered data the VetBiobank data base automatically generates the SPREC code and allows a quick search for selected criteria to check the appropriateness of samples to be included in specific types of analysis.

Ethics Statement

Patient owners agree on the admission form, that biological material collected from animal patients as part of diagnostic and therapeutic measures, which is not needed for diagnosis, can be used for research projects at the Vetmeduni. Since 2018, the consent has been changed from a right to use into a transfer of property rights to the Vetmeduni. Additionally, we offer our expertise and infrastructure after consultation with the ethics committee and the animal welfare board, to provide animal model user services to store backup samples and to share left-over materials in accordance to the 3R principles.

All sample applications are checked to be in accordance to the ethical principles and values of the Vetmeduni Vienna to promote the health and welfare of the animal and the benefit of the public and are approved by the Access Committee, including one representative of the Ethics and Animal Welfare Commission.

There is no time limit for sample using or sample storing.

Constraints

Only sample applications, which are in accordance to the values of the Vetmeduni Vienna to promote the health and welfare of the animal and the benefit of the public, can be approved. Applications intended to reduce animal experiments in terms to the principles of 3Rs (Replacement, Reduction, Refinement) are particularly preferred.

(3) Bioresource description

Object name

VetBiobank

Bioresource name

VetBiobank, University of Veterinary Medicine, Vienna

Bioresource location

As the VetBiobank is a part of the Core Facility of the Vetmeduni, Vienna, Austria, it is a non-profit organization. It is not linked to any institute or clinic of the Vetmeduni Vienna, but is under direct supervision of the Vice rector of research.

Bioresource contact

VetBiobank
University of Veterinary Medicine
Veterinärplatz 1
1210 Vienna, Austria
Phone: +43 125077
Email: VetBiobank@vetmeduni.ac.at

Bioresource URL

<http://www.vetmeduni.ac.at/vetbiobank>

Identifier used

N/A

Bioresource type

The VetBiobank is an animal biobank, focusing on three major sample strategies:

- Residual specimens of the clinics after routine treatment (mainly tissue samples from cats and dogs, including a large number of tumor- and “healthy” reference tissues) – samples for retrospective application.
- Collection of samples assigned to a predetermined study or research project (prospective sampling of tissues or body fluids (plasma, serum, urine, feces).
- Residual specimens from animal experiments (often tissue samples from mice).

Type of sampling

- Diseased based/Clinical based: residuary material after diagnosis/therapy e.g. tumor collection
- Collection of healthy reference material
- Collection of samples derived from animal experiments

Anatomical site

Several anatomical sites are available, depending on collection type (**Table 1**: Healthy reference tissues, **Table 2**: Tumor tissues).

Disease status of patients/source
Available.

Clinical characteristics of patients/source

Patient related information e.g. species, breed, gender, age, anamnesis, kind of surgery, medication, date of surgery, localization, arterial clamping time, time of collection, diagnosis and ICD code are available for most

archived samples on request. No specific inclusion criteria are defined, despite some quality criteria (e.g. time for making samples available for the VetBiobank).

Vital state of patients/source

Biospecimens are collected from living animals during medical treatment (surgery, biopsy) and post mortem from euthanized animals (sample collection within 6 hours after time of death).

Table 1: Healthy reference tissues.

Control tissues	cat	dog	horse	rat	hamster	guinea pig	pig	goat	monkey	mouse
Adrenal gland	X	X	X	X			X		X	
Bone	X	X	X					X	X	X
Bone marrow	X	X	X				X			
Brain	X	X	X		X		X	X	X	X
Cartilage		X					X			
Cornea	X									
Ductus epididymidis			X							
Esophagus	X	X	X	X		X	X	X	X	X
Fat tissue		X	X							X
Heart	X	X	X	X	X		X	X	X	X
Hoof			X							
Intestine	X	X		X	X		X	X	X	X
Kidney	X	X	X	X	X		X	X	X	X
Liver	X	X	X	X	X		X	X	X	X
Lung	X	X	X	X	X		X	X	X	X
Lymph node	X	X	X				X	X	X	X
Mamma		X							X	
Muscle	X	X	X		X		X	X		X
Ovary	X	X	X						X	X
Pancreas	X	X		X			X	X	X	X
Parotid gland	X									
Prostate										X
Salivary gland	X	X	X	X	X			X		X
Skin		X	X				X		X	X
Spleen	X	X	X	X	X		X	X	X	X
Stomach	X	X		X			X	X	X	X
Tendon		X	X				X			
Testis	X	X	X	X				X		X
Thymus		X		X	X		X		X	X
Thyroid gland		X					X		X	
Tongue	X	X			X		X		X	X
Trachea	X	X	X	X			X	X		X
Urinary bladder	X	X	X		X		X	X		X
Uterus	X	X	X		X		X		X	X

Table 2: Tumor tissues.

Tumor types	Localisation
Adenocarcinoma	intestine, kidney, lung, mamma, uterus, skin (metastasis);
Adenoma	adrenal gland, hepatoid gland, intestine, skin, thyroid gland;
Ameloblastoma	bone (jaw) enamelum
Chondrosarcoma	scapular
Carcinoma	adrenal gland, bladder (urinary), circumanal gland, hepatoid gland; liver, lung, lymph node, mamma, metastases, pancreas, prostate gland, skin, thyroid gland, tonsils;
Fibrosarcoma	skin (limbs, shoulder, thorax, neck, perianal), abdomen, lung, spleen;
Hemangiopericytoma	skin;
Hemangiosarcoma	liver, spleen, skin (thorax, neck, limbs);
Histiocytoma	skin (limbs, scapula);
Insulinoma	pancreas;
Leiomyoma	stomach, uterus, vagina;
Leiomyosarcoma	intestine, kidney, maxilla;
Leydig cell tumor	testicle;
Lipoma	subkutis (mamma, muscle, skin, vessel);
Lymphoma	intestine, liver, lymph node, spleen, stomach;
Mastcell tumor	skin (limbs, ear, thorax, hip); lymph node (metastasis);
Melanoma	skin (limbs), oral, lymph node (metastasis), lung (metastasis);
Meningioma	brain, spine;
Myelolipoma	spleen;
Osteosarcoma	bone (limbs, rib, shoulder, jaw), lung (metastasis), mamma, skin;
Peripheral nerve sheath tumor (PNST)	brain, skin;
Phaeochromocytoma	adrenal gland;
Sarcoid	skin;
Sarcoma	lymph node, spleen, palate, skin (limbs);
Seminoma	testicle;
Spindle cell sarcoma	ear, jaw, lung, spleen;
Thymoma	thymus, mediastinum;

Clinical diagnosis of patients/source

The VetBiobank database contains a minimal set of clinical data, including information about the patient (species, breed, gender, and age), localization, tumor type or disease according to the pathologic report and ICD-10 code.

Samples are linked to the Clinical Data Registry; any further or more detailed information can be provided by clinical staff members on request.

Pathology diagnosis

Samples are linked to pathology diagnosis.

Control samples

Tissues from healthy animals e.g. testicles and ovaries after castration and not affected tissues from diseased animals after surgery or euthanasia are collected as control tissues.

Some tumor samples are stored with matching normal tissue samples.

Biospecimen type

The vast majority of biospecimens stored at the VetBiobank are tissue samples, processed to FFPE blocks, cryo blocks (embedded in O.C.T.TM) and small tissue pieces (0.3 × 0.3 cm) in cryotubes with and without RNAlaterTM. Additionally, whole blood, plasma, serum, urine and feces are archived according to single project purposes.

Size of the bioresource

The VetBiobank is a part of the central research infrastructure of the University of Veterinary, a permanent institution to support internal research. The number of full time equivalents is 4, the head count is 5. Currently (August 2020), about 58000 samples are archived, originated from about 1300 animal donors.

Release date

Data and samples are permanently available.

Access criteria

The VetBiobank provides samples and data worldwide to researchers from academia and industry. Information about sample application is offered on the website <https://www.vetmeduni.ac.at/vetbiobank>, including the access policy, the access procedure the form for sample application and the lists of control and tumor tissues (**Tables 1 and 2**), giving an insight into the range of already stored samples. For sample request, just complete the sample application form and send it to the VetBiobank. This will start a standardized approval workflow, including the final decision by the Vetmeduni Vienna Access Committee, a board, which is supported by the advisory ethics committee and the rectorate. Reply to this request will take place within 3 weeks. In case of positive evaluation, a MTA/DTA has to be entered before sample/data release, specifying terms of use and cost recovery. Calculated costs cover shipping and handling fees, no patient samples or data are sold for profit. Researchers commit to acknowledge the VetBiobank for provision in case of any scientific communication.

(4) Reuse potential

The VetBiobank does not take back any released samples, as it cannot be verified, if the sample material was maintained (e.g. storage temperature) according to internal specifications. Hence, received biological biospecimens are divided, stored and distributed in “single use” aliquots to avoid a waste of surplus material. Only sections of paraffin and cryo blocks are forwarded, while FFPE and cryo blocks remain at the VetBiobank for further applications. Additionally, small single cryo pieces (<0.3 × 0.3 cm, with or without RNAlater) are distributed. There are no exclusive rights for aliquots derived from one primary biospecimen, consequently, remaining aliquots from already released samples are not withheld for other use.

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Competing Interests

The authors have no competing interests to declare.

Author Roles

Walter Ingrid, Head of the VetBiobank
 Burger Stefanie, Expert in tissue preparation and (immuno) histological stainings
 Stargardt Melanie, Project coordinator, Expert in molecularbiological examinations
 Kummer Stefan, IT administrator, Expert in image processing and image analyses
 Wieser Monika, Quality management, BBMRI.at coordinator

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