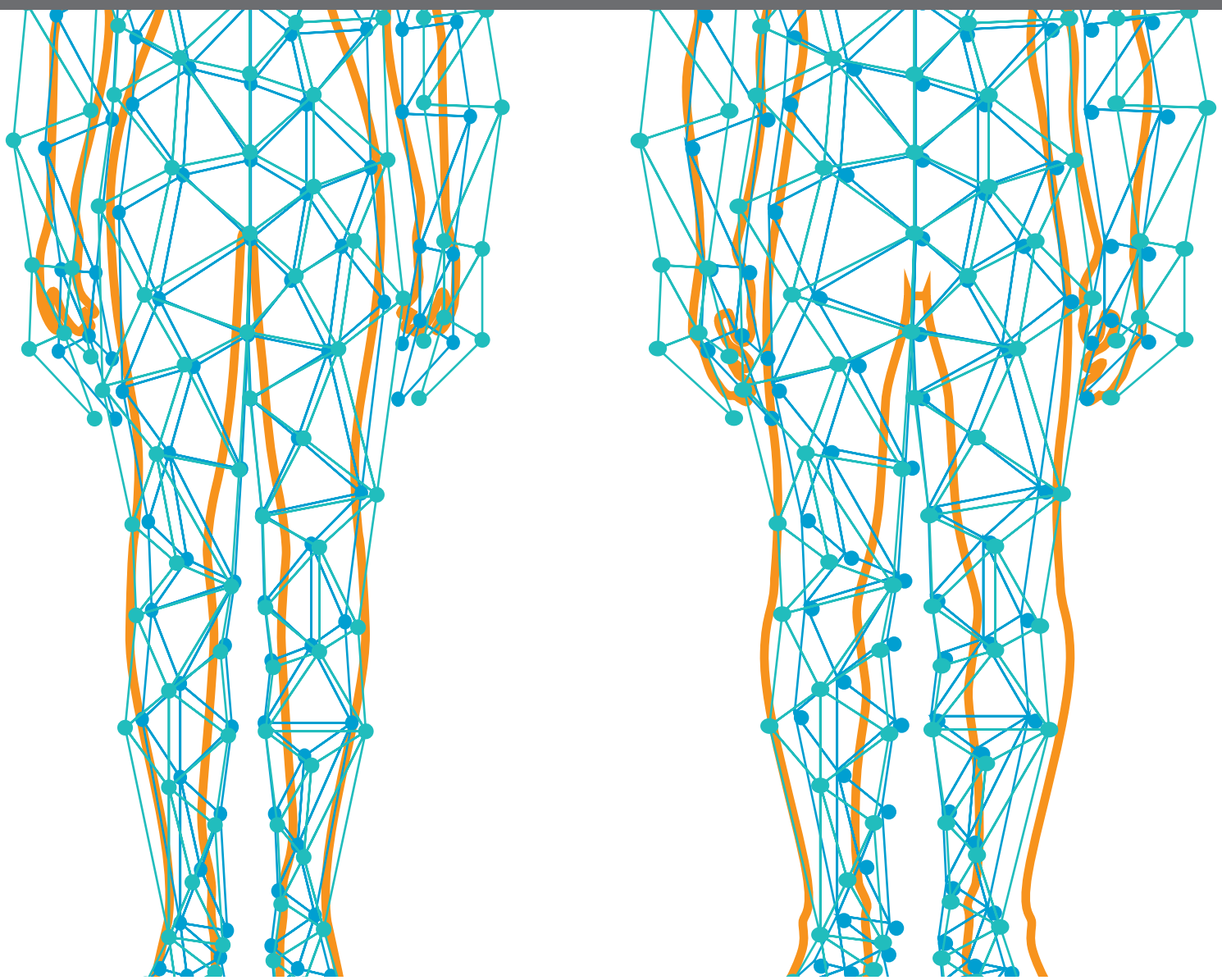
BIOBANKS AS ESSENTIAL TOOLS FOR TRANSLATIONAL RESEARCH: THE BELGIAN LANDSCAPE

EDITED BY: Sofie J. S. Bekaert, Annelies Debucquoy, Veronique T'Joen, Laurent Georges Dollé and Loes Linsen

PUBLISHED IN: Frontiers in Medicine



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88966-016-2
DOI 10.3389/978-2-88966-016-2

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BIOBANKS AS ESSENTIAL TOOLS FOR TRANSLATIONAL RESEARCH: THE BELGIAN LANDSCAPE

Topic Editors:
Sofie J. S. Bekaert, Ghent University, Belgium
Annelies Debucquoy, Belgian Cancer Registry, Belgium
Veronique T'Joen, Ghent University Hospital, Belgium
Laurent Georges Dollé, Biothèque wallonie bruxelles, Belgium
Loes Linsen, University Hospitals Leuven, Belgium

Citation: Bekaert, S. J. S., Debucquoy, A., T'Joen, V., Dollé, L. G., Linsen, L., eds. (2020). Biobanks as Essential Tools for Translational Research: The Belgian Landscape. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88966-016-2

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frontiers in Medicine

EDITORIAL
published: 28 July 2020
doi: 10.3389/fmed.2020.00378

Editorial: Biobanks as Essential Tools for Translational Research: The Belgian Landscape

Annelies Debucquoy[1], Loes Linsen[2], Veronique T'Joen[3], Laurent Dollé[4] and Sofie Bekaert[5,6] on behalf of BBMRI.be*

[1] BBMRI.be, Belgian Cancer Registry, Brussels, Belgium, [2] AC Biobanking, University Hospitals Leuven, Leuven, Belgium, [3] ESBB, Brussels, Belgium, [4] Biothèque Wallonie Bruxelles, Brussels, Belgium, [5] Department of Public Health and Primary Care, Faculty of Medicine and Health Sciences, Gent, Belgium, [6] Manager Translational Program VIB, Gent, Belgium

Keywords: biobank, samples, data, biobank network, BBMRI

Editorial on the Research Topic

Biobanks as Essential Tools for Translational Research: The Belgian Landscape

BBMRI.be (1), the Belgian biobank network and Belgian National Node of the European biobank infrastructure BBMRI-ERIC (2), was set up in order to support the ever-increasing need for human biospecimen samples for research guaranteeing quality control, access, transparency, and interconnectedness of biobanks (3). The BBMRI.be network was initiated by uniting the three existing Belgian network biobank initiatives i.e., Belgian Virtual Tumourbank (BVT) (4) project assigned to the Belgian Cancer Registry, Biothèque Wallonie-Bruxelles (BWB) (5) and the Flemish Biobank Network (CMI). From 2013 to 2019, BBMRI.be has matured into a solid partner network of 16 biobanks in Belgium and has proven to reach out to a broader community beyond the founding partners. From 2019 onwards, BBMRI.be invites all Belgian biobanks with translational research potential as well as biobank user organizations that are seeking structural research collaborations to join the BBMRI.be network. The strong representation of several members of BBMRI.be in working groups of local, regional, and national decision-making organizations covering ethical, legal and other aspects of biobanking [FAMPH (6), BAREC (7), VLIR (8), BVT (4), BWB (5), NBN (9)…] as well as the active participation in international biobank networks and associations [ESBB (10), ISBER (11), BBMRI-ERIC (2), 3C-R (12), ISO (13) …] and regional health/life Sciences Clusters [BioWin (14), LifeTech Brussels (15), Flanders.bio (16)] assures a good cross-fertilization on all levels and pushes forward the development of the biobank community.

With the current Research Topic, we focus on the challenges local biobanks and biobank networks are facing along the road toward implementation and sustainability and how these can be overcome. We also share some success stories illustrating how, over a decade, the BBMRI.be biobank network has managed to build strong cornerstones and become a fertile substrate for human biospecimen samples management and access for translational research purposes.

First, we highlight the new processes and strategies implemented by the Belgian biobanks to optimize their biobank activities in light of new quality standards and changing national laws legislation. The first manuscript describes the evolution of the University Biobank Limburg (UBiLim) from an archival sample collection into a federated biobank structure, supporting translational research, dissecting the major challenges at each stage (Linsen et al.). A campus-wide cell line dataset was developed in the biobank of the Ghent University Hospital

Edited and reviewed by:
Hans-Dieter Volk, Charité Medical University of Berlin, Germany

****Correspondence:***
Annelies Debucquoy
annelies.debucquoy@kankerregister.org

Specialty section:
This article was submitted to Translational Medicine, a section of the journal Frontiers in Medicine

Received: *20 March 2020*
Accepted: *18 June 2020*
Published: *28 July 2020*

Citation:
Debucquoy A, Linsen L, T'Joen V, Dollé L and Bekaert S (2020) Editorial: Biobanks as Essential Tools for Translational Research: The Belgian Landscape. Front. Med. 7:378. doi: 10.3389/fmed.2020.00378

(T'Joen et al.), to enhance cell line data quality and its usability in the translational research community. The third paper describes the extensive setup and validation process of two automated sample storage and retrieval systems at the UZ KU Leuven biobank (Linsen et al.), detailing the problems encountered and the efforts needed to obtain successful implementation.

As already illustrated above, quality in biobanking is crucial for the long-term sustainability of the biobank and for the reproducibility of the downstream research. This message is further emphasized by work from the Quality Working Group of BBMRI.be which assessed and demonstrated a solid quality approach and mindset in the Belgian biobanks (Linsen et al.). Another illustration thereof is depicted by Craciun et al., with a hands-on example on how the quality of samples can be assured by implementing quality control schemes in the biobank, either by internal quality control test or by participating to external quality test such as the ISBER Proficiency Testing.

The BBMRI.be biobank network hosts a treasure of very valuable collections, a flavor of which is presented in this Research Topic, where we share some success stories from collections stored in our biobanks.

The Belgian Virtual tumorbank, described by Vande Loock et al., connects the tumor biobanks from 11 Belgian hospitals. While all biobanks store the residual human tumor samples locally, the data is centrally registered at the Belgian Cancer Registry and available for researchers in the field of oncology. The manuscript describes the setup of the virtual network, the quality checks performed on the data and gives an overview of the samples and associated data available for research.

The Inflammatory bowel disease collection (Cleynen et al.) was built up as a collaboration between three Belgian IBD centers (University Hospitals Brussels, Ghent and Leuven) and has evolved over the years into a valuable source of material from patients with IBD and normal controls. The paper details the setup of this collection and demonstrates its added value by sharing some success stories that were obtained with samples and data collected within this framework.

The Cardiogeneticsbank@UZA biobank (Alaerts et al.) and the collection on viral hepatitis (Ho et al.) are both integrated in the biobank of Antwerp. The Cardiogenetics biobank collected samples and data of patients with a cardiogenetic disorder. In the manuscript, several research projects are described to illustrate the potential of these valuable collections and the prospects for future research. The Viral Hepatitis collection is a unique collection that was established by collecting samples from hepatitis patients collected both in-hospital and during community outreach screenings. The publication describes the setup and associated challenges of both the in-hospital as community collections, the samples that were obtained and some research results that were acquired with these samples.

Van den Heuvel et al. focus on the VITO biobank and illustrate the potential of a population biobank. This biobank, with about 70.000 biological samples from the general population in Flanders, was set up to answer research questions related to health and environment. Samples were collected within different human biomonitoring studies and are linked with extended data on the lifestyle, environment, and health status of the donor.

The manuscripts assembled in this Research Topic clearly illustrate the value of the Belgian biobanks as catalyst for translational and clinical research. However, biobanks are to the best of their means the custodians of the most precious human biospecimen samples donated by patients and healthy volunteers. The input of these important stakeholders should therefore be implemented into the biobanking process. The manuscript of Broes et al., in which patients were questioned about their view on re-use of clinical trial samples and data is an excellent example of how sharing knowledge and engaging with patients, might help to push forward the biobank community.

This Research Topic gives an impression of the numerous research opportunities with human biospecimen samples and data from the Belgian biobanks and illustrates how biobanks are an essential tool for translational research.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 31 May 2019
doi: 10.3389/fmed.2019.00120

The Belgian Virtual Tumorbank: A Tool for Translational Cancer Research

Kim Vande Loock[1], Eva Van der Stock[1], Annelies Debucquoy[1], Katia Emmerechts[1], Nancy Van Damme[1] and Etienne Marbaix[2] on behalf of the Steering Committee of the Belgian Virtual Tumorbank*

[1] Belgian Cancer Registry, Brussels, Belgium, [2] Service d'Anatomie Pathologique, Université Catholique de Louvain, St-Luc University Hospital, Brussels, Belgium

Background: Biobanks play a critical role in cancer research by providing high quality biological samples for research. However, the availability of tumor samples in single research institutions is often limited, especially for rare cancers. In order to facilitate the search for samples scattered among different Belgian institutions, a nationwide virtual tumorbank project was launched and is operational since February 2012. The Belgian Virtual Tumorbank (BVT) network encompasses the tumor biobanks from eleven Belgian university hospitals that collect and store residual human tumor samples locally and is coordinated by the Belgian Cancer Registry.

Materials and Methods: A web application was developed and consists of two modules. The registration module (BVTr) centralizes the tumor sample data from the local partner biobanks. The catalog module (BVTc) allows researchers to trace the tumor samples in the 11 tumor biobanks. The BVTc contains patient, medical and technical data, but excludes identifying information to ensure privacy of individuals. Automatic and manual controls guarantee high quality data on the samples requested by scientists for research purposes in oncology. A major advantage of the BVT network is that the available data can be linked to the data of the Belgian Cancer Registry for quality control purposes.

Results: Currently, more than 92,000 registrations are available in the catalog. Twenty-seven percent of the residual primary tumor samples originate from breast tissue, but also less frequent localisations such as head and neck (4%), male genital organs (1.7%), and urinary tract (1%) are available. In addition to the residual tumor tissue samples, also other available material can be stored and registered by the local biobanks. The most common type is corresponding normal tissue (19%).Other frequently available materials are plasma, blood, serum, DNA, and buffy coat. Even PBMCs, RNA, cytology, and urine are available in some cases.

Discussion and Conclusion: The BVT catalog is a valuable source of information for oncology research and the ultimate goal is to promote multidisciplinary cancer research (i.e., pathogenesis, disease prediction, prevention, diagnosis, treatment, and prognosis) for the benefit of all cancer patients.

Keywords: catalog, tumorbank, data quality, virtual, cancer

OPEN ACCESS

Edited by:
Enrico Capobianco, University of Miami, United States

Reviewed by:
Jing He, Guangzhou Women and Children's Medical Center, China
Marco Diego Dominietto, Paul Scherrer Institut (PSI), Switzerland

****Correspondence:***
Kim Vande Loock
kim.vandeloock@kankerregister.org

Specialty section:
This article was submitted to Translational Medicine, a section of the journal Frontiers in Medicine

Received: *26 February 2019*
Accepted: *15 May 2019*
Published: *31 May 2019*

Citation:
Vande Loock K, Van der Stock E, Debucquoy A, Emmerechts K, Van Damme N and Marbaix E (2019) The Belgian Virtual Tumorbank: A Tool for Translational Cancer Research. Front. Med. 6:120. doi: 10.3389/fmed.2019.00120

INTRODUCTION

Cancer registration in Belgium has evolved from a number of regional initiatives in the late nineties toward a national and centralized population based cancer registry with a firm legal basis. In 2003, the Royal Decree on the oncological care programs describing reimbursement of the multidisciplinary team meeting, was enacted (1). Later on, in 2006, the specific law on the Cancer Registry was created, making cancer registration compulsory for the oncological care programs and for the laboratories for pathological anatomy (2). The Belgian Cancer Registry (BCR) is a population-based registry that reports regularly on cancer patterns and trends in incidence and cancer survival, giving insights in the role, objectives and dataflow of the Cancer Registry (3–11). The patient's unique national social security identification number (SSIN) enables linkage with other medical and/or administrative data sources and allows the patient's vital status follow-up (12). In addition to describing cancer incidence and survival, the BCR is also involved in clinical registration projects (13, 14), in the evaluation of quality of care in oncology (15), in the registration of all tissue samples taken for early diagnosis and screening for breast, colorectal and cervical cancer (16) and in the centralization of the data on residual human tumor samples stored in local biobanks for scientific research purposes.

A biopsy or resection of tissue for diagnostic or therapeutic purpose might result in left over tissue or residual tissue. Instead of discarding this valuable material, this residual tissue can be stored at the local biobank together with associated clinical data and can be used at a later timepoint for scientific research. According to the Belgian Royal Decree on biobanks (17), every patient admitted to the hospital must be informed about the potential use of this residual tissue in scientific research. This is often mentioned in the welcome brochure of the hospital (= presumed consent). In case of explicit explanation and signing of a document, this is called an informed consent. Opposition needs to be communicated to the treating physician after which all residual tissue and associated data from the involved patient will be destroyed.

Different aspects of modern biobanking were recently highlighted by Paskal et al. (18) and the critical role of biobanking in cancer research by providing high quality biological samples for research has been shown by various papers (19–21).

In Belgium, a first biobank network was created in 2007 as a collaboration between 5 university hospitals. The network gathered pathologists and oncologists to discuss and evaluate the biobanking situation. This first consortium adopted the model of a virtual biobank and set objectives in order to extend the project to all major university hospitals in Belgium. In the course of the next years the network expanded, leading to the current network of 11 university hospitals. These hospitals all have a local biobank which stores human residual material, including tumor samples.

In March 2008, the National Cancer Plan (NCP) was launched by the former federal minister of Social Affairs and Public Health (Minister L. Onkelinx). One of the funded initiatives was the creation of a Belgian Virtual Tumorbank (BVT) in order to promote translational cancer research and the collaboration between different cancer researchers in Belgium[1]. Coordination of the Belgian Virtual Tumorbank was assigned to the Belgian Cancer Registry. A Steering Committee was setup with representatives of all biobanks for the strategic management of the Belgian Virtual Tumorbank. The criteria for recognition and conditions for the hospitals to be financed by this initiative are stated in the Royal Decree of September 20th 2009 (22).

The aim of the BVT is to facilitate the search for tumor samples scattered among different institutions by centralizing the data of residual human tumor samples in one database. A coded version of this central database is made available in an online application, which is called the BVT catalog (BVTc), and is accessible to researchers in the broad field of oncology. This application allows the researchers to perform queries based on specific search criteria to locate the samples of their interest in the different Belgian local tumorbanks. Afterwards, the researchers can contact the involved biobanks to get access to the samples. Since the BVT is fully integrated in the Belgian and European Biobank Network (BBMRI.be and BBMRI-ERIC), researchers that do not find suitable samples for their research in the BVT catalog, can be directed to the BBMRI-ERIC Directory of European biobanks[2] and the linked Negotiator service.

This paper gives an insight in the dataflow of the Belgian Virtual Tumorbank and the different quality control steps that are performed in order to guarantee high quality of data about human tumor samples in the BVT.

MATERIALS AND METHODS

Data Flow and Quality Control

For the setup of the BVT a custom-made online application was developed, consisting out of 2 modules; the registration module (BVTr) or central database and the catalog module (BVTc). Both applications can only be accessed by authorized users after identification and authentication by a user and access management (UAM) system to allow highly secure handling of medical data. The use of medical data in this initiative is authorized by the Data Protection Authority (23).

Before researchers are allowed to trace tumor samples of their interest at the local biobanks, three steps need to be performed (**Figure 1**). The first one is registration of the necessary data regarding the residual tumor samples stored at the local biobank in one central database. The second step involves processing of this data, including quality control, to allow publication in the coded database of the Belgian Virtual Tumorbank catalog (BVTc). In the third step, the researchers need to request access to the BVT catalog. By following a strict quality control (QC) incorrect data on biospecimens, which could crucially influence the research output, is limited to a minimum. Data quality of the BVT includes control measures at every stage of the data process guaranteeing a high quality of the data on the biospecimens requested for research purposes. Each of these steps will be elucidated in the next paragraphs, including the automatic and manual quality controls.

[1] www.virtualtumourbank.be
[2] https://directory.bbmri-eric.eu/

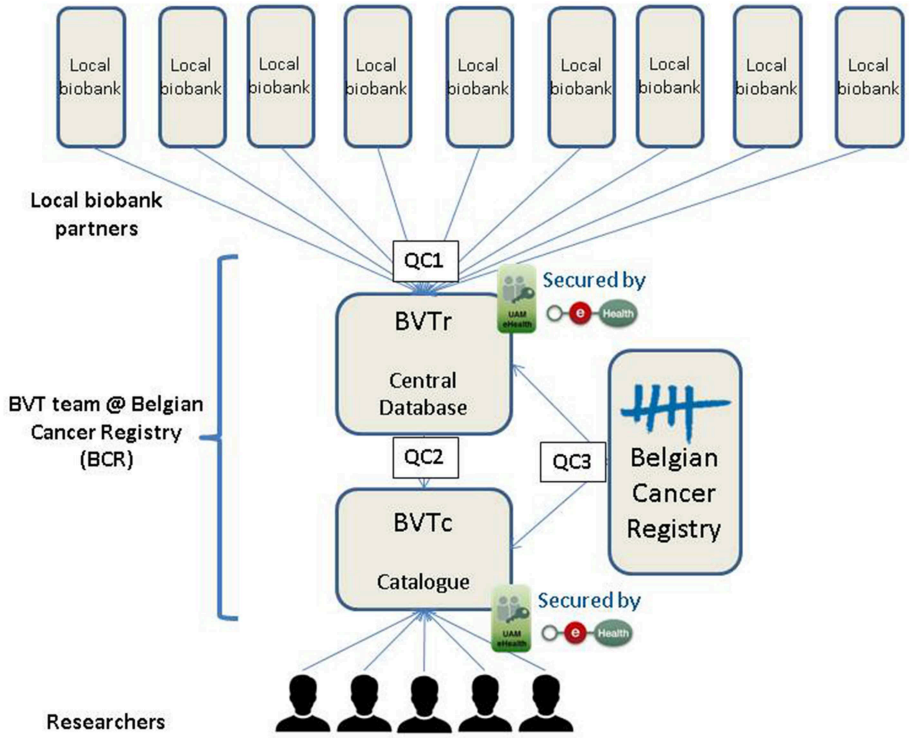

FIGURE 1 | Data flow and quality control steps of the Belgian Virtual Tumorbank.

Registration of Data in the BVT (BVTr)

Local biobanks store residual tumor samples as well as relevant clinical and technical data regarding the samples. The residual tumor samples are collected under the condition of presumed consent in accordance with the Belgian law. For associated materials (e.g., plasma, serum, …), patients sign an informed consent in the hospital linked to the local biobank. If the patient opposes, the patient needs to inform the treating physician and all samples and data of the patient are removed.

A standard set of variables that needs to be completed for every tumor was defined by the BVT Steering Committee in 2010 (**Table 1**). The local biobanks can collect additional data (epidemiological data, molecular data, imaging data,…), but this data is not collected centrally in the catalog, since the authorization of the Data Protection Authority only allows the collection of the data mentioned in **Table 1**. Samples are registered via the online web application, which is restricted to authorized users only, because of the sensitive medical data that are available in the application. The registration module (BVTr) allows the local biobanks to upload registrations, query and update their own data if necessary. Local biobanks can enter one single registration or upload multiple registrations in batch. Most biobanks use their own local registration system to store relevant data concerning the stored residual tumor samples in a structured way. By exporting their data to a standardized template for batch upload (.csv-file) via an extraction algorithm, these biobanks can easily upload this extracted file in the central database of the BVT.

On the moment of uploading the data an automatic quality check is performed by the application. This first data quality control step (QC1) includes format checks, content checks and a few basic cross checks. A format check implies that the format of the variable will be verified e.g., social security identification number (SSIN) should contain 11 digits. During the content checks, the application will verify whether the variables contain the predefined content. One of the cross checks performed by the application is checking whether the birth date of the patient precedes the sample date. If a variable is incorrect or if a mandatory variable is not filled in, the registration will not be accepted by the application and either appear in an error file visible for the local biobank (in case of a batch upload) or generate an error message (in case of submission of one registration in the BVTr-application). After this automatic validation, the registered data appears in the central database of the BVT.

Processing of Data From the Central Database (BVTr) to the Catalog (BVTc)

Once data are registered in the central database, BCR employees authorized to have access to the BVTr can search the uploaded data from all the local biobanks and perform a manual quality control of the registered data before this data becomes available in the catalog. During this second quality control step (QC2), the expert data manager at BCR will manually verify all variables using advanced crosschecks and registration rules to check for internal inconsistencies. When inaccuracies are noticed, the

TABLE 1 | Overview of the set of variables per database.

Category	Local biobank	BVTr	BVTc
Source		Laboratory	Laboratory
		Creation date	
		Update date	
		Reference ID	Reference ID
Patient	SSIN	SSIN	
	Gender	Gender	Gender
	Birth date	Birth date	
		Age	Age
	Patient opposition	Patient opposition	
Technical	Sample ID	Sample ID	Sample ID
	Biopsy number	Biopsy number	
	Sample date	Sample date	Sample year
	Conservation mode	Conservation mode	Conservation mode
	Conservation delay	Conservation delay	Conservation delay
	Vital state of the patient (at the time of resection)	Vital state of the patient (at the time of resection)	Vital state of the patient (at the time of resection)
	Available material	Available material	Available material
	Technical remarks	Technical remarks	
Oncological	Sample type	Sample type	Sample type
	Sample localization	Sample localization	Sample localization
	Localisation of the primary tumor in case of metastasis	Localisation of the primary tumor in case of metastasis	Localisation of the primary tumor in case of metastasis
	Laterality	Laterality	Laterality
	Morphology	Morphology	Morphology
	Behavior	Behavior	Behavior
	Degree of differentiation	Degree of differentiation	Degree of differentiation
	pTNM	pTNM	pTNM
	pTNM prefix	pTNM prefix	pTNM prefix
	Oncological remarks	Oncological remarks	
	Other		

records are rejected. Rejected registrations need to be verified and corrected or confirmed by the local biobank and can then be uploaded again into the central database. If the quality of all data is good, records are published into the catalog. Publication of the data implies that the registrations will be coded and become available in the catalog.

Access to the Catalog of the BVT (BVTc)

The catalog contains technical, oncological and patient details but excludes identifying information; SSIN, technical and oncological remarks are removed, birth date is converted into age and sample date into sample year. In accordance with the central database of the BVT, access to the catalog is restricted to authorized users only. The BVT catalog is accessible to Belgian researchers working in the oncology field, that have a research project approved by an ethical committee, after authorization of the BVT steering committee as described in the authorization of the Data Protection Authority (24).

The BVT catalog allows researchers to perform queries based on specific search criteria and trace the samples of interest located at different local biobanks of the BVT network. Upon request from the researcher or local biobank, a third quality control step (QC3) can be performed on data of specific samples selected for their study. Via the unique patient identifier (SSIN), the expert data manager at BCR can crosslink the overlapping information present in the BVT database and the database of the Belgian Cancer Registry. The BCR database is restricted to data of malignant tumors with a structural delay of 2-years in incidence year to allow the programs to provide the compulsory information and perform treatment of the data at the BCR. If SSIN is not available (foreign patient) or in case of mismatch the patient can be traced by laboratory, biopsy number, and sample year.

Common variables between the BVT central database and the BCR cancer registration database are SSIN, birth date, gender, sample date, biopsy number, sample type, sample localization, laterality, morphology, behavior, differentiation grade, pT, and pTNM prefix. Post-surgical histopathological classification of the primary tumor according to TNM classification (pT), laterality, differentiation grade and pTNM prefix are non-mandatory fields in the BVT. Comparison of the overlapping information allows to distinguish recurrent tumors from primary tumors and provide additional information that might be of importance for the researcher like neoadjuvant treatment or the staging parameter that indicates the extent of the tumor after clinical diagnosis (cTNM). The database of the Belgian Cancer Registry is estimated to be more than 95% complete (25). Incompleteness of the database is more likely in case of elderly patients with very poor prognosis at diagnosis and patients with clinical diagnosis only.

RESULTS

Description of the Tumor Sample Registrations in the BVT Catalog

Currently, the BVT catalog contains 92,164 registrations from 48,756 patients. There are 53,142 registrations from female patients and 39,022 from male patients. Twelve percent of the registrations (11,102) origin from metastases and 84.9% from primary tumors (78,664). The primary tumors can be divided into malignant (70%), *in situ* (1.5%), borderline (2.4%), and benign tumors (11%). For 55.6% of the registrations only residual tumor tissue is stored at the local biobank (**Table 2**). For some patients, paired samples of other material types are stored and registered besides tumor tissue. The most common type is corresponding normal tissue (19.4%), followed by blood (7.6%), plasma (7.4%), and serum (6.4%).

Of all registrations, 69.3% (69,813) are stored at −80°C and 28.8% (28,980) are included in paraffin blocks. A small fraction (1.9%) of the fresh frozen samples are stored in liquid nitrogen. Conservation delay, time between excision of the residual tumor tissue and storage of the sample, is <30 min for

TABLE 2 | Overview of available material.

Available materials*	Number of registrations
Only tumor tissue	62,263
Corresponding normal tissue	21,698
Blood	8,517
Plasma	8,303
Serum	7,115
DNA	2,595
Buffy coat	1,256
PBMC	144
RNA	71
Urine	68
Cytology	32
Total	112,062

**multiple other materials can be indicated.*

17% of the registrations (15,915) and more than 30 min for 25.7% of the registrations (23,691). For 57% of the registrations the conservation delay is unknown.

Third Quality Control on Data of Breast Tumor Samples

This part focusses on the third step of the data quality control: comparison of the data available in BVT and in the BCR cancer registry database. Additional quality control checks have been performed on various tumor types like breast, kidney and esophagus. In this article, the results of the most recent QC3 analyses on data of breast tumor samples will be highlighted.

As far as the distribution sample localization is concerned, every localization is well-represented in the catalog of the BVT (**Table 3**). Breast tumor samples comprise more than one fourth (26%) of all primary tumor samples available. Therefore, we decided to perform a quality control study on data from primary breast tumor tissue samples, including patients from all 11 local biobanks. Taking into account the most recent complete and available database of the Belgian Cancer Registry at the moment of study set-up, it was decided to select patients with samples collected in 2014. A search using sample localization (breast C50), sample year (2014), behavior (malignant /3) and sample type (primary tumor) as criteria resulted in the retrieval of 2,358 tumor sample registrations. Only published registrations (i.e., available in the BVT catalog) were taken into account. Next, a random selection of 20 patients per local biobank resulted in a final study population of 197 patients. This means that not for all biobanks 20 patients were available.

On the final study population, a quality control was performed for common variables between BVT and BCR registration database: patient variables (SSIN, gender, and birthdate), technical variables (sample date and biopsy number) and oncological variables (sample type, sample localization, morphology, differentiation grade, and pT).

Mean age of the study population is 62 years (**Table 4**). Seven of the registered breast tumor samples originated from male patients. The majority of the tumor samples (77.2%) were stored at −80°C and for 15.7% of the samples the time between excision of the sample and storage was <30 min. For 59.4% of the patients ($n = 117$) other material is stored at the local biobank in addition to residual tumor tissue, including corresponding normal tissue (87.2%), blood (15.4%), serum (12%), and plasma (12%).

Comparison of 13 variables available in both BCR and BVT database resulted in the retrieval of additional information for 17 patients and correction of data for 19 patients (**Table 5**). In total, the variables of 33 (16.8%) out of 197 registrations (patients) contained 1 ($n = 30$) or 2 ($n = 3$) errors. Patient variables (SSIN, birth date, and gender) were identical between both databases. Sample type and differentiation grade data were in concordance between BCR and BVT database. Technical variables revealed 3

TABLE 3 | Overview of the topology groups.

Sample localization*	Number of registrations
Breast	20,458
Central Nervous System	7,646
Colorectal	6,684
Other Digestive Organs	5,741
Soft Tissue	5,702
Lung	5,251
Lymph Nodes	4,186
Kidney	3,762
Endocrine Organs	3,754
Female Genital Organs	3,219
Head and Neck	3,060
Bone Marrow and Spleen	2,468
Skin	2,032
Male Genital Organs	1,329
Other Intrathoracic Organs	1,199
Bone and Articular Cartilage	1,079
Urinary Tract	845
Unknown	249
Total	78,664

**Calculated on primary malignant tumors.*

TABLE 4 | General description of the study population.

Variables	*n* (%)	mean ± S.D.
Age	197 (100)	62 ± 13.50
Gender (female/male)	197 (96.4/3.6)	–
Conservation mode (Paraffin/ −80°C)	197 (22.8/77.2)	–
Conservation delay (≤30 min/ >30 min/unknown)	197 (15.7/31.5/53.3)	–
Available material (only tumor tissue/other material*)	197 (40.6/59.4)	–
*Corresponding normal tissue	102 (87.2)	–
*Blood	15 (15.4)	–
*Serum	14 (12)	–
*Plasma	14 (12)	–

n, number of patients; S.D., standard deviation; min, minutes.

TABLE 5 | Overview of the comparison of the overlapping data between BCR and BVT.

Variables	Registered in BVT *n* (%)	Corrections needed in BVT *n* (%)
SSIN*	197 (100)	0
Birth date*	197 (100)	0
Gender*	197 (100)	0
Sample date*	197 (100)	1 (0.5)
Biopsy number*	197 (100)	2 (1.0)
Sample type*	197 (100)	0
Sample localization*	197 (100)	1 (0.5)
Laterality	168 (85.3)	3 (1.8)
Morphology*	197 (100)	10 (5.1)
Behavior*	197 (100)	1 (0.5)
Differentiation grade	136 (69.0)	0
pT	166 (84.3)	1 (0.6)
Additional information (pTNM prefix)		**pTNM prefixes to be added in the BVT *n* (%)**
Recurrent tumors (rpTNM)		4 (2.0)
Tumor resection after neoadjuvant therapy (ypTNM)		13 (6.6)

*n, number of patients; *mandatory variable.*

typing errors, 2 in biopsy number and 1 in sample date. For the oncological variables, a distinction is made between mandatory and non-mandatory variables.

For the mandatory variables the error rate is the highest in morphology with discordances in 10 out of 197 patients. Sample localization and behavior showed each only 1 error. For one patient the tissue sample concerned a skin tumor instead of a breast tumor as was registered in the BVT database.

As far as the non-mandatory variables are concerned, the pT was incomplete for 31 out of 197 patients: for 17 the pT variable was empty, for 14 patients pTx was indicated and for 1 the completed pT value was incorrect. Comparison of the laterality between both databases revealed a mistake for 3 patients out of 168 where the variable was specified. Linkage the BVT database to BCR database resulted in retrieval of additional information to complete the BVT database. For 4 patients the stored tissue concerned a recurrent tumor, while 13 patients received neoadjuvant therapy prior to resection.

DISCUSSION AND CONCLUSION

The national virtual tumorbank has been set up in order to facilitate the search for samples scattered among different Belgian institutions. To achieve this an online BVT application was developed consisting out of two modules; the central database (BVTr) and the catalog (BVTc). The central database (BVTr) allows centralization of patient, technical and oncological data of human residual samples stored locally in a harmonized and standardized way while the catalog (BVTc) enables researchers to localize the samples required for their oncology research. Implementation of automatic and manual data quality control steps guarantees a high quality of associated data from residual tumor samples.

Establishment of a data standard enables biobanks to integrate within a network and allows communication not only between biobanks but also between initiatives and most importantly with researchers (26). Within BVT the standard set of variables regarding the residual tumor samples stored at the local biobanks include patient, oncological and technical information. In the BVT catalog identifying data is excluded to ensure that core information related to the patient and the sample can be found by the researcher while maintaining confidentiality of the patient. Overview of the data available in the BVT catalog shows coverage of a broad range of sample types with samples originating from primary malignant tumors, *in situ*, borderline and benign tumors as well as samples from metastases. Most of the registered samples are stored at −80°C while a significant smaller fraction is stored as paraffin-embedded blocks. This can be explained by the fact that at some local biobanks the paraffin-embedded blocks are stored at the anatomopathological department and therefore not registered in the BVT database. In addition, not all of the local biobanks have the facility to create paraffin-embedded blocks on site at the biobank. In this last years, the frozen and paraffin-embedded tumor samples and corresponding normal tumor tissue collections are more and more complemented by matched samples of blood, serum, plasma, and other body materials.

The value of successful linkage of data in biobanks and cancer registries has been elaborated in various studies (27, 28). The Belgian Cancer Registry is a population-based cancer registry collecting information on all cancer cases diagnosed in Belgium provided by the oncological care programs in all hospitals and services for pathological anatomy. Validity and quality of the data are ensured by an extended set of automated and manual validation procedures based on the IARC guidelines (29). Comparison of the overlapping information between the BVT and the BCR database revealed a good quality of sample data. Moreover, this comparison resulted in the retrieval of additional information on recurrent breast tumors and neoadjuvant therapy prior to resection of the breast tumor which might be important information for researchers. These results indicate the relevance of a joint evaluation of biobank and cancer registry information to guarantee a high quality of associated data from biospecimens used in translational cancer research. Harmonized storage of clinical and other associated data in combination with the good quality of the data might facilitate further linkage to additional information in the future. Linkage of samples to relevant clinical and (molecular) pathological information enables researchers to further understand tumor development, response to treatment and clinical outcomes (19, 20, 30).

One biobank cannot always provide sufficient numbers of samples and therefore the number of data sharing initiatives increases, enabling researchers to find suitable number of available samples and associated data (18, 19, 31–34). The developed online BVT application is a dedicated mechanism for researchers to localize their residual tumor samples of interest and associated data stored at the 11 local biobanks. The system takes into account the balance between the burden of data entry

for the biobank manager providing adequate information for the researcher to find and localize their sample of interest while maintaining the confidentiality of the patient. An advantage is that the quality of the sample data of all 11 biobanks is verified in a uniform way and that the standards are extended to the application using automatic and manual quality checks. This combination of automatic and manual quality checks guarantees a high quality of the data.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the supplementary files.

AUTHOR CONTRIBUTIONS

EV is mostly involved in data management of the BVT project and helpdesk for the application. AD supervises the coordination of the BVT project and is consulted as a biobank expert. NV was consulted and contributed as an expert of the Belgian Cancer Registry. EM is the president of the BVT steering committee and consulted as a pathology expert. KE supervises coordination of the BVT project. KV is involved in BVT project management and took the lead in writing the manuscript with input, comments, and critical feedback provided by all other authors mentioned. All authors contributed to the final version of the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 25 June 2019
doi: 10.3389/fmed.2019.00137

Rationalized Development of a Campus-Wide Cell Line Dataset for Implementation in the Biobank LIMS System at Bioresource Center Ghent

Veronique T'Joen[1], Lieven Vaneeckhaute[2], Sara Priem[1], Steven Van Woensel[1], Sofie Bekaert[3], Elke Berneel[1] and Catherine Van Der Straeten[4]*

[1] Bioresource Center Ghent, Health, Innovation and Research Center, Ghent University Hospital, Ghent, Belgium, [2] Data Management Unit, Health, Innovation and Research Center, Ghent University Hospital, Ghent, Belgium, [3] Department of Public Health and Primary Care, Faculty for Medicine and Health Sciences, Ghent University, Ghent, Belgium, [4] Health, Innovation and Research Center, Ghent University Hospital, Ghent, Belgium

The Bioresource center Ghent is the central hospital-integrated biobank of Ghent University Hospital. Our mission is to facilitate translational biomedical research by collecting, storing and providing high quality biospecimens to researchers. Several of our biobank partners store large amounts of cell lines. As cell lines are highly important both in basic research and preclinical screening phases, good annotation, authentication, and quality of these cell lines is pivotal in translational biomedical science. A Biobank Information Management System (BIMS) was implemented as sample and data management system for human bodily material. The samples are annotated by the use of defined datasets, based on the BRISQ (Biospecimen Reporting for Improved Study Quality) and Minimum Information About Biobank data Sharing (MIABIS) guidelines completed with SPREC (Standard PREanalytical Coding) information. However, the defined dataset for human bodily material is not ideal to capture the specific cell line data. Therefore, we set out to develop a rationalized cell line dataset. Through comparison of different datasets of online cell banks (human, animal, and stem cell), we established an extended cell line dataset of 156 data fields that was further analyzed until a smaller dataset—the survey dataset of 54 data fields—was obtained. The survey dataset was spread throughout our campus to all cell line users to rationalize the fields of the dataset and their potential use. Analysis of the survey data revealed only small differences in preferences in data fields between human, animal, and stem cell lines. Hence, one essential dataset for human, animal and stem cell lines was compiled consisting of 33 data fields. The essential dataset was prepared for implementation in our BIMS system. Good Clinical Data Management Practices formed the basis of our decisions in the implementation phase. Known standards, reference lists and ontologies (such as ICD-10-CM, animal taxonomy, cell line ontology…) were considered. The semantics of the data fields were clearly defined, enhancing the data quality of the stored cell lines. Therefore, we created an essential cell line dataset with defined data fields, useable for multiple cell line users.

Keywords: biobank, cell line, data management system, dataset, quality, data quality

OPEN ACCESS

Edited by:
Enrico Capobianco, University of Miami, United States

Reviewed by:
Nicholas Ekow Thomford, University of Cape Coast, Ghana
Xiaoguang Li, Johns Hopkins Medicine, United States

****Correspondence:***
Veronique T'Joen
veronique.tjoen@uzgent.be

Specialty section:
This article was submitted to Translational Medicine, a section of the journal Frontiers in Medicine

Received: *28 March 2019*
Accepted: *04 June 2019*
Published: *25 June 2019*

Citation:
T'Joen V, Vaneeckhaute L, Priem S, Van Woensel S, Bekaert S, Berneel E and Van Der Straeten C (2019) Rationalized Development of a Campus-Wide Cell Line Dataset for Implementation in the Biobank LIMS System at Bioresource Center Ghent. Front. Med. 6:137. doi: 10.3389/fmed.2019.00137

INTRODUCTION

In the last two decades, biobanks—specialized infrastructures that store, annotate, and distribute biospecimens—have emerged and professionalized through the implementation of quality and data management systems based on harmonized minimal datasets which allow sharing of samples between researchers and thus enhancing progression of clinical research (1).

In 2015, the Bioresource center Ghent—formerly known as Bimetra Biobank (2)—established a central high quality biobanking facility at Ghent University hospital. This hospital-integrated biobank brought together multiple decentralized biobank initiatives into a professionalized biobank, with implemented quality management system.

Local strategic prospective collections, important historical collections, and interuniversity focus collections are operationally managed within the biobank through an implemented biobank information management system (BIMS), named SLims[1]. Current minimal datasets for these collections reflect recommended fields from known guidelines (3–6) or standards (7, 8) complemented with quality parameters, by use of the "Standard Pre-analytical Code" (SPREC) (9, 10) or the "Biospecimen Reporting for Improved Study Quality" (BRISQ) system (11, 12), as harmonization of datasets is still ongoing at the European ("Biobanking and Biomolecular Resources Research Infrastructure—European Research Infrastructure Consortium" (BBMRI-ERIC)[2]) and international (driven by the International Society for Biological and Environmental Repositories (ISBER)[3]) level. The samples are collected in a project-based manner and can be used for fundamental basic research studies, in preclinical screening phases and in actual clinical trials.

The Bioresource center Ghent is part of the "Health, innovation and research institute" of Ghent University Hospital, which is a central contact point, service provider and knowledge center for biomedical translational and clinical research and health care innovation. The goal of translational biomedical science—an interdisciplinary field—is to expedite health care progress in prevention, diagnosis and treatment by combining disciplines, resources, expertise and techniques (13). The mission of the Bioresource center Ghent is to operate as a central contact point, knowledge center and high-quality service provider for all aspects related to biobanking.

Translational biomedical science is a clinical domain supported by three main pillars: bench side, bedside and the community. The translation of "bench side" observations into actual clinical applications is a long and elaborate process. Before actual clinical trials can be initiated, several basic research and preclinical research phases have to be completed. In preclinical screening phases, potential chemical compounds are often screened in the lab on cell lines. Both human and animal derived cell lines are considered as representative model systems for studying numerous biological mechanisms and serve as important preclinical models for drug target discovery and rapid assessment of toxicity profiles (14).

Cell line annotation, authentication as well as the quality of the cell line are pivotal for determining the reliability and reproducibility of these preclinical tests. The lack of attention given to these preclinical data is an underestimated problem in biomedical science, leading to delays and increased costs in drug discovery studies (15, 16). Vast warehouses of cell line samples are available in commercial and academic settings. However, the datasets pertaining to these cell line samples differ massively in content and information (e.g., cell line origin, processing history) leading to cell line misidentification, misuse, mismatching, and the use of mixed clones by culture mix-ups (17, 18). Remarkably, SPREC and BRISQ do not cover specific data fields for cell lines, as they are categorized as complex derivatives, whose isolation requires usage of multiple steps and/or addition of chemical substances (3).

As multiple cell line collections are present on our campus, we set out to develop a uniform, campus-wide essential cell line dataset that tackles the issues regarding misidentification, annotation and poor culture follow-up. Our experience with cell lines indicated that a comprehensive cell line dataset should ideally contain three large categories of information.

First of all, general information regarding the origin and culture of the cell line, such as cell line name, type of tissue, derivation method, relevant clinical, and demographic information, cell line passage, current culture/freezing/thawing protocols and cell line aging information is paramount (19, 20).

Secondly, information for clear authentication of the cell line should be included. Cell line authentication relies on comparing samples derived from the same donor (16) by Short Tandem Repeat (STR) profiling and Single Nucleotide Polymorphisms analysis. To our current knowledge, there is no general approved standard or centralized online reference database for cell line authentication using Single Nucleotide Polymorphisms analysis (18, 21), leading to inaccuracy (22), although there is a general consensus on the need to establish this for cell line authentication.

Thirdly, quality data should be available, such as information regarding control of bacterial, viral, fungal of mycoplasma contaminations of the cell cultures (23, 24).

Thus, we set out to develop a comprehensive cell line dataset which would enhance cell line quality and their usability in translational research..

MATERIALS AND METHODS

Establishment of the "Extensive Cell Line Parameter Dataset"

Relevant articles regarding cell line datasets were searched in PubMed®. Additionally, several cell line companies, vendors and a large cell line locator[4] were identified through a general website search. A selection of frequently used and mentioned cell banks was made, taking into account that human (15 cell banks), animal (15 cell banks), and stem cell lines (3 cell banks) were represented

[1]Genohm, https://www.genohm.com/
[2]http://www.bbmri-eric.eu/
[3]http://www.isber.org
[4]https://www.labome.com/method/Cell-Lines-Companies.html

within the selected banks. All data fields found in the cell banks were listed, forming the "Extensive cell line data field set."

Evaluation of the "Extensive Cell Line Data Field Set" and Establishment of the "Survey Cell Line Data Field Set"

The usability of the "Extensive Cell line data field set" for different cell types was evaluated by subdividing the dataset fields into 6 nominative categories (named "basic cell line," "administrative information," "clinical and demographic," "cell culture," "genetic" and "quality/validation data" fields) and comparing the presence of each data field per cell line type, thus for human, animal, and stem cell lines. Data fields that were hardly present in any cell line database were eliminated from the survey. Subsequently, a redundancy strategy was applied to the dataset in order to eliminate data fields in which similar and overlapping information was captured. To identify similar and overlapping information, the selected 15 human cell banks were searched for a particular widely used human kidney (HEK 293, immortalized human embryonic kidney cell line) and cancer (HeLa, immortalized cell line from cervical cancer cells) cell line. The 15 selected animal cell banks were searched for a particular well-known animal cell line [MC 3T3, osteoblast precursor cell line derived from Mus musculus (mouse) calvaria cell line]. The obtained information in each data field was listed and compared. Subsequently, the most appropriate name for the data field was selected to provide an as clear as possible content for the field.

REDCap (Research Electronic Data Capture) Survey

The "Survey Parameter Set" formed the basis of a REDCap survey (25). Survey data were collected and managed using REDCap electronic data capture tools hosted at Ghent University Hospital[5]. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing: (1) an intuitive interface for validated data entry; (2) audit trails for tracking data manipulation and export procedures; (3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources. The survey was distributed "campus-wide" to research groups that have cell lines to their disposition. The survey was constructed in such a way that for each type of cell line (i.e., human, animal, cancer, and stem cell lines), researchers could indicate responses, thus allowing the identification of essential differences in the data fields per type of cell line. The researchers were asked which parameters they retain for their cell lines at present and if they annotate their cell lines. Additionally, they were asked which data fields they would find relevant to be mentioned in the campus-wide minimal dataset.

REDCap Survey Analysis

The returned survey data were thoroughly evaluated per cell line type (human/animal/stem cell). The data fields were as described before, regrouped in nominative categories to allow efficient analysis of the data: Basic cell line data, clinical and demographic data fields, cell culture data, genetic data, quality, and validation data and administrative data. Data fields were considered as highly relevant if more than 50% of the responders indicated it. Fields were considered as not relevant if more than 50% of responders indicated it. Global analysis of the REDCap survey results led to the inclusion of data fields in the rationalized "essential cell line dataset."

Essential Dataset: Defining the Cell Line Dataset Template

The data fields obtained in the "essential cell line dataset" were further evaluated and defined to allow the actual development of a cell line dataset template. Several steps were initiated and each data field was individually reviewed. Some fields from the essential dataset were split into multiple fields so that one type of data would be recorded per data field and not a combination of data, which is a general "Good Clinical Data Management Practice (GCDMP)" rule. This was also applied for registering units accompanying their specific values.

The "label" or "field name" of the data fields were screened for synonymy for which a correction was made by selecting the least ambiguous term as "survivor." If, after selecting the least ambiguous term, the need for a better label still persisted, a new label was proposed and presented to a panel consisting of biobank data managers and two quality managers, which have extensive experience with cell lines and cell culture. The newly proposed terminology was compared to literature to ensure its validity. Next, the essential data fields were defined by using the best existing description and thus introducing definitions to the data fields. Definitions were chosen from SPREC (10), BRISQ (12), MIABIS (6), PubMed (MeSH) or by adjusting existing definitions (26, 27) to best suit a biobanking/clinical context in concurrence with propositions from the Good Clinical Practices and the General Data Protection Regulation[6]. If no suitable existing definition could be found, a new definition would be postulated. Because of the great value of consistency in semantics, existing definitions were always favored above newly established definitions and if necessary, existing terms were divided into better definable sub labels.

A full list of the withheld data fields and their definitions can be found in **Table 3**.

Implementation of the Essential Cell Line Dataset Through Use of Ontologies and Lists Within the BIMS

Following the creation of essential data field labels and providing a definition for the desired content of the field, the actual field options for filing in the fields were reviewed in order to obtain clear and consistent data. To implement and secure good data practices, optimal use was made of "fixed choice" data fields, with the addition of options as "not performed," "unknown" or "missing data."

[5]https://www.uzgent.be/nl/home/Paginas/home.aspx

[6]https://eugdpr.org/

Several known standards and ontologies were evaluated for implementation: the "Cell Line Ontology" (28), "International Classification of Diseases for Oncology (29)" and the "Biological Classification (Taxonomy) for Class, Order and Species." For date and time stamps, the ISO 8601 standard[7] was implemented and all units were collected through the use of the "International System of Units" (30). Preference was given to known standards if these were practical in use. If no appropriate standard could be found or was deemed suitable for our setting, data fields with a well-defined fixed choice list were implemented. By the combination of standards and ontologies, all elements of essential sample information were collected and stored in a structured and well-approved manner.

RESULTS

Establishment of the Different Datasets

The Extensive Cell line parameter dataset was established as described in the Materials and methods section. Datasets used by different companies and described in articles were extracted and listed for comparison. This led to a dataset of 156 different data fields, visible in **Table 1**. All data fields in **Table 1** are listed alphabetically. Subsequently, we divided the data fields in nominative categories ("basic cell line," "administrative information," "clinical and demographic," "cell culture," "genetic", and "quality/validation data") and a redundancy strategy was applied to reduce overlapping data. This led to a reduction of 65% of fields, resulting in 54 remaining data fields. The most appropriate name was chosen for overlapping data fields and the resulting set formed the survey cell line parameter dataset (**Table 2**).

Survey Results

A REDCap survey was designed using the survey cell line parameter dataset. The survey results cover the global responses of 17 different research groups on our campus. **Figure 1** gives an overview of the received responders according to cell type origin. Responses showed that 57.1% of the respondents exclusively store human cell lines and 14.3% exclusively work with animal cell lines. 28.6% of the respondents work with both human and animal cell lines. Within the respondents that work with human cell lines (85.7%), all respondents have human cancer cell lines and 14.3% work additionally with human stem cell or induced pluripotent stem cell lines. Within the respondents that work with animal cell lines (42.9%), 7.1% work with animal stem cell or induced pluripotent stem cell lines.

As cell line authentication is essential for good cell line practices, we also inquired if cell line authentication was performed before use of cell lines in experiments. **Figure 2** shows their perspective regarding their performance of cell line authentication practices. This demonstrates that <35% of the responders authenticates the cell lines they are using.

Next, the survey data field list was analyzed per type of cell line, i.e., human, animal, and stem cell line. In order to be able to compare the results, the data fields were subdivided in 6 grouped categories: "basic cell line information," "clinical and demographic data," "cell culture information," "genetic characteristics, quality," "validation and administrative information." The responders had the option to indicate if they found a field "Highly relevant," "neutral" or "not relevant." A cut-off point was set at 50%, meaning that if more than 50% of the responders found a field "highly relevant," it should be included into the final dataset. Furthermore, if more than 50% of the responders found the field "not relevant," it will not be include in the final dataset.

Figure 3 gives an overview of the relevance scores for the basic cell line data fields. A line was used for indicating the 50% relevance cut-off point. Analysis of the basic cell line data fields shows that most fields are considered as highly relevant, regardless of the cell line type. As can be seen, the fields "Cell type," "Organism" and "Tissue origin" got the overall best relevance score. "Cell type" was the only field with a perfect score over the 3 types of cell lines. The field "derivation" is considered as neutral for human and animal cell lines, though highly relevant for stem cell lines. "Amount and cell conc" divides researchers of animal cell lines between "Neutral" and "Highly relevant." Overall could be noted that these fields are extremely relevant for stem cell lines. Eight of the ten fields received a 100% highly relevant score, however all fields received a good to excellent score for all three types of cell lines.

Figure 4 gives an overview of the clinical and demographic data fields. Within the clinical and demographic data, regardless of cell line type, "Illness, Age and Gender" are considered as highly relevant fields. Differences in relevance of the datafields can be seen depending on the type of cell line. Ethnicity is only viewed as highly relevant for stem cell lines, and as neutral for human and animal cell lines. Additional clinical and demographic data fields are seen as neutral.

Figure 5 gives an overview of the cell culture datafields. Differences can be observed between the cell line types. Most data fields (15 out of 18) are considered as highly relevant for human cell lines, except for anticoagulant use, growth medium, and freezing medium composition that are considered as neutral. Data analysis for animal cell lines is almost identical with the sole exception that growth medium additives are also considered as neutral. The relevance of cell culture data fields for stem cell lines differs, showing that only 9 out of 18 data fields are considered as highly relevant. Neutral data fields are: anticoagulant use, lot number registration, supplier registration, subculture protocol, freezing medium composition, freezing storage temperature, cryovial type, thawing method, and culture temperature.

Figure 6 gives an overview of the genetic data fields. There is overall variation in fields that are considered as relevant between all cell line types. In general, for each cell line type, half of the data fields is considered as relevant, the other half as neutral.

Figure 7 gives an overview of the quality and validation data fields. Microbial screening status and mycoplasma screening are considered as highly relevant for all cell types. Viral quality control is also considered as highly relevant for human cell lines and stem cell lines. STR profile is also rated as highly relevant for animal cell lines and stem cell lines. Additionally, DNA

[7]https://www.iso.org/obp/ui#iso:std:iso:8601:-1:dis:ed-1:v1:en

TABLE 1 | Extensive cell line data field set.

General cell line information	Administrative information	Clinical and demographic information	Culture method information	Validation and quality control information	Genetic information
Achor-dependancy	Analyse certificate	Age	Acclimatation of cells	Bacteria	Antigen expression
Advantages	Applications + advice	Age at collection	Antibiotic resistance	Biosafety guidelines	Antigen expression (surface)
Alias	Available product formats	Case history	Antibiotics	Biosafety level	Cell line stability
Animal	Catalog number	Clinical data	Anticoagulant	DAPI	Cytogenetics
Brief description	Cell culture images	Diagnosis information	Atmosphere	Flow cytometry	Details karyotype
Cell line alias	Comments	Disease	Cell density (cells/cm²)	Fungi	DNA Fingerprint
Cell line biological properties	Compliance with regulations	Donation frequency	Cellular products	Hazard	ELISA
Cell line description	Compliance with standards	Donor criteria	CO2 concentration	Health hazards of liquid nitrogen	Genes expressed
Cell line origin	Delivery forms	Ethnicity	Complete growth medium	Microbiological culture	Genetic alteration
Cell type	Distribution	Ethnicity information	Cryovial	MSDS file	Genetics
Clonality	Effects	Gender	Culture conditions	Mycoplasma	Immunology
Genus	Images	Harvest of cells	Derivation	Personal protective equipment	Isoenzymes
Identity	Limited use	Histopathology	Doubling time	Safety precautions	Karyotype
Lifespan	Limited warranty	Metastasis	Freeze concentration	Sterility	Mutational status
Morphological character	MTA agreement	Organ of metastasis	Freeze medium	Sterility tests	Oncogene
Morphology	Name of depositor	Pathology	Incubation	Storage precautions	Pathway activation
Organism	Originator	Preparation organ	Medium	Tryptan-Blue exclusion	PCR assay
Species	Ownership + patents	Race	Medium renewal frequency	Validation assay	Profile
Species validation	Permissions And Restrictions	Screened before donation	Passage	Viable cell count	Receptor expression
Strain	Price	Sex	Passage number	Viruses	Receptors
Tissue	Provider	Tissue form	Protocol for cell culture		Reprogramming method
Tissue origin	References	Weight	Protocol for cell thaw		Reverse transcriptase
	Register		Protocol for culture medium preparation		RNA hybridization
	Regulation		Protocol for freezing cells		STR profile
	Related products		Protocol for maintenance		Transformation
	Shipped in		Protocol for subculturing		Tumorigenic
	Shipping table + distribution notes		Quantity and concentration		
	Video + resources		Required materials		
	Year of origin		Split ratio		
			Storage conditions		
			Storage temperature		
			Subcultivation ratio		
			Subculture routine		
			Subculturing		
			Subculturing protocol		
			Temperature		
			Thawing method		

fingerprinting is also highly relevant for stem cell lines. The other data fields are considered as neutral.

Figure 8 gives an overview of the administrative data fields. For human and animal cell lines, these are generally considered as neutral. For stem cell lines, the Material Transfer Agreement (MTA) is highly relevant. However, conformity with regulations is regarded as not relevant.

As analysis of the relevance of the data fields between human, animal, and stem cell lines showed that no parameters are deemed completely irrelevant in either type of cell line and a large overlap

TABLE 2 | Survey cell line data field set.

Data field	Nominative category	Data field	Nominative category
Adhesion	Basic cell line	Growth medium Additives	Cell culture
Age	Clinical and demographic	Growth medium Composition	Cell culture
Amount and cell conc	Basic cell line	Illness	Clinical and demographic
Antibiotic resistance	Cell culture	Immunology	Genetic
Antibiotics	Cell culture	Isoenzyme validation	Quality/validation data
Anticoagulant use	Cell culture	Lot number registration	Cell culture
Antigen expression	Genetic	Medium renewal (sub cultivation)	Cell culture
Biosafety	Basic cell line	Microbial screening status	Quality/Validation data
Cell line stability	Cell culture	Morphology	Basic cell line
Cell type	Basic cell line	MTA agreement	Administrative information
Cellular products	Genetic	Mutational Status	Genetic
Clinical data	Clinical and demographic	Mycoplasma Screening	Quality/validation data
Conformity with regulations	Administrative information	Organism	Basic cell line
Cryovial type	Cell culture	Passage number	Basic cell line
Culture atmosphere	Cell culture	Patents and properties	Administrative information
Culture temperature	Cell culture	Receptor expression	Genetic
Cytogenetics	Genetic	Reprogramming method	Genetic
Derivation	Basic cell line	Short tandem repeat profile	Quality/validation data
Details karyotype	Genetic	Sub cultivation ratio + cell density	Cell culture
Dna fingerprint	Quality/validation data	Subculture protocol	Cell culture
Doubling time	Cell culture	Supplier registration	Cell culture
Ethnicity	Clinical and demographic	Thawing method	Cell culture
Freezing medium composition	Cell culture	Tissue origin	Basic cell line
Freezing storage temperature	Cell culture	Transformation	Basic cell line
Gender	Clinical and demographic	Tumor details	Genetic
Gene expression	Genetic	Tumor formation	Genetic
GMO status	Genetic	Viral quality control	Quality/validation data

in relevance exists. Thus, we concluded to develop one dataset, useable for all three types of cell lines. All fields were retained and categorized into four levels. The basic and crucial data fields (level 1) consist of highly relevant fields, mandatory for all cell lines. The fields containing data related to certain procedures, quality processes or performed analysis can be found in level 2 and are considered as optional to fill in. Level 3 data is data pertaining to biobanking activities, such as operational, administrative and storage information. These data fields are completed by biobank staff members, and are also considered mandatory to fill in. Finally, all data that can be calculated or automatically filled in by the BIMS system has been classified as level 4 data.

From a GCDMP perspective, the data fields, regardless of their level, were further reviewed one by one,: units were separated from numbers and fields in which grouped data responses were expected were divided in multiple fields to capture one type of data per field. This led to an increase in amount of data fields in the dataset, though a better resulting data quality. Next, a clear and understandable "label" or "field name" was selected and a definition was added, to clarify the intended data response. Overall, this approach resulted in multiple changes in the dataset.

The "Organism" field has been relabeled to "Species" reducing ambiguity. In addition two extra fields -"Class" and "Order"— were added, which are automatically filled in when the species is selected into the "Species" field. Further, the fields "Tissue origin" and "Morphology" were evaluated and encompassed by the following fields: "Cell type," "Cell line name" and "Anatomical location." The field "Biosafety" was relabeled to "Biosafety level," "Illness" to "Disease" and the fields "Age," "Gender," "Passage number" and "Ethnicity" were preserved. "Amount and cell concentration" were split into the fields "Amount (volume)," "Amount (volume) unit," "Amount of cells," "Amount of cells unit," and two calculated fields "Cell concentration" and "Cell concentration unit."

Multiple cell culture data fields were adapted for good data capture. "Adhesion" was relabeled as "Growth mode" as this field captures information regarding adherent or suspension culture and the label was deemed more appropriate. The field "doubling time" was split into a field capturing the number and the time unit. The fields "Culture atmosphere," "Culture temperature" and "Antibiotic resistance" were kept as is and the field "Antibiotics" was changed to "Antibiotic addition" and "Medium renewal"

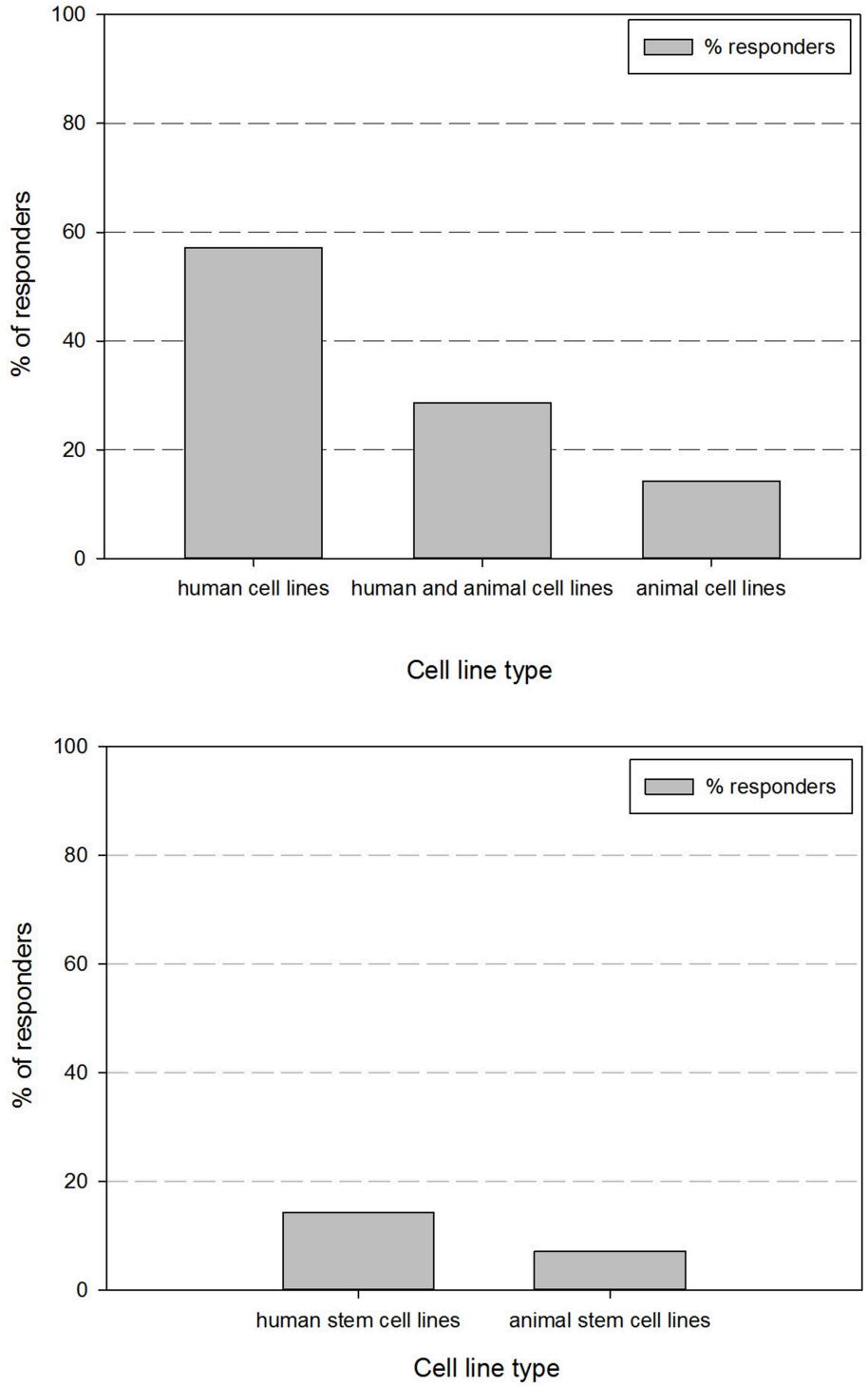

FIGURE 1 | Overview of the percentage of responders vs. the type of cell line they manage.

was relabeled to "Medium renewal frequency" in order to avoid misinterpretation. The fields "Growth medium composition" and "growth medium additives" were subdivided in multiple fields labeled: "Basal culture medium," "Serum (or alternative)," "% of Serum (or alternative)," "Growth medium additives," "Growth factors" and "Remarks on culture medium." "Sub cultivation ratio" was renamed to "Split ratio" and "subculture protocol" was renamed to "Cell dissociation agent or technique." A

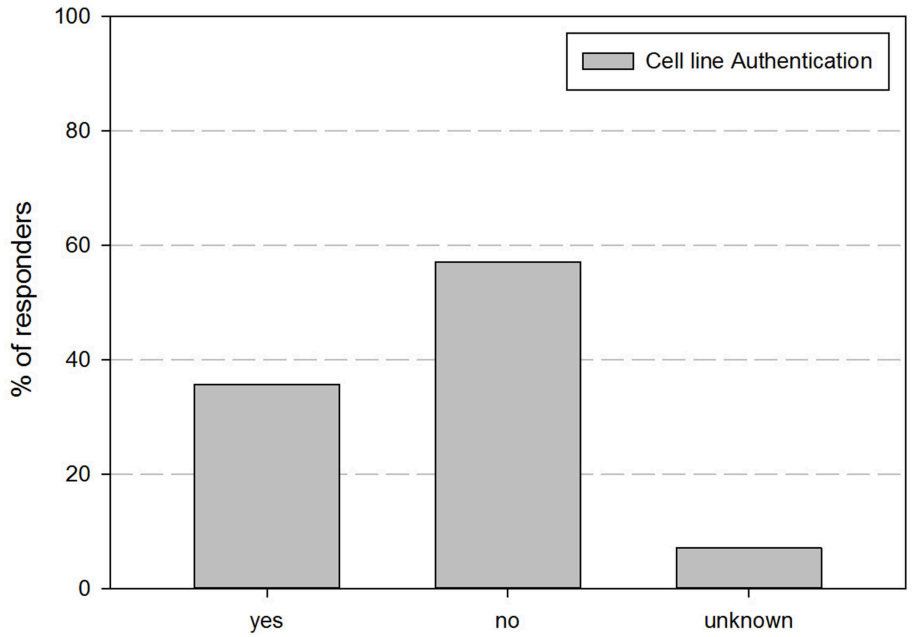

FIGURE 2 | Overview of the percentage of responders that indicated to perform cell line authentication on their cell lines.

similar approach was applied to the field of "Freezing medium composition." The following fields were created to encompass all data: "Basal Freezing medium," "Serum (or alternative) in freezing medium," "Cryoprotectant," "% of cryoprotectant" and "Freeze protocol." "Freezing storage temperature" is renamed to "Storage temperature" and "Cryovial type" to "Storage container." A date and time stamp "Freeze date and time" was also added to enhance the data value. The field "Thawing method" was split into "Basal thawing method," "Serum (or alternative) in thawing medium," "% of serum (or alternative) in thawing medium, "Thawing stabilizer," "% of thawing stabilizer" and "Thawing temperature."

To obtain clear data in the database, the fields related to quality control and genetic information were often split in multiple fields where the first field indicated if the analysis was performed and the second field with which technique/method, e.g., "Mycoplasma screening" became "Mycoplasma screening" and "Mycoplasma screening method." This applied for "Antigen expression," "DNA fingerprint" and "Viral quality control." The field "Gene expression" was split into multiple fields, as mentioned above however as this field encompassed more complex information, 5 fields were created to capture this in a structured way. The fields "Cytogenetics/karyotype," "GMO status" and "Tumor formation" were defined and by the use of fixed options there was no need to further separate the fields.

Before the cell line dataset was released for use on our campus, some additional fields were added to allow the practical implementation. The field "provider" is a field to identify who is bringing in the samples. Each collaborator of the biobank receives a unique number from the Bioresource center upon signing of the service level agreement. Additionally, the collaborators can use the fields "Biobank subcollection ID," "study specific patient ID," "Adremanumber," "Reference ID," "collection center" and "Visit number" to further define specific information regarding their collection. The fields "Status" and "date and time of registration" are filled in by the biobank personnel, as is the information regarding the location of the samples which are defined by the fields "Location path," "Location," "Row" and "Column." Some open text fields are added to capture important additional information: "Remark of group," "Sample remarks" and "Comment."

A section of fields to encompass information regarding 2D and 3D culture on biomaterials was also included to be filled in optionally, as there is a large biomaterial and tissue engineering consortium present on our campus which uses multiple cell lines for their experiments.

To have an easily fillable, consistent and searchable database, the use of "fixed choice fields" was introduced. If ontologies and standard classifications/lists were available, the user-friendliness was reviewed. E.g., for designing a list of cell types a concise selection of different cell types was made out of the "Cell Line Ontology: CLO" (28, 31) as reference. These cell types will be combined with their anatomical location in an additional data field, based on the topology code of the "International Classification of Diseases for Oncology" (29) and for ease of use the high level of anatomic location was implemented ("Lip" instead of "External upper lip" etc.). The applied ontologies and standard lists that were considered, can be found in **Table 3**, under column "Standards/principles for data quality."

The resulting cell line data set consists of a total of 101 data fields. The majority of these fields (58 out of 101 data fields) are level 1 fields, thus mandatory to complete by the

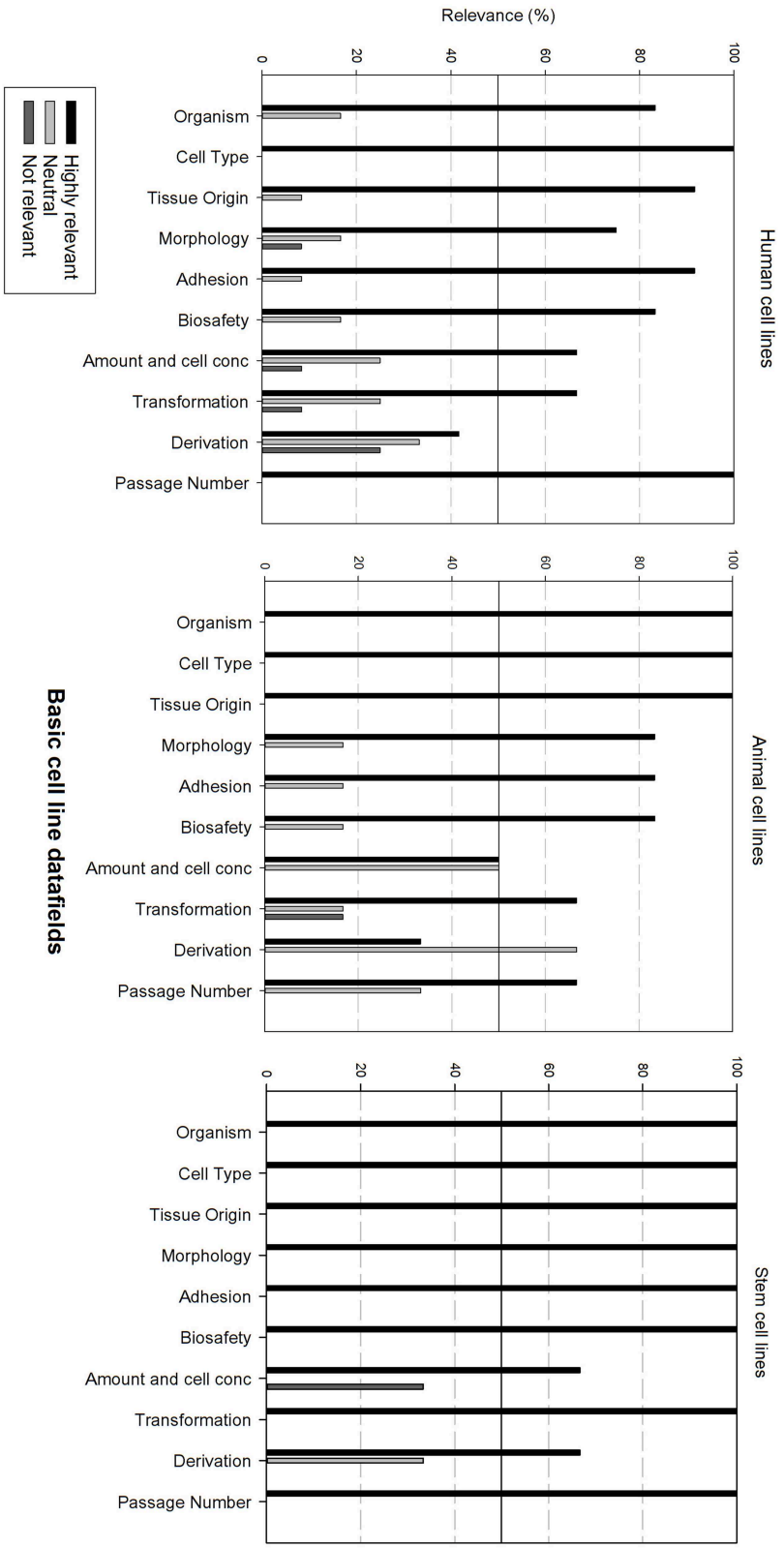

FIGURE 3 | Overview of the relevance score of basic cell line data fields for each type of cell line. A cut-off line at 50% is visible, indicating which data fields are generally considered as highly relevant or as not relevant.

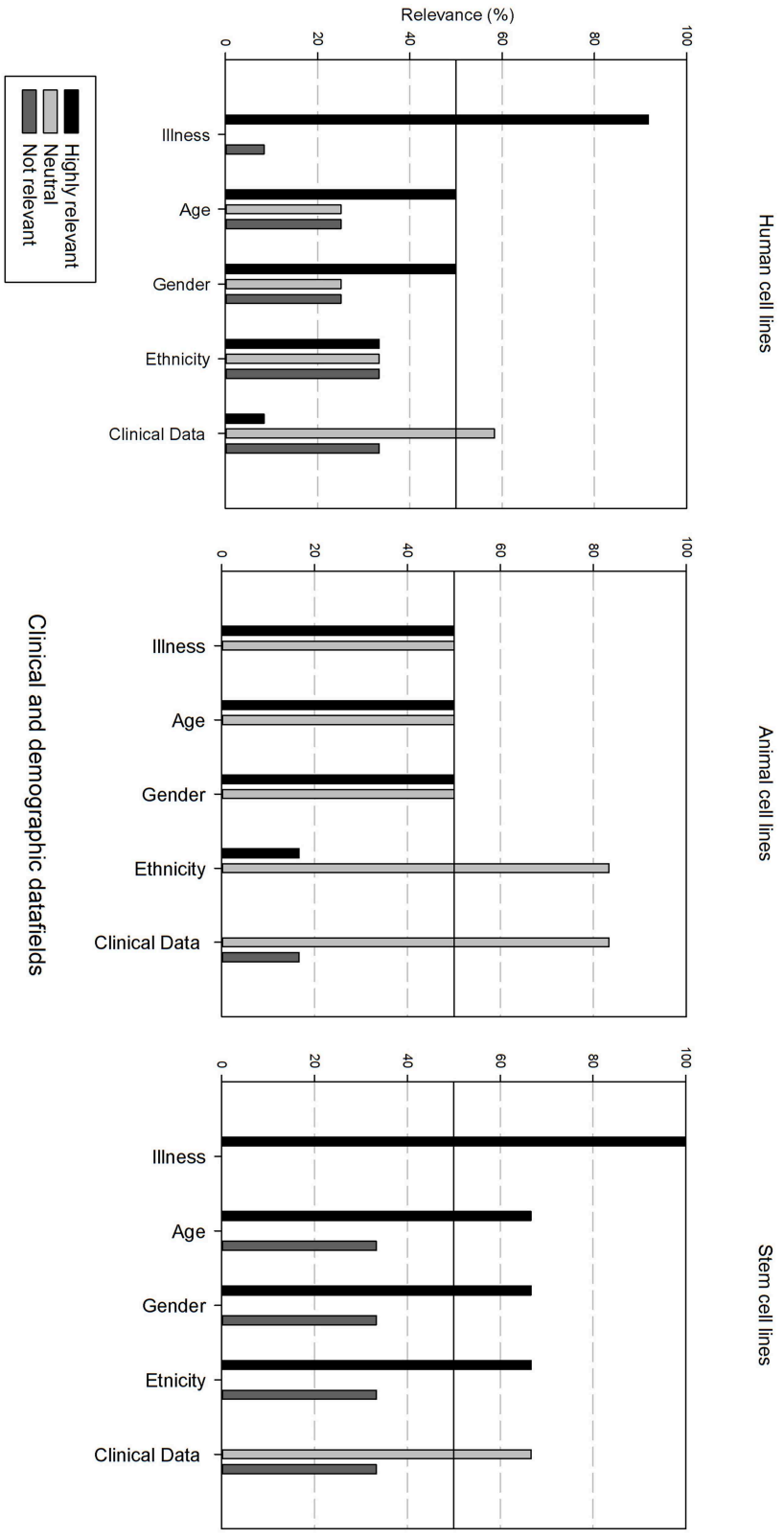

FIGURE 4 | Overview of the relevance score of clinical and demographic data fields for each type of cell line. A cut-off line at 50% is visible, indicating which data fields are generally considered as highly relevant or as not relevant.

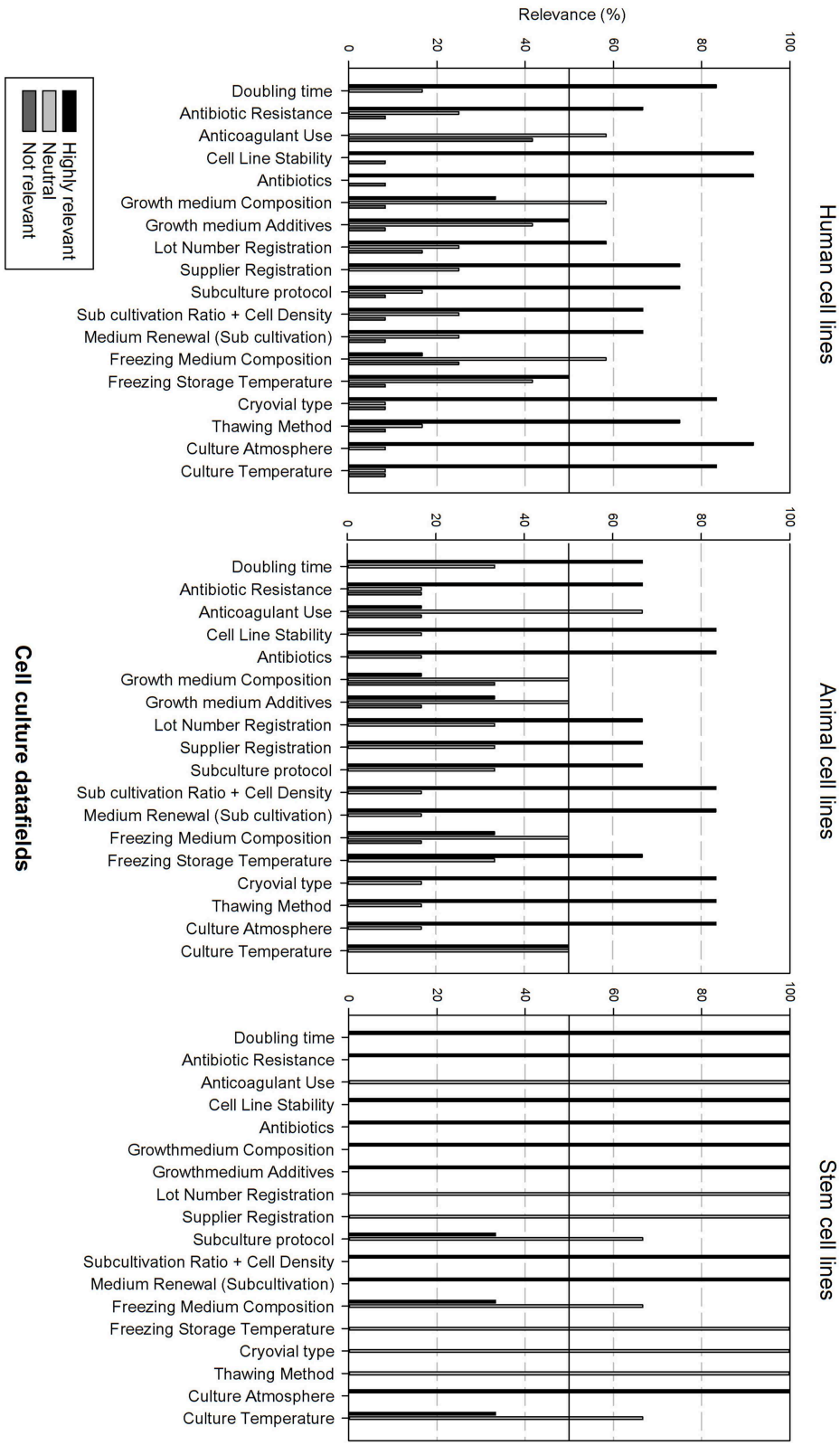

FIGURE 5 | Overview of the relevance score of cell culture data fields for each type of cell line. A cut-off line at 50% is visible, indicating which data fields are generally considered as highly relevant or as not relevant.

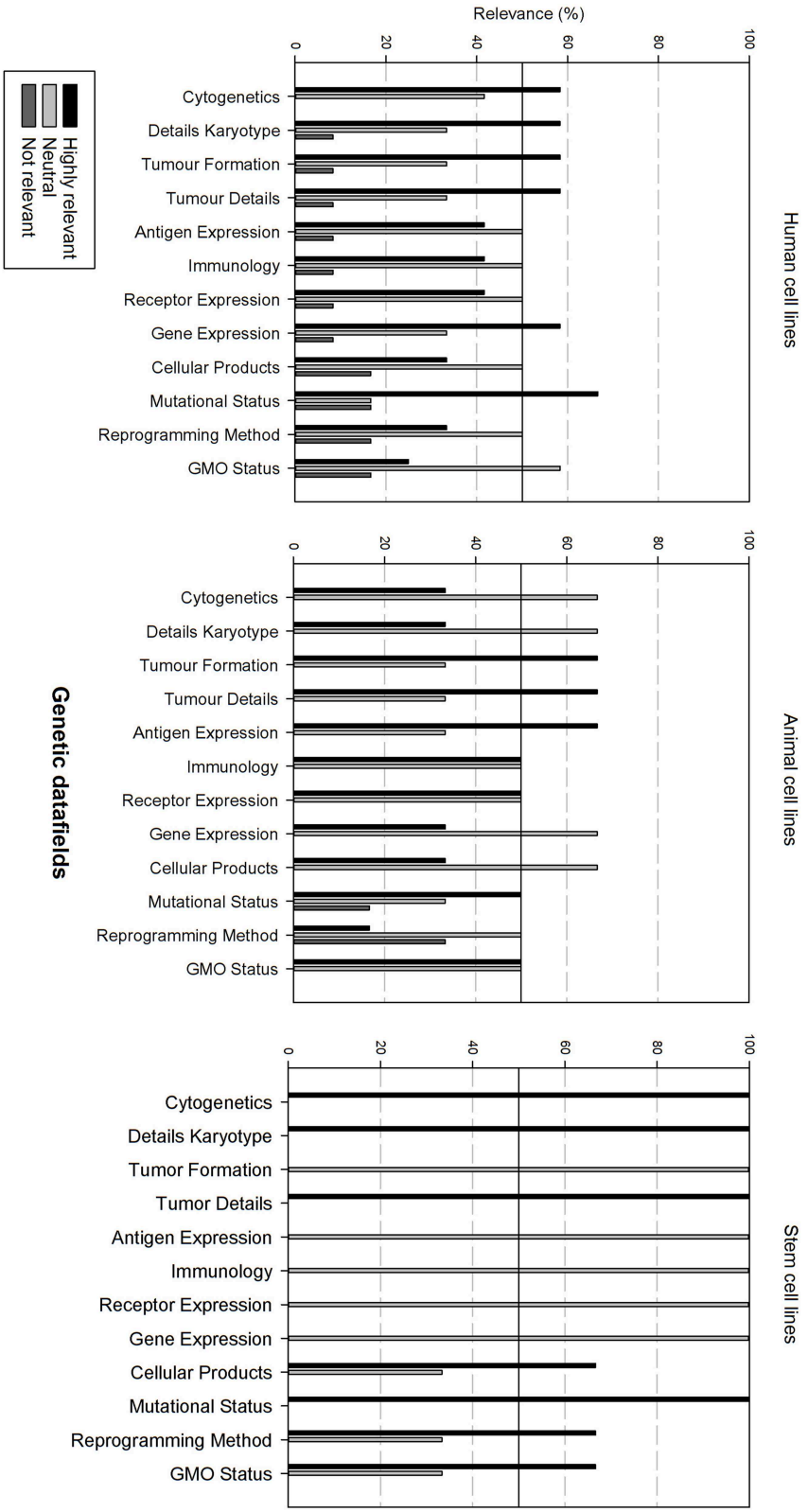

FIGURE 6 | Overview of the relevance score of genetic data fields for each type of cell line. A cut-off line at 50% is visible, indicating which data fields are generally considered as highly relevant or as not relevant.

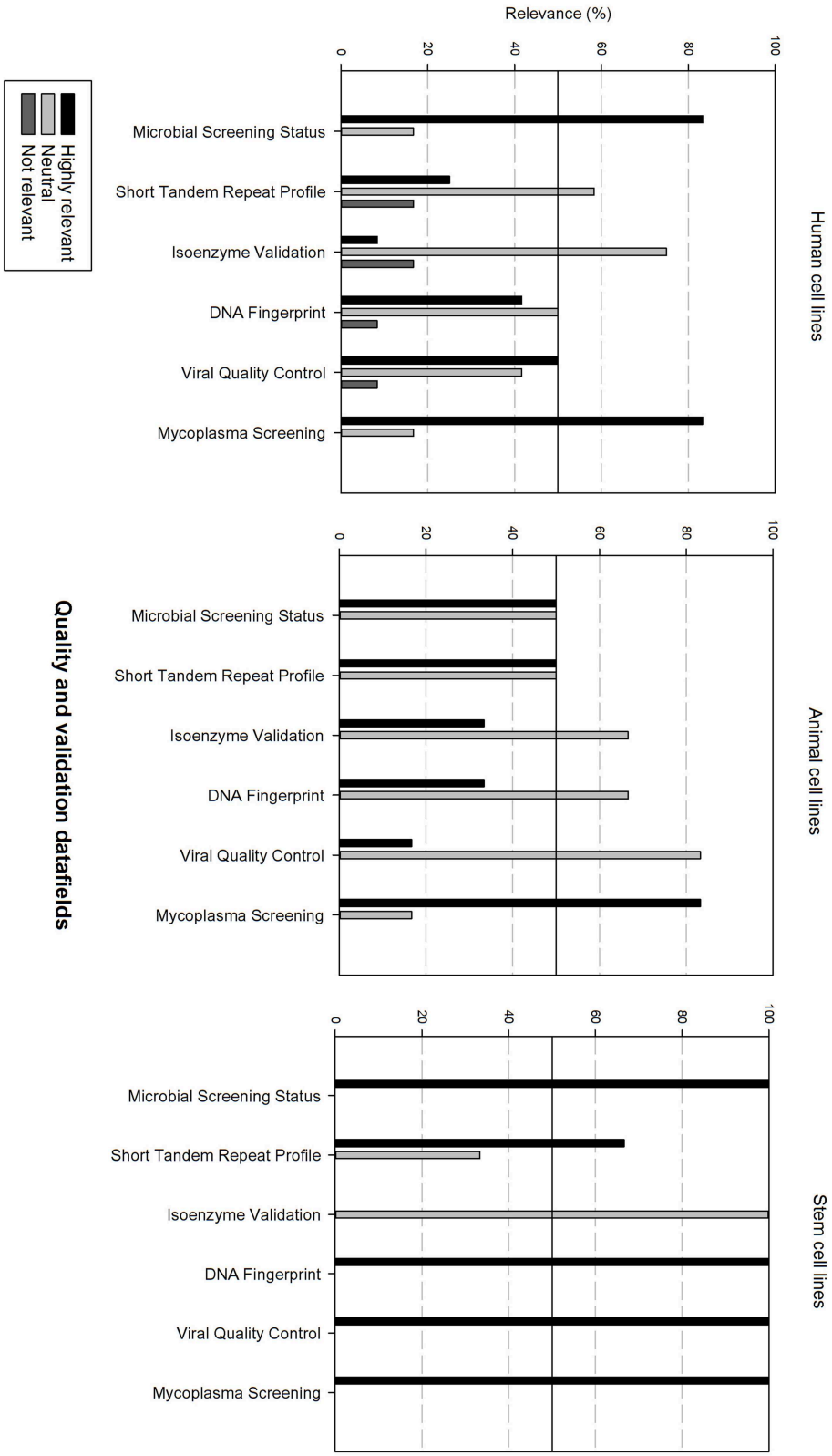

FIGURE 7 | Overview of the relevance score of quality and validation data fields for each type of cell line. A cut-off line at 50% is visible, indicating which data fields are generally considered as highly relevant or as not relevant.

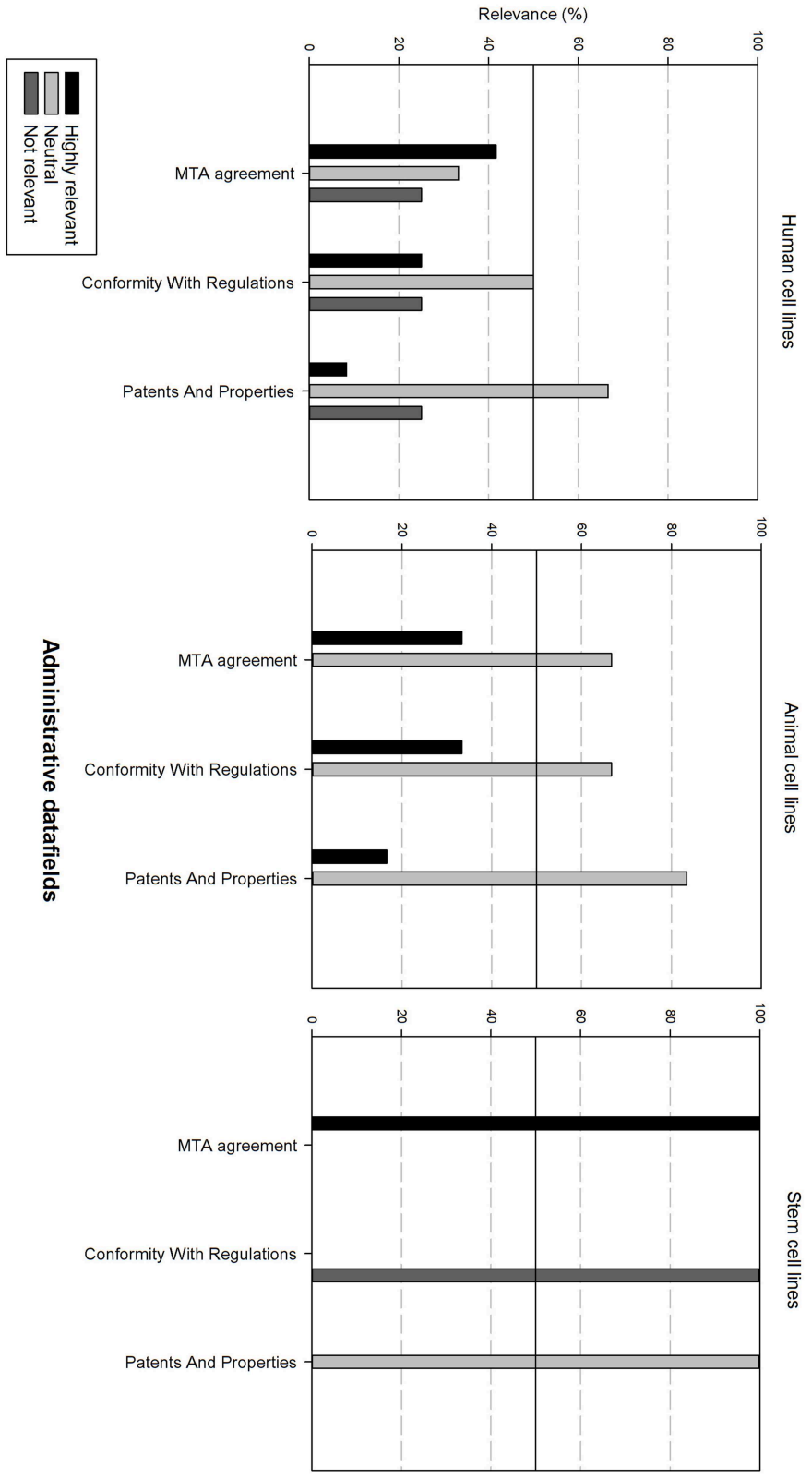

FIGURE 8 | Overview of the relevance score of administrative data fields for each type of cell line. A cut-off line at 50% is visible, indicating which data fields are generally considered as highly relevant or as not relevant.

TABLE 3 | Essential cell line dataset.

Data field label	Level	Data type	Principles for data quality	Definition
Species	1	Fixed choice	Biological classification (taxonomy) for class, order and species	Species from which the animal cell line was derived
Class	4	Automatic completion	Biological classification (taxonomy) for class, order and species	
Order	4	Automatic completion	Biological classification (Taxonomy) for class, order and species	
Cell type	1	Fixed choice	Cell Line Ontology (CLO)	Cell line cell type.
Cell line name	2	Free text field	The International Cell Line Authentication Committee (ICLAC)	Name of the (commercial) cell line.
Tissue origin/anatomic location	1	Fixed choice		Anatomical location/origin of the sample.
Biosafety level	1	Whole number		Biological safety levels are ranked from one to four and are selected based on the agents or organisms on which the research or work is being conducted. Each level builds up on the previous level, adding constraints and barriers. The classification of your organism can be checked at https://www.biosafety.be/content/tools-belgian-classification-micro-organisms-based-their-biological-risks
Growth mode	1	Fixed choice		Growth mode of the cell culture.
Disease	1	String (restricted format)	Diseases for Oncology (ICD-O)	ICD10 code of the studied disease where for the sample was collected, https://icd.who.int/browse10/2016/en
Gender	1	Fixed choice		This indicates the gender of the participant/animal. "Unknown" means information about the gender was missing, "Other" stands for transgender/gender neutral participants.
Ethnicity	2	Fixed choice		A large group of people who have the same national, racial, or cultural origins, or the state of belonging to such a group.
Study specific patient ID	2	Free text field		The link to the patient (according to the patient identification log) (pseudonomized).
Adremanumber	2	String (restricted format)		Directly identifying patient identification code provided by UZ Gent.
Reference Id	2	Free text field		
Collection center	2	Fixed choice		This field contains the location where the sample was collected from the patient. It allows identification of multiple collection centers (e.g., hospitals or general practice centers).
Collection date and time	2	Date	ISO 8601	Date and time of collection.
Consent status	1	Fixed choice		The consent status of the participant regarding the sample.
Sample status on arrival	1	Fixed choice		Status of your sample at arrival in the Biobank facility.
Visit number	2	Whole number		This contains the visit number. E.g., 0 stands for the baseline visit. 1 is the first visit after the baseline visit.
Type	1	Fixed choice		This describes the content type of the sample.
Passage number	1	Whole number		A record of the number of times the culture has been subcultured, i.e., harvested and reseeded into multiple 'daughter' cell culture flasks.
Amount (volume)	1	Whole number		
Amount (volume) unit	1	SI units	International system of units (SI)	
Amount of cells	1	Whole number		

(Continued)

TABLE 3 | Continued

Data field label	Level	Data type	Principles for data quality	Definition
Amount of cells unit	1	SI units	International system of units (SI)	
Cell concentration	4	Calculated field		
Cell concentration unit	4	SI units	International system of units (SI)	
Culture atmosphere	1	Fixed choice		The controlled atmosphere in which the cells are cultivated (CO_2/O_2 levels).
Culture temperature	1	Fixed choice		The controlled temperature at which cells are cultivated.
Basal culture medium	1	Fixed choice		The basic unsupplemented medium which promotes the growth of many types of cells.
Serum (alternative)	1	Fixed choice		Serum or alternative that contains a complex array of protein components, essential for cell culture.
% of serum (alternative)	1	Decimal number		Percentage of serum used in culture medium.
Growth medium additives	1	Fixed choice		Additional supplements to the basic culture medium that provide optimal growth conditions for the specific cell line.
Growth factors	1	Fixed choice		Additional growth factors to the basic culture medium that provide optimal growth or differentiation conditions for the specific cell line.
Antibiotic addition	1	Fixed choice		Antibiotics that are added to routine culture medium.
Antibiotic resistance	1	Fixed choice		Antibiotics for which the cell line is resistent.
Remarks culture medium	2	Free text field		Extra information concerning the culture medium.
Cell dissociation agent or technique	1	Fixed choice		Agent or technique used for dissociation of cells.
Split ratio	1	Fixed choice		The divisor of the dilution ratio of a cell culture at subculture, e.g., 1/5.
Doubling time	2	Time	ISO 8601	The period of time required for the cells to double in amount.
Doubling time unit	2	SI units	International system of units (SI)	
Seeding cell density	1	Whole number		Density/concentration at which the cells are seeded after passaging.
Seeding cell density unit	1	SI units	International system of units (SI)	
Medium renewal frequency	1	Fixed choice		Frequency of culture medium renewal.
Cell Line Stability	2	Fixed choice		Indication of cell line stability.
Basal Freezing Medium	1	Fixed choice		The basic unsupplemented medium which forms the essential part of the freezing solution.
Serum (alternative) in freezing medium	1	Fixed choice		Serum or alternative that contains a complex array of protein components, used to supplement the basic freezing medium.
% of serum (alternative) in freezing medium	1	Decimal number		Percentage of serum used in freezing medium.
Cryoprotectant	1	Fixed choice		A cryoprotectant is a substance used to protect biological tissue from freezing damage.
% of cryoprotectant	1	Decimal number		Percentage of cryoprotectant used in the freezing medium.
Freeze protocol	1	Fixed choice		Technique used for freezing the sample.
Conservation	1	Fixed choice		
Basal thawing medium	1	Fixed choice		The basic unsupplemented medium which forms the essential part of the thawing solution.
Serum (alternative) in thawing medium	1	Fixed choice		Serum or alternative that contains a complex array of protein components, used to supplement the basic thawing medium.

(Continued)

TABLE 3 | Continued

Data field label	Level	Data type	Principles for data quality	Definition
% of serum (alternative) in thawing medium	1	Decimal number		Percentage of serum used in thawing medium.
Thawing stabilizer	1	Fixed choice		Supplements added to the thawing medium to stabilize the cells during the thawing process.
% of thawing stabilizer	1	Decimal number		Percentage of thawing stabilizer in thawing medium.
Thawing temperature	1	Fixed choice		Temperature at which the samples are thawed.
Adapted to 3D culture	1	Y/N; Fixed choice		Has the cell line been adapted to 3D culture?
Feederlayer	1	Y/N; Fixed choice		Is a feeder layer needed for cell culture of the cell line?
Feederlayer determination	2	Free text field		Which feeder layer is needed for maintaining the cell culture of the cell line?
Biomaterial (basic composition)	2	Fixed choice		
Biomaterial modification	2	Fixed choice		
Biomaterial coating	2	Fixed choice		
Remark of group	2	Free text field		Extra remarks related to the cell line.
Sample remarks (QC)	2	Free text field		Remarks concerning the quality of the specific sample.
Comment	2	Free text field		General comment (cannot contain identifying data).
Storage temperature	1	Fixed choice		Temperature at which sample is stored.
Storage container	1	Fixed choice		Type of container in which the sample is store for long term storage.
Freeze date and time	1	Date	ISO 8601	Date and time of freezing of the sample.
Cytogenetics/karyotype	1	Fixed choice		Method used for karyotyping/ Cytogenetic procedures.
Antigen expression	1	Fixed choice		Is there antigen expression within the cell line sample?
Type of antigen expression	2	Fixed choice		What type of antigen expression can be observed?
Method of antigen expression	2	Fixed choice		Method used for determining the antigen expression profile.
Reprogramming method performed	1	Fixed choice		Was the cell line obtained through a reprogramming process?
Reprogramming method	2	Fixed choice		Which method was used for reprogramming the cell/line.
GMO status	1	Fixed choice		Is the cell line classified as a genetically modified organism?
Microbial screening status (microbial contamination)	1	Fixed choice		Was the sample screened for microbial contamination and what was the result of this screening?
DNA fingerprint	1	Fixed choice	The American National Standards Institute (ANSI), American Type Culture Collection (ATCC)	Has a DNA fingerprinting method been performed?
DNA fingerprint (method)	2	Fixed choice	The American National Standards Institute (ANSI), American Type Culture Collection (ATCC)	Which screening method was used for DNA fingerprinting?
Viral quality control	1	Fixed choice		Has the cell line been screened for viral contamination?
Viral quality control (method)	2	Fixed choice		Which method was used for the viral quality control?
Mycoplasma screening	1	Fixed choice		Has the cell line been screened for mycoplasma contamination?
Mycoplasma screening (method)	2	Fixed choice		Which screening method was used for the Mycoplasma detection?

(Continued)

TABLE 3 | Continued

Data field label	Level	Data type	Principles for data quality	Definition
Tumor formation	2	Fixed choice		Ability for tumor formation.
Gene expression level	2	Fixed choice		
Gene expression level (test)	2	Free text field		
Gene expression analysis method	2	Fixed choice		
Gene expression overexpression method	2	Fixed choice		
Gene expression inhibition method	2	Fixed choice		
Provider	1	String (restricted format)		Provider number given by Bioresource center Ghent (unique for the biobank)
Biobank subcollection ID	2	Free text field		Field that can be used to indicate specific subprojects in which the samples are collected
Status	3	Fixed choice		Operational status of sample (Bioresource center Ghent)
Date and time of registration	3	Date	ISO 8601	Date of registration in the biobank
Location path	3	Fixed choice		Location of the sample including subdivisions (freezer, shelf, rack…)
Location	1/3	Fixed choice		Box number
Row	1/3	Fixed choice		Row within the box in which the sample is located
Column	1/3	Fixed choice		Column within the box in which the sample is located
Cell line formation	2	Free text field		Description creation of the cell line.

researcher. There are 32 optional (level 2) data fields that allow to enhance the data quality, 7 level 3 fields that are filled in by the biobank staff and four automatic calculated fields (level 4). The dataset fields were configured in our BIMS system. Through an Excel template with the configured option lists, the information can easily be received from the researchers and put into the BIMS system.

DISCUSSION

Cell lines are essential in translational biomedical science. Misidentification, culture mix-ups, authentication and annotation issues often occur, hampering and delaying the reliability and reproducibility of preclinical tests, which are mandatory before the initiation of actual clinical trials. Accurate documentation of cell line data in a state-of-the-art database system is critical to ensure the credibility, reproducibility, and translation of data and results from cell culture-based experiments (17).

The need for international standards to close multiple gaps in this field is obvious. In order to resolve this issue, the first steps to harmonization are being initiated as new standards are arising [human cell line STR profiling (32) (ASN-0002), DNA barcoding for animal cell lines (13) (ASN-0003)]. Additionally, an effort was made to make cell line misidentification more conspicuous with the establishment of "The International Cell Line Authentication Committee (ICLAC)." They established controlled vocabularies and ontologies for already existing cell lines. However, a reduction in complications and redundancies in the literature concerning cell lines didn't seem attainable (15).

At our campus, multiple cell lines are kept in biobanks. The need for a uniform, campus-wide cell line dataset that tackles issues regarding misidentification, annotation and poor culture follow-up is high. We initiated this process by a large-scale literature and public database review of cell line datasets. There is an enormous lack of clear information in literature regarding cell line datasets and the fields these contain. A compiled extensive dataset was established as described in the Materials and Methods section. There is a massive difference in available information in the datasets pertaining to cell lines, as some vendors/repositories only list 8 data fields and others over 50 data fields regarding the same cell line. Further analysis through the redundancy strategy approach, revealed additionally a lack of standardization in terminology and definitions of the data field and the use of divergent labels for identical field information. It is clear that currently, different cell line repositories have established their own divergent sets of data fields without any verification or mutual agreement on which data should be recorded.

Through use of the redundancy strategy, a concise set of 54 data fields could be compiled for the survey dataset. As our aim was also to examine which data researchers are currently registering and which quality checks they are performing, we included these questions into the REDCap survey. The REDCap

tool allows integration of all these parameters in a survey of reasonable length, which can be completed in a user-friendly way by the researchers. No comments about the setup of the survey or any remarks about difficulties completing the survey were received. Responses from human, animal, and stem cell line users were received, which allowed us to evaluate different expectations and needs regarding the datasets for human, animal and stem cell lines. Remarkable, over 75% of the cell line users do not authenticate their cell lines.

A general consensus could be observed regarding the high relevance of the basic cell line data fields, which was expected. These fields were also present in the datasets of the majority of all vendors/cell line depositors. The relevance of the clinical and demographic related data fields varies more, but is considered more as neutral. Cell culture information is considered as highly relevant or neutral. The pattern of relevance for human and animal cell lines is quite comparable. There is more distinction with stem cell lines, where certain parameters are considered as either very relevant or completely neutral. Some vendors/cell line depositors give only minimal information related to cell line culture parameters, or allow for the upload of culture protocols to be distributed upon request of the cell line.

Genetic data information is in general less prominent in datasets of vendors/cell line depositors, and is also mostly considered as neutral to relevant. Genetic information is prominent available when buying specific animal cell line clones, engineered for certain research purposes. It is clear however, that information related to the mutational status is necessary for stem cell lines. Quality and validation information is rarely available. It is assumed that some large vendors have quality and validation procedures in place, though no specific information can be given upfront, only upon request. One exception clearly standing out is the German collection of Microorganisms and cell cultures, hosted at the Leibniz Institute[8]. Clear information regarding the performed tests and results can be found online, e.g., Mycoplasma screening by PCR, DNA fingerprinting and type of performed PCR for revealing the STR profile, PCR analysis for several viral contaminants. This is crucial information when the cell lines are used in pre-clinical assays. Administrative information detailing the proposed use, warranties, limitations and restrictions of the material are considered as neutral. From a legal perspective, however, this is an exceptional important part of information and essential to keep track of, therefore these data fields will be kept in the developed dataset. Our analysis showed that the development of one dataset for the different types of cell lines would be applicable and usable for our local cell line users.

A list of data fields was compiled, based on the REDCap survey. Within this list, every single entry was subsequently evaluated based on score, relevance and multiplicity. In the survey, certain broad terms were used to describe the content of data field. These fields were split in more clearly definable sub-data fields according to GCDMP rules.

Two essential aspects to obtain good structured data are clear and unambiguous naming of the defined data fields and thoughtfully chosen definitions of the data fields.

[8]DSMZ: https://www.dsmz.de/home.html

The definitions implemented were based upon terminology used in three known biobanking data categorizing systems: SPREC, BRISQ, and MIABIS. SPREC and BRISQ are both proposed as a set of recommendations for reporting data elements of human biospecimens used in biomedical research with the difference that SPREC allows generating a code based on the pre-analytical processing of the samples (33). MIABIS, as its name suggests, is an attempt to unify sample data in a way that simplifies communication and exchange of samples (and sample information) in a clear and non-ambiguous way (6). The applicable definitions out of these standards were retained for implementation, although sometimes the formulation was simplified. Additionally, specific definitions that were not present in the standards were designed (e.g., especially for cell culture specific fields) based upon general accepted definitions out of histology and cell culture handbooks (34, 35).

The data quality was further enhanced through the implementation of standards and ontologies, and through the use of fixed fields, thus allowing limited options per field. Another measurement undertaken to maintain good data practice was the possibility to distinct missing data from empty "not filled in" data fields. For instance, within the selection list of the fixed choice data fields an option for missing data, e.g., not performed, unknown etc., was included. It is taken into account, however, that certain options might be to restraining to be able to input the data, thus, if this would occur, the biobank collaborators have the possibility to request additional options for a field. The request will be reviewed by a cell line advisory board, consisting of data managers and cell line experts. This is especially true for new and rapid expanding fields of research, for example the use of (bio)polymers in tissue engineering. Bearing this in mind, the complete data set will be implemented on campus with notification of the possibility to propose relevant additions to fixed choice fields. The complete set of defined options can be procured by contacting the Bioresource center manager.

The essential dataset consists of certain fields that are linked to each other, e.g., "Class," "Order" and "Species." To enhance the user-friendliness of the dataset, it was decided, through gathered experience weighed against literature-based study[9] (36), to include the most commonly used organisms in research studies and not entire ontology lists. Additionally, when selecting the "Species" in the dataset, the related "Class" and "Order" are automatically completed in the database. This automatic completing of fields is also the case for the calculation of "cell concentration" and the unit based on "volume" and "amount of cells".

For ethnicity (37), which is more important for human based cell lines, a compact classification was designed based on existing classifications[10] (38) and the most common nationalities in Belgium (39–41). Statistics concerning citizens with a different ethnical background residing in Belgium can be found online, provided by the government. We finally designed a compact

[9]https://www.thermofisher.com/be/en/home/technical-resources/cell-lines.html
[10]https://www.ethnicity-facts-figures.service.gov.uk/ethnicity-in-the-uk/ethnic-groups-and-data-collected

list of 29 different ethnicity options adapted to the Belgian population including persons who identify themselves with more than one social group. In case the Belgian population changes, extra options may be added. Other fixed choice lists were based on existing data fields from other data capturing systems.

Although useful as they are, and taking into account GCDMP guidelines, most of the withheld SPREC data fields needed to be subdivided to create clean and unambiguous data. For example, the SPREC field "Long term storage" which contained the temperatures at which a sample can be stored, the methods of storing the sample (Liquid Nitrogen (LN), Ultra low temperature (ULT) freezing, …), as well as the type of container used for storing the sample had to be split. It was subdivided into "Conservation," encompassing the type of conservation (LN, ULT,…), "Storage temperature" en "Storage container." In the same way the fields "Type of collection" (SPREC) and the non SPREC fields "Mode of transportation" and "Thawing procedure" were divided into numeric (temperature, time, …) fields and full text (protocol) fields. The use of these existing standards by researchers globally increases harmony and quality in biospecimen reporting in general.

Only minimal clinical and demographic information is present in our cell line dataset. We do, however, recognize the importance of this type of data relating to biobank samples but chose to collect these data in clinical registries, linked with the BIMS system. For clinical registries, REDCap, the tool that was used to perform the survey, can also be used. It allows capturing clinical and demographic information in a structured and easy way, by designing multiple forms that can be filled in at different time points. Additionally, sample data can be linked to the relevant clinical and demographic information present at that specific time point.

We chose to combine a system for sample data with one for clinical and demographic information, thus storing all the relevant data necessary for research in divergent but interconnected systems. This differs from other data capturing methods such as BRISQ where both sample and clinical/demographic information are encompassed within all three tiers (levels of importance to report) in one system. The main advantage of this type of data collection lies in the fact that all data concerning the sample is linked to the sample itself. The downside however, is that potentially large amounts of data are stored per sample. Additionally, many of the fields within the BRISQ tiers allow for free text data input, opening the possibility of clouding the data through less than optimal data management practices.

Furthermore, in Europe, addition of clinical and demographic data could make it possible to identify patients by specific information, such as birthdate, specific disease etc., which is not conform the General Data Protection Regulation guidelines. Thanks to the REDCap software, a clear distinction can be made between clinical and demographic data and the essential pre-analytical data concerning the sample itself in accordance with local legislation. This allows for researchers to use the REDCap tool as a Case Report Form and put an extra focus on sample specific data, which all too often is only an afterthought in the data capturing process. Altogether, a separation between clinical/demographic information and pre-analytical sample specific data will elevate sample-specific data quality and improve reproducibility.

The cell line dataset that was created, captures the most important information related to cell lines. The database allows distinction between clones of cell lines cultured in different settings, frozen and thawed with different procedures, products and methods and those that were kept on feeder layers, coatings or biomaterials. Additionally, the inclusion of genetic information and quality information makes the dataset extremely valuable.

Capturing this informations assures that cell line reactions observed in preclinical tests are in reality related to the performed test and not to "metadata," meaning to possible contamination of the cell line, misidentification of the cell line or culture mix-ups. Clear cell line identification, in which genetic parameters of the cell lines are included, also lead to the correct use of the cell lines combined with certain specific test substances created for personalized medicine approaches or disease-specific solutions.

The dataset will be evaluated after one year of use. A customer satisfaction survey will be sent out to all our cell line users, who hopefully will be enthusiastic about the changes made. Based upon their feedback, additional changes could be made to the survey. As our biobank is part of the Biobanking and Biomolecular Resources Research Infrastructure of Belgium[11], we will discuss spreading this dataset within our network to allow a broader use within Belgium as part of the ongoing harmonization strategies related to data management and quality.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the supplementary files.

AUTHOR CONTRIBUTIONS

VT and LV devised the project, the main conceptual ideas, and proof outline. VT, LV, SV, SP, and EB worked out the details of the essential dataset, researching ontologies, known standards, and other literature. LV, SV, and SP worked out the actual technical implementation in the BIMS system upon agreement on the dataset in all its details. SB and CV supervised the project. VT wrote the main manuscript. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

ACKNOWLEDGMENTS

I would like to acknowledge the work of Sara Phlypo, research student, in the preliminary searches regarding the need for a cell line dataset. Additionally, I would like to acknowledge the Data Management Unit of the Health, Innovation and research institute (HIRUZ) of Ghent University Hospital for support regarding the RedCap survey and analysis tool.

[11] https://www.bbmri.be/

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 26 June 2019
doi: 10.3389/fmed.2019.00141

Biobank Quality Management in the BBMRI.be Network

Loes Linsen[1†], Veronique T'Joen[2†], Catherine Van Der Straeten[2], Kristel Van Landuyt[1], Etienne Marbaix[3], Sofie Bekaert[4] and Nadine Ectors[1] on behalf of the BBMRI.be Quality Working Group*

[1] Biobank, University Hospitals Leuven, Leuven, Belgium, [2] Bioresource Center Ghent, Health Innovation and Research Institute, University Hospital Ghent, Ghent, Belgium, [3] Pathology Department, de Duve Institute, Saint-Luc University Clinics, Catholic University of Louvain, Brussels, Belgium, [4] Department of Public Health and Primary Care, Faculty for Medicine and Health Sciences, Ghent University, Ghent, Belgium

From as early as 2005, different guidelines and quality standards covering biobank activities and sample handling methods have been developed to improve and guarantee the reproducibility of biomarker research. Ten years on, the BBMRI.be Quality working group wanted to gauge the current situation of these aspects in the biobanks of the BBMRI.be network. To this end, two online surveys were launched (fall 2017 and fall 2018) to the biobank quality managers in the BBMRI.be network to determine the status and setup of their current quality management system (QMS) and how their QMS and related practices have evolved over a 14 month time period. All biobanks addressed by the two surveys provided a complete response (12 and 13, respectively). A QMS was implemented in 85% of biobanks, with 4 standards emerging as primary basis. Supplementary guidelines were used, with a strong preference for the ISBER best practices for biobanks. The Standard Preanalytical Code—an indicator of the preanalytical lifecycle of a biospecimen impacting the downstream analysis results—was already implemented in 50% of the biobanks while the other half intends future implementation. To assess and maintain the quality of their QMS, 62% of biobanks used self-assessment tools and 71% participated in proficiency testing schemes. The majority of biobanks had implemented procedures for general and biobank specific activities. However, policies regarding the business and sustainability aspect of biobank were only implemented in a limited number of biobanks. A clear desire for a peer-review audit was expressed by 69% of biobanks, with over half of them intending to implement the recently published biobank standard ISO20387. Overall, the biobanks of the BBMRI.be network have actively implemented a solid quality approach in their practices. The implementation of ISO 20387 may bring further professionalization of activities. Based on the needs expressed in this survey, the Quality working group will be setting up an audit program for the BBMRI.be biobanks, to enhance, harmonize and streamline their activities. On the whole, the biobanks in the BBMRI.be network are able to substantially contribute to translational research, as a primary facilitator guaranteeing high quality standards and reproducibility.

Keywords: biobank, quality, survey, audit, BBMRI.be, ISO 20387, QMS

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Ondrej Viklicky, Institute for Clinical and Experimental Medicine (IKEM), Czechia

Reviewed by:
Xiaoguang Li, The Johns Hopkins Hospital, Johns Hopkins Medicine, United States
Jan-Eric Litton, Karolinska Institute (KI), Sweden

***Correspondence:**
Nadine Ectors
nadine.ectors@uzleuven.be

[†]*These authors have contributed equal to this work*

Specialty section:
This article was submitted to Translational Medicine, a section of the journal Frontiers in Medicine

Received: *08 April 2019*
Accepted: *07 June 2019*
Published: *26 June 2019*

Citation:
Linsen L, T'Joen V, Van Der Straeten C, Van Landuyt K, Marbaix E, Bekaert S and Ectors N (2019) Biobank Quality Management in the BBMRI.be Network. Front. Med. 6:141. doi: 10.3389/fmed.2019.00141

INTRODUCTION

Irreproducibility of results has been identified as a major undermining factor for translating research results into clinical applications (1). Different categories of errors contribute to this irreproducibility, with biological reagents and reference materials having the biggest impact (2). It has also been shown that standardization and auditing of biological materials—through biological resource centers or biobanks—can enhance cumulative production of scientific knowledge by improving both availability and reliability of research inputs (3). This need for biospecimen handling standards and the professionalization of biobanking practices to improve research outcome was recognized more than a decade ago. As early as 2005, the "International Society for Biological and Environmental Repositories" (ISBER) published the first version of their best practices in order to support the increasing demands for specific high quality biological material (4). Concurrently, different organizations, biobank networks and national initiatives all worked on best practices and guidelines to address the need for more professionalized biobanking practices and quality management systems (QMS) (5–9). Additionally, significant effort has been put in creating technical standards for pre-examination processes such as those developed within the SPIDIA project (10, 11), for capturing pre-analytical factors such as the Standard Pre-analytical Code (12, 13) and standardized data collection (14–16) to allow fit-for-purpose biological sample management. Finally, educational programs for biobank personnel have been set-up to further professionalize the discipline (17–19).

At the same time, three biobank networks were established in Belgium: the Flemish Biobank Network (FBN) [formerly known as the Center for Medical Innovation (CMI)], the Belgian Virtual Tumorbiobank (BVT), and the Biothèque Wallonie Bruxelles (BWB). A common goal of these networks is to improve and/or harmonize the quality of the biospecimens for the purpose of high-quality collaborative research, albeit through a different approach. The FBN was initiated in 2010 by the Center for Medical Innovation (CMI, Flemish government). The CMI was established to stimulate translational biomedical research and to reach a significant economic value in Flanders by setting up 4 clinical research centers within the Flemish universities and university hospitals. The initial focus lay on advancing biobank professionalization and harmonization within Flanders for five focus disease domains (inflammatory bowel disease, rheumatoid arthritis, diabetes type I, sudden cardiac death and hepatological/hepatotropic diseases). Apart from defining local ethical and legal guidelines, a key result of the CMI initiative was the publication and implementation of the uniform CMI biobank quality guidelines. These were based on the ISO 9001:2008 standard and the OECD guidelines for biorepositories and allowed standardization within the Flemish biobanks. All biobanks of the network were peer-review audited in 2014 according to the CMI quality guidelines. In parallel, a minimal data set of 14 attributes was defined to enable the setup of a centralized virtual catalog for sample query to facilitate collaboration within the five focus domains. In analogy to the FBN network, the BWB project was set up in 2012, incorporating the academic biobanks in Wallonia and Brussels, with the objective of providing a virtual catalog of biospecimens to facilitate translational research. The BWB also established QMS guidelines for biobanks, initially based on the guidelines previously defined by the FBN, which have been used by the BWB biobanks as a basis for their QMS. The BVT was created in 2008 as part of the Belgian National Cancer Plan, which intended to fight cancer by integrating all aspects of the fight against the disease. The aim of the BVT is to centralize standardized and curated data of available residual human tumor samples, collected in the university hospitals and liaised laboratories, in an easy lookup tool. The pseudonymized database is accessible for researchers to query and trace their samples of interest to the local biobanks of the network, where the samples can be released for research projects. Additionally, the BVT also strives to optimize quality by creating awareness about data quality and sample collection by incorporating these elements in the requested standardized dataset.

Since 2013, these three biobank networks are participating in BBMRI.be, the national node of the European Research Infrastructure BBMRI-ERIC, effectively gathering 13 biobanks within one network. Within this national node of BBMRI.be, a Quality working group was established with the aim to define a consensus approach to harmonized QMS systems, biobank sample flows and procedures based upon existing international, European, Belgian and regional requirements. Ten years after the start-up of the Belgian biobank networks, the BBMRI.be Quality working group wanted to gauge the current quality status of the connected biobanks, to define the areas of improvement within the biobanks and to develop tailored support by the Quality working group. To this end, we launched 2 online surveys (fall 2017 and fall 2018) to the biobank quality managers (12 and 13, respectively) in the BBMRI.be network to determine the status and setup of their current QMS and how their QMS and related practices have evolved over a 14 month time period.

MATERIALS AND METHODS

First Survey

Fall 2017, a short, high level questionnaire made using the SurveyMonkey™ tool was distributed to the quality managers of biobanks linked to BBMRI.be to assess the general status and activities with respect to the QMS. The survey was distributed to the 12 biobanks of the BBMRI.be network, by providing a link in an explanatory email. For those biobanks which did not have a separate quality manager (e.g., due to limited biobank staff size), the general biobank manager was addressed. The survey questions are available in the **Supplementary Material**.

Second Quality Survey

The second, more detailed Quality survey was designed using REDCap electronic data capture tools hosted at Ghent University Hospital. REDCap (Research Electronic Data Capture) (20) is a secure, web-based application designed to support data capture for research studies, providing: (1) an intuitive interface for validated data entry; (2) audit trails for tracking data

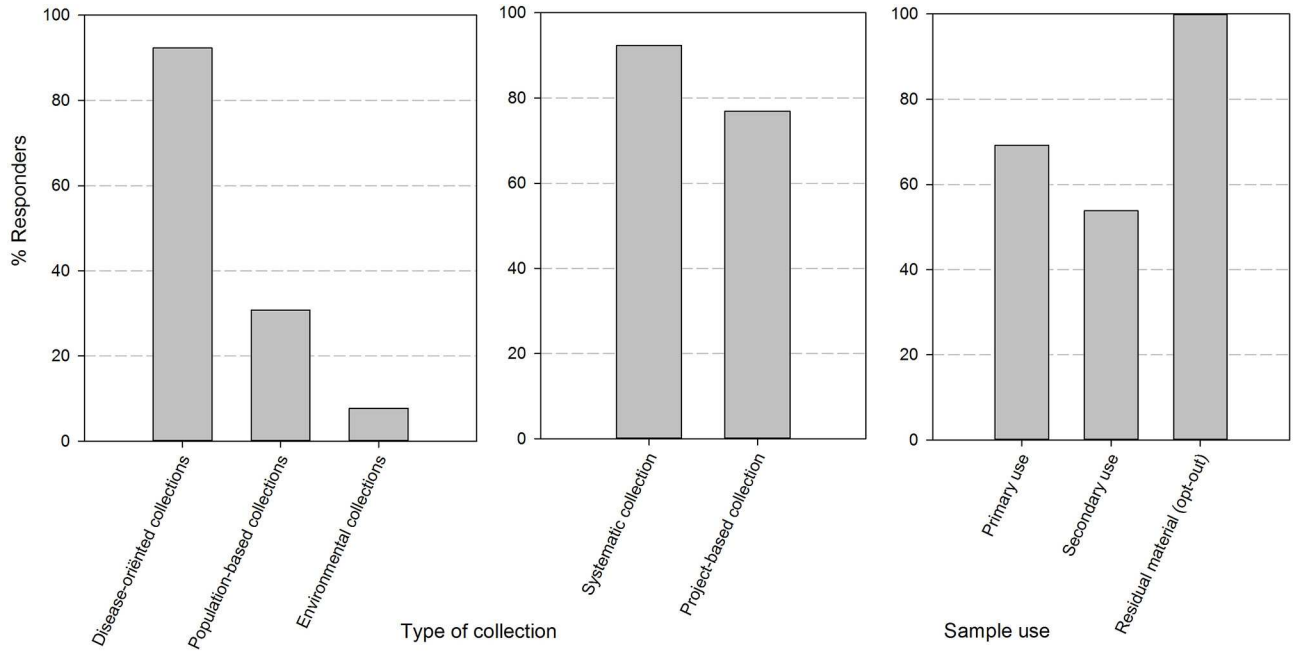

FIGURE 1 | The set-up and composition of human body material collections in biobanks of the BBMRI.be network. Responses were divided by the nature of the human body material collection (collection with a disease orientation, population-based or on environmental ground), by the method of collection of the material (systematic i.e., by sampling at occasions within the clinical path of the patient or project-based i.e., by sampling in the framework of a specific study/project at pre-defined and study-based time points) and by the type of use as defined in the Belgian legislation (primary use: the use to which the donor has explicitly consented to at the time of collection; secondary use: any other use than the one consented to at the time of collection; residuary material: the portion of human bodily material that has been taken from the donor for diagnostic or treatment purposes which, when a sufficient sample is safeguarded for refinement or completion of these purposes, is superfluous to these purposes and as such could be destroyed—presumed consent applies).

manipulation and export procedures; (3) automated export procedures for seamless data downloads to common statistical packages; and (4) procedures for importing data from external sources. The survey was distributed to the 13 (2018) biobanks of the BBMRI.be network, by providing a link in an explanatory email. The survey consisted of a dynamical questionnaire, visualizing additional questions dependent on the responses given to deepen the answers given to the core set of questions. The content focused on three main sections. The first section captured the general information of the biobank and the QMS system. The second part focused on the specific procedures present in the QMS system and the supportive systems used. The third part addressed the needs related to Quality of the BBMRI.be biobank community. The survey questions are available in the **Supplementary Material**.

Survey Analysis

The survey data of both surveys was exported into a spreadsheet and data analysis was performed using the REDCap data analysis tool combined with Sigmaplot for graph design.

RESULTS

Properties of BBMRI.be Biobanks

After sending out the surveys, all quality/biobank managers (respectively, 12/12 in 2017 and 13/13 in 2018) targeted in the mailing submitted a complete set of responses for the two online questionnaires. The majority of biobanks were university hospital integrated (9/13). The responders also included 1 academic biobank, 1 general hospital-based biobank and 2 biobanks of research institutions (type non-profit organization or association without lucrative purpose). The responders are biobanks with multiple types of collections in their catalog, as can be seen in **Figure 1**. These collections are mainly disease-oriented (92%) and originate from both systematic (92%) and project/study based (77%) approaches to sample collection. All of the biobanks collect residuary material cleared by presumed consent, in compliance to current Belgian legislation. In addition, 69% of biobanks collect samples for primary use and 54% of biobanks also distribute samples for secondary use (defined by Belgian law as any use different from that to which was consented by the donor at the time of collection of the specimen).

Of the 13 responders, 8 biobanks are part of a certified/accredited lab environment (60% ISO 15189, 10% ISO 17025). Three biobanks had obtained an ISO 9001 certificate specifically for their biobanking activities. Sixty two percent of the responders receive samples of an accredited lab environment [Pathology lab (ISO 15189), Clinical-Analytic lab (ISO 17025) or Medical Genetics lab (ISO 15189) or JACIE accredited facility].

QMS Status and Sources of Belgian Biobanks

At the time of the first survey in the fall of 2017, 11 out of 12 participating biobanks had implemented a QMS. By the time

of the second survey, about 1 year later, 85% of the biobanks have implemented an operational QMS system. The remaining 15% of responders is currently in the process of implementing a QMS system. Four guidelines stand out as primary basis for the QMS: ISO 9001 (31%), CMI quality guidelines (31%), ISO 15189 (23%), and the BWB quality guidelines (15%) (**Figure 2**). Apart from one biobank, all biobanks use additional guidelines for their QMS (**Table 1**). The most frequently used are the ISBER guidelines for biobanks (69%), the ISO 9001 standard (67%), and the OECD guidelines for biorepositories (54%). Fifty percent of responders applied the Standard Pre-analytical Code, either automatically (33%), or manually (17%), while the other half intended to implement SPREC in the future. Only 1 responder had implemented BRISQ and only 1 responder intended to implement it in the future (data not shown).

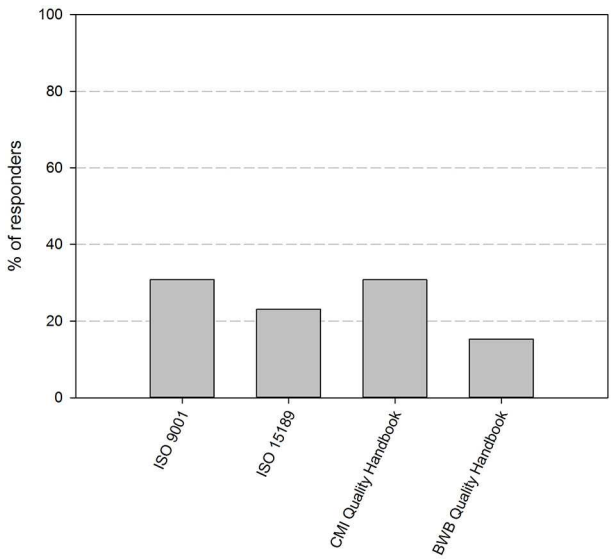

FIGURE 2 | Quality standards or guidelines used as primary basis for the quality management system of the biobanks in the BBMRI.be network. Participants were asked to select the standard or guideline used as primary basis for their QMS from a list of nine standards/guidelines (ISO 9001, ISO 15189, ISO 17025, ISO 20387, CMI QMS Guidelines, BWB QMS Guidelines, ISBER Best Practices, OECD Recommendations for Biorepositories, French Biobanking standard NF S96-900). Only one option could be selected. Only four standards/guidelines were indicated by the responders to be used as primary basis for QMS systems, as displayed in the figure. QMS, quality management system.

At the time of the first survey, a majority (73%) had never taken part in freely available online self-assessment surveys (SAS) (9% had taken the ISBER SAS, 18% the BBMRI-ERIC general QMS SAS or the BBMRI-ERIC SAS to check compliance to the CEN technical standards for pre-examination processing of biospecimens). One year on, 62% of responders are using one or more self-assessment tools. The BBMRI-ERIC general QMS SAS (63%) and the ISBER SAS (50%) are the most commonly applied tools in the BBMRI.be network (**Figure 3**).

Fall 2018, 54% of the responders was participating in yearly external proficiency testing for their testing or processing methods. Of these responders, 71% is using biobank specific proficiency testing programs (such as the IBBL proficiency testing program), which is an increase compared to the number reported the year before (46%). Biobanks embedded in accredited laboratories can participate in laboratory related proficiency schemes and 43% of the biobanks make use of this possibility (data not shown). Several reasons were given for not participating in the biobank specific proficiency testing schemes: (i) it is not requested by the providers or customers (4/6), (ii) the high cost of participation (2/6), (iii) the lack of added value for the biobank (1/6), and (iv) the adequacy of available biobank testing schemes (1/6).

TABLE 1 | Primary and secondary QMS standards and guidelines used in Belgian biobanks.

Responder	ISO 9001	ISO 15189	ISO 17025	ISO 20387	CMI QMS guidelines	BWB QMS guidelines	ISBER	OECD	NF S96-900
1	Prim	Sec	–	Sec	–	Sec	Sec	Sec	–
2	Prim	Sec	Sec	Sec	Sec	–	Sec	Sec	Sec
3	Prim	–	–	–	–	–	–	–	–
4	Prim	–	–	–	–	Sec	–	–	–
5	Sec	Prim	Sec	–	Sec	–	Sec	Sec	–
6	Sec	Prim	–	Sec	–	Sec	Sec	Sec	Sec
7	–	Prim	–	–	–	Sec	Sec	Sec	Sec
8	Sec	–	–	–	Prim	–	–	–	–
9	Sec	–	–	Sec	Prim	–	Sec	–	–
10	Sec	Sec	–	Sec	Prim	–	Sec	Sec	Sec
11	–	Sec	Sec	–	Prim	–	Sec	Sec	–
12	–	–	–	–	–	Prim	Sec	–	–
13	Sec	–	–	–	-	Prim	–	–	–
Total # of QMS standard use	10	7	3	5	6	6	9	7	4
# of secondary QMS standard use	6	4	3	5	2	4	9	7	4

Prim, standard/guideline used as primary basis for QMS; Sec, standard/guideline used as supplementary basis for QMS; QMS, Quality Management System.

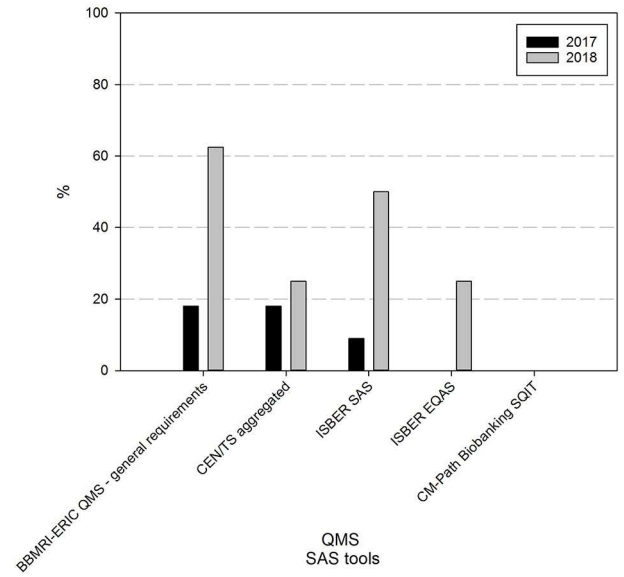

FIGURE 3 | Use of self-assessment tools in biobanks in the BBMRI.be network in 2017 and 2018. Participants were asked to indicate from a list which self-assessment survey (SAS) tools were used in their biobank to assess the status of the overall quality management system (BBMRI-ERIC QMS—general requirements and ISBER SAS), the compliance to standard specifications for pre-examination processes (CEN/TS aggregated: 10 individual SAS tools developed by BBMRI-ERIC, participation in one or more of these SAS tools was considered as 1 positive reply), the overall quality status of the pre-analytical processes (ISBER EQAS) or awareness of factors in tissue collection, processing and storage that may impact sample quality (CM Path Biobanking SQIT). Black bars indicate participants' responses in 2017, gray bars indicate participants' responses in 2018. CEN/TS, European Committee for Standardization Technical Standards; EQAS, pre-analytical external quality assessment survey; SQIT, CM-Path Biobanking Sample Quality Improvement Tool.

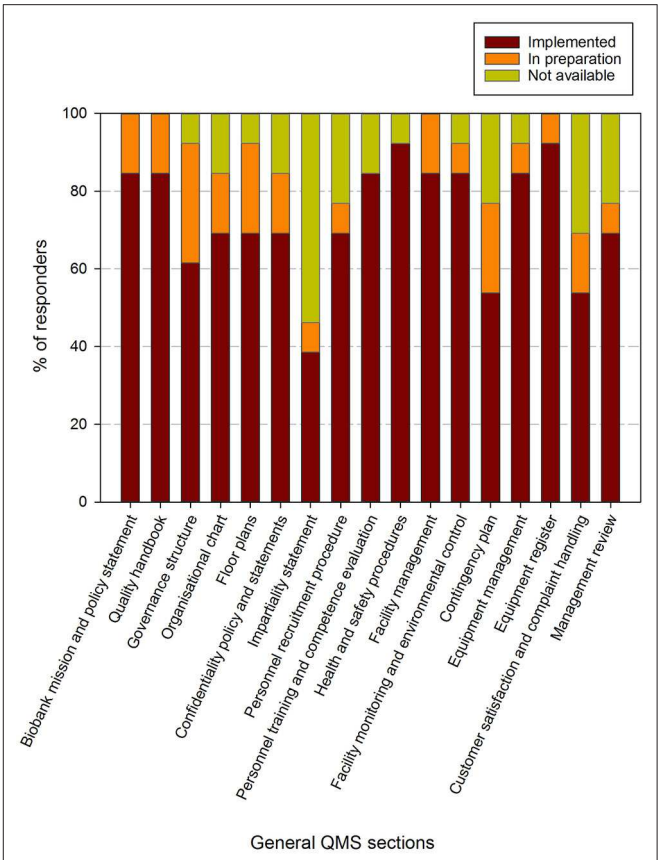

FIGURE 4 | Implementation status of general procedures in the quality management system in biobanks in the BBMRI.be network. Participants were requested to indicate for each item in a list of general procedures/items whether these were fully implemented (brown), in preparation (orange) or not available (lime-green). The items listed were collected and aggregated from the ISBER best practices for biorepositories, the ISO 20387 and ISO 9001 standards. All participants responded to all items listed. The list of items asked is displayed in the X-axis.

Status of QMS and Biobank Requirements in BBMRI.be Biobanks

In the questionnaire, an aggregated list of required procedures for ISO 9001:2015, ISO 20387:2018, and the ISBER best practices (fourth edition) was presented to the participants. An overview of the responses regarding the general and biobank specific procedures and requirements is shown in **Figures 4**, **5**, respectively.

Eight out of 17 general QMS requirements are fulfilled by more than 80% of responders and an additional 6 have been implemented by 60–80% of responders. Two items have been implemented by 40–60% of responders (i.e., contingency plan and customer satisfaction & compliant handling) and 1 item was available in <40% of responding biobanks (i.e., impartiality statement). When also taking the responders into account where the procedures were in preparation, 12 out of 17 requirements were being addressed by over 80% of responders. Again the impartiality statement was the least addressed, whereas the contingency plan, customer satisfaction and complaint handling, personnel recruitment and management review were the procedures that were proportionally less present (**Figure 4**).

Regarding the biobank specific requirements, 8 out of 16 items had been implemented by >80% of responders at the time of the survey, 5 by 60–80%, 1 by 40–60% (contracts for collaborators) and 2 by 20–40% (quality control of methods/processes and quality control of data). The procedure for storage of human bodily material was present in all biobanks. When taking into account the responders that are in implementation phase for the procedures, 16 out of 17 items were being addressed in more than 80% of biobank QMS. The remaining item, quality deviations or corrective/preventive actions, was reported in 60–80% of responders (**Figure 5**). One biobank indicated that quality control of human bodily material or quality control of methods/processes was not applicable for their activities.

Supportive systems used to handle these processes contained in these procedures are present in at least 60% of responders, although only document management and audit follow up systems are used at over 80% of biobanks (**Figure 6**). Risk management systems and provider/customer management

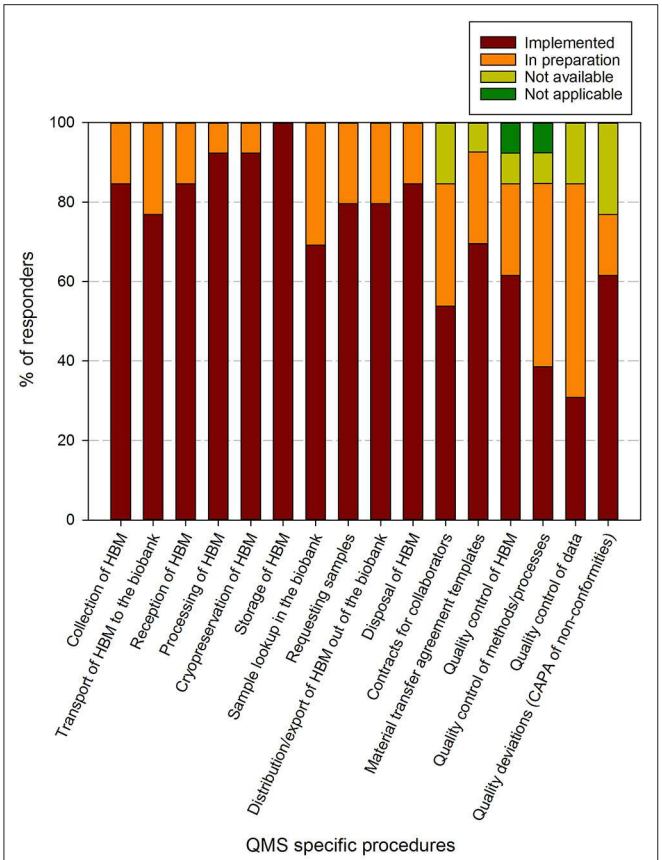

FIGURE 5 | Implementation status of biobank specific procedures in biobanks in the BBMRI.be network. Participants were requested to indicate for each item in a list of specific procedures/items whether these were fully implemented (brown), in preparation (orange), not available (lime-green), or not applicable (dark green). The items listed were collected and aggregated from the ISBER best practices for biorepositories, the ISO 20387 and ISO 9001 standards. All participants responded to all items listed. The list of items asked is displayed in the X-axis.

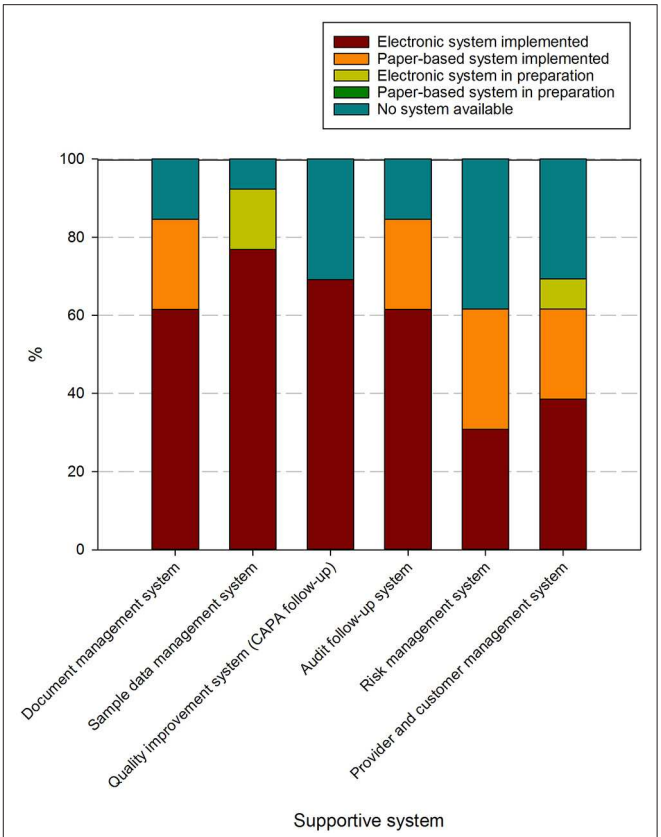

FIGURE 6 | Overview of systems used to support activities in biobanks in the BBMRI.be network. Participants were asked to indicate in a list for which supportive tools for the QMS an electronic system was implemented (brown), a paper-based system was implemented (orange), an electronic system was in preparation (lime-green), a paper-based system was in preparation (dark green), or no system was available (petrol blue). All participants responded to all items listed. The list of items asked is displayed in the X-axis.

systems are the least available. Predominantly electronic/digital systems are utilized, although paper-based systems still occur for document management, audit follow-up, risk management and provider/customer management.

Future Goals and Needs of the Belgian Biobanks

Finally, the intentions and perspectives of Belgian biobanks were assessed. Sixty nine percent of the responders indicate to strive for biobank certification and/or accreditation within 2 years, as can be seen in **Figure 7**. Although the percentage of biobanks intending to acquire certification is similar to the one indicated the year before (75%), the intended certification has shifted. In 2017, 42% of biobanks aimed for both ISO 9001 and ISO 20387 certification, 25% for ISO 20387 and 8% for ISO 9001 certification, while in 2018 this has focused to nearly 38% intending ISO 20387 certification, 23% ISO 9001, and only 8% of responders still intending to acquire both ISO 9001 and ISO 20387 certification. About 31% of responders did not intend to acquire certification.

The majority of responders (77%) indicated a clear need for a Belgian SAS tool, adapted to the national biobank law which has come into effect on November 1st 2018. The need for a national proficiency program, on top of already existing schemes, was put forward by 54% of the responders (data not shown).

An overwhelming majority (12/13) of the responders expressed a need for the setup of a national peer-review audit program. Several types of peer-review audit programs were suggested: 75% of the responders would prefer a two-phase audit, consisting of an initial administrative/documentary audit (off-site) of the biobank procedures followed by a site visit 1 year later. Twenty five percent of the responders would prefer a full on-site audit straightaway.

DISCUSSION

Irreproducibility of results, also originating from the biospecimens used, has been identified as a major undermining

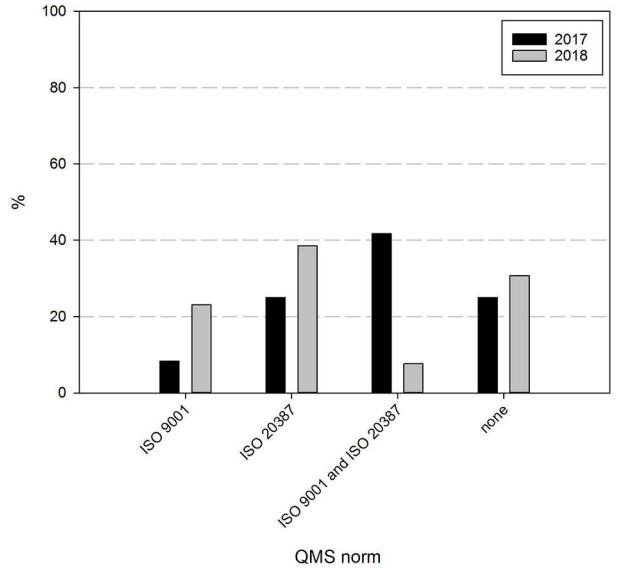

FIGURE 7 | Certification intentions biobanks in the BBMRI.be network. Participants were asked to indicate which if any certification/accreditation they intended to acquire within 2 years. Black bars show participants' responses in 2017, gray bars show participants' responses in 2018. All participants responded to all items listed.

factor for translating research results into clinical applications. Biobanks can play an important role by providing fit-for-purpose (human) bodily material, governed through a professional QMS according to evidence-based guidelines and/or standards. Since 2013, the BBMRI.be network brings together the large academic/non-profit research biobanks in Belgium and strives through working groups for harmonization on multiple levels. The BBMRI.be Quality working group setting new goals in alignment with BBMRI-ERIC to further professionalize qualitative biobanking in Belgium. To this end, the Quality working group performed two surveys over the course of a year to gauge the current quality status of the connected biobanks, to define the areas of improvement in the biobanks themselves and in the support delivered by the Quality working group.

The surveys were targeted to the Quality managers of the biobanks or in absence of a dedicated person due to biobank size, to the overall operational manager of the biobank. One completed survey response was requested and received per biobank. The biobanks show a big diversity in nature and type of their collections. In the Belgian biobank legislation, residual material can be obtained and used for research via an opt out system (21), and a third of the BBMRI.be biobanks consist entirely of residuary material collections. The use of residuary material is not the default situation within the global biobanking community, but it can show an impressive track record of valuable results, provided certain quality and ethical conditions are met (22). The results of this survey mainly reflect the activities of academic research biobanks, which are currently the main members of BBMRI.be. However, BBMRI.be intends to represent the complete Belgian biobank landscape by also reaching out to other institutional and/or commercial/private biobanks in the near future, in an effort to address commonly encountered biobank related issues. Uniting the Belgian biobanks through BBMRI.be may also further improve and harmonize biospecimen quality and consequentially contribute to an increased reproducibility of translational research.

The survey results show that the BBMRI.be related biobanks have a highly developed "quality" mindset, as the grand majority of the biobanks have implemented a formal QMS and the remaining biobanks are in progress of implementing a system. The primary basis of the implemented QMS system is different between the responders. This divergence has both historical and organizational reasons. The FBN and the BWB were already active and had published their quality handbooks before the BBMRI.be community was set up. Additionally, biobanks that are integrated in a ISO 15189 or ISO 17025 accredited laboratory prefer to take this standard as primary basis of their QMS system. Even so, all of the aforementioned guidelines/standards used as a primary basis have a direct reference to ISO 9001, the commonly accepted standard for general quality management systems, indicating that the Belgian biobank QMS's contain a similar general basis for their activities and procedures.

The majority of biobanks take additional norms/guidelines into account to develop their QMS. The variety of widely available biobank guidelines is mirrored in the responses of the participants, with the ISBER guidelines, the OECD guidelines and ISO 9001 being the most popular. This diversity is likely caused by the initial absence of a relevant international norm covering all of the activities of a biobank (23), a gap recently filled by the biobank centered ISO 20387 standard (24). Despite its recent publication, it is already being picked up by the biobanks in the BBMRI.be network. However, the ISO 20387 standard was developed to be complementary to and to be used with the existing biobank guidelines from the start to strengthen a biobank's pursuits for quality management (25). This allows biobanks to tailor their own needs but also leaves room for deviation based on the guidelines used.

The majority of procedures for general and biobanking specific activities, aggregated from the ISBER Best Practices and the ISO 9001 and ISO 20387 standards, were present in the BBMRI.be connected biobanks. The items with the lowest compliance rates were either requirements from the recently published biobanking ISO standard (e.g., impartiality statement) or items that had received more focus due to the publication of the standard (e.g., quality control of biospecimens and/or methods/processes). The latter however are actively being implemented by the non-compliant biobanks, indicating that these have gained importance in the biobank activities. In comparison to the results of the ISBER self-assessment participants, the BBMRI.be biobanks score better at several of the commonly asked items, both general and biobank specific, again emphasizing the quality-mindset within the participants (26).

We acknowledge that the results reported in this study are self-reported and may therefore overestimate the actual status of the responders' QMS. However, the lagging of the business

aspects of biobanking is in line with data from a recently performed international study on biobank business operations (27). Although this part of biobanking is an important factor for success, it is a known gap in the community and the subject has recently gained more visibility to professionalize this side of biobank operations (28). Given the raised awareness regarding this aspect in literature and biobank standards, we expect to see progress in this area in future surveys.

Self-assessment tests and participation in proficiency testing schemes are recognized ways to monitor the QMS controlled activities and define areas for improvement. Initially these tools were not very well-known within the biobanks of BBMRI.be, but their participation rate has greatly increased over the 14 month period covered by the surveys. One factor explaining this success might be the indirect education through the surveys and the presented first survey results.

Although most of the BBMRI.be biobanks indicate to use the ISBER Best Practices as inspiration for their QMS, only half of them have already taken the ISBER self-assessment survey. Still, this is an increased proportion compared to the 62 global biobanks that completed the full survey in the period 2015–2017 (26). The biobanks might consider the self-assessment as a premature activity, since some of the essential biobank processes might still be in the implementation phase. Additionally, diagnostic self-assessment surveys have lost a bit of their appeal, with external audits gaining a more apparent value. This is also reflected by the fact that nearly all participants were in favor of setting up a national peer-review audit program. Similar audit initiatives have been and are being set-up in different national biobank networks (29, 30), emphasizing the need felt by biobanks to comply with the audit requirements stated by guidelines and standards. Although these within-network audits have the advantage of allowing to assess local legislative and regulatory requirements, it may also introduce quality differences between these networks. The European research infrastructure BBMRI-ERIC therefore intends to setup a peer-review audit program framework to be used by the member states, leaving room for local peculiarities while maintaining an independent comparative evaluation.

Peer-review audits can also serve as preparation for intended certification or accreditation activities. With about three quarter of participants intending to acquire certification for ISO 9001, ISO 20387, or both within 2 years, the implementation of a peer-review audit program might support the BBMRI.be biobanks in achieving this goal. This intention is in line with the ongoing evolution to "biobanking 3.0," with an increased focus on operational standardization of processes (31). A key element initially unavailable to biobanks in this respect has been quality assessment by an external organization (32). Two currently available international programs show a high overall success rate (33, 34). Furthermore, the new standard ISO 20387 will allow biobanks to pursue accreditation or certification for their activities, formalizing their competence (25). The recent publication of this standard is also likely to explain our observed shift of the combined ISO 9001 & ISO 20387 intention toward the majority opting for ISO 20387 in the second survey.

The participants expressed a clear need for a national peer review program and a self-assessment survey fit to the Belgian legislation. These requests can be addressed by the BBMRI.be Quality working group, by developing add-ons to existing or starting international initiatives in order to harmonize to the global community. Given the resources available, the initial focus will be put on the implementation of the audit program, building on the FBN peer review audit. About half of the responders indicated a need for a national proficiency testing scheme. It has been shown that repeated participation in biobank proficiency schemes can indeed lead to global improvement of performance (35, 36). However, about half of the responders are currently not participating in already available proficiency schemes. It is therefore opted by Quality working group to educate the biobanks regarding the existence and advantage of proficiency testing programs as a more valuable first step.

Overall, the biobanks of the BBMRI.be network have actively implemented a quality approach in their daily practices, though room for improvement exists. The implementation of ISO20387 may bring further professionalization of activities. Based on the current needs expressed in this survey, the Quality working group will be setting up a novel audit program for the BBMRI.be biobanks, to enhance, harmonize and streamline activities. Additionally, raising further awareness about self-assessment tools that are freely available, proficiency testing schemes and the value of performing these tests will be on the agenda in the coming months and years. On the whole, the biobanks in the BBMRI.be network are able to contribute to better translational research through a sustained quality approach.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the **Supplementary Files**.

AUTHOR CONTRIBUTIONS

LL, VT, CV, KV, EM, SB, and NE conceived of the presented idea. LL and VT designed the surveys, analysed the results and wrote the article. All authors critically reviewed the manuscript.

ACKNOWLEDGMENTS

The authors wish to thank all quality and biobank managers that have participated in the two surveys.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2019.00141/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

METHODS
published: 20 August 2019
doi: 10.3389/fmed.2019.00183

Biobanking for Viral Hepatitis Research

Erwin Ho[1,2†], Stijn Van Hees[1,2†], Sofie Goethals[3], Elke Smits[3], Manon Huizing[3], Sven Francque[1,2], Benedicte De Winter[2], Peter Michielsen[1,2] and Thomas Vanwolleghem[1,2,4]*

[1] Department of Gastroenterology and Hepatology, Antwerp University Hospital, Antwerp, Belgium, [2] Laboratory of Paediatrics and Experimental Medicine, University of Antwerp, Antwerp, Belgium, [3] Biobank Antwerp, Antwerp University Hospital, Antwerp, Belgium, [4] Department of Gastroenterology and Hepatology, Erasmus Medical Center, Rotterdam, Netherlands

Introduction: Viral hepatitis is a worldwide, important health issue. The optimal management of viral hepatitis infections faces numerous challenges. In this paper, we describe how biobanking of biological samples derived from viral hepatitis patients collected both in-hospital and during community outreach screenings provides a unique collection of samples.

Materials and Methods: All samples and materials were provided with a study code within the SLIMS system Study protocols and an informed consent form were approved by the Antwerp University Hospital/University of Antwerp Ethical Committee. Systematic biobanking was initiated in October 2014. Collected sample types include: (1) serum and plasma of all newly diagnosed HBV, HCV, HDV, and HEV positive patients; (2) left-over serum and plasma samples from all PCR analyses for HBV and HCV performed in the context of routine clinical care; (3) left-over liver tissue not needed for routine histological diagnosis after liver biopsy; and (4) additional virus-specific, appropriate sample types using a scientific rationale-based approach. A community outreach screening program was performed in three major Belgian cities. Serum, EDTA, Tempus Blood RNA and BD Vacutainer CPT were collected. CPT tubes were centrifuged on-site and mononuclear cells collected within 24 h.

Results: Concerning community screening: 298 individuals supplied all 4 sample types. Samples were stored at −150°C and were logged in the biobank SLIMS database. Samples were used for HBV-related immunological and biomarker studies. DNA isolated from plasma samples derived from chronic HBV patients was used to investigate Single Nucleotide Polymorphism rs 1790008. Serum samples collected from chronic hepatitis C patients were used to assess the efficacy of HCV treatment. Peripheral Blood Mononuclear Cells (PBMC) isolated from chronic HBV patients and healthy controls were used for different immunological study purposes. Virus isolated from biobanked stool of a chronic hepatitis E patient was used to establish a mouse model for Hepatitis E infections, allowing further HEV virology studies.

Conclusion: The establishment of a biobank with samples collected both in-hospital and during community-outreach screening resulted in a unique, continuously expanding collection of biological samples which provides an excellent platform for prompt answers to clinically and translational relevant research questions.

Keywords: viral hepatitis, SLiMs, biobank, screening, immunology, B cells

OPEN ACCESS

Edited by:
Laurent Georges Dollé, Biothèque Wallonie Bruxelles, Belgium

Reviewed by:
Helmuth Haslacher, Department of Laboratory Medicine, Medical University of Vienna, Austria
Carla Osiowy, National Microbiology Laboratory, Canada

****Correspondence:***
Erwin Ho
erwin.husin@gmail.com

†These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Translational Medicine, a section of the journal Frontiers in Medicine

Received: *13 May 2019*
Accepted: *29 July 2019*
Published: *20 August 2019*

Citation:
Ho E, Van Hees S, Goethals S, Smits E, Huizing M, Francque S, De Winter B, Michielsen P and Vanwolleghem T (2019) Biobanking for Viral Hepatitis Research. Front. Med. 6:183. doi: 10.3389/fmed.2019.00183

INTRODUCTION

Viral hepatitis is a worldwide, important health issue, mostly caused by five different primary hepatotropic viruses: The Hepatitis A Virus (HAV), Hepatitis B Virus (HBV), Hepatitis C virus (HCV), Hepatitis Delta Virus (HDV), and Hepatitis E Virus (HEV). Infected patients are at increased risk of developing liver-related events, including liver failure, liver cirrhosis and hepatocellular carcinoma, ultimately culminating in liver-related death (1–3). As such, viral hepatitis accounts for an estimated 1.45 million deaths annually, 90% of which are attributed to chronic HBV and HCV infections (3). Importantly, this number is on the rise, ranking viral hepatitis among the most important causes of death worldwide (3).

HBV and HCV replicate non-cytopathically in human hepatocytes. As such, liver damage caused by both viruses incurs primarily through host immune responses (4–6). Chronic infection develops in 10% of adult HBV and approximately 80% of HCV infected patients (4–6). The immunopathogenesis of both infections is, however, not fully understood (1, 4–6). As of now, HCV is curable, but HBV is not. The Hepatitis B Virus forms a stable genomic "reservoir" within the nucleus of infected hepatocytes. It integrates part of its genetic code in the host genome and forms covalently closed circular DNA (cccDNA), which acts as a mini-chromosome. Current standard of care treatment suppresses viral replication, but fails to clear the genomic "reservoir" (1). Additionally, HDV infections are seen in up to 5% of chronic HBV patients, leading to more aggressive liver disease, not seldom presenting with liver complications before the fourth decade of life (7).

HEV infections are mostly self-limiting in immunocompetent hosts, but chronic infections may develop in immunosuppressed or HIV coinfected hosts. Little is known on its pathogenesis (8). Management of chronic infections involves lowering the dosage of immunosuppressants with addition of ribavirin treatment if needed, which results in viral clearance in up to 80% of the infected patients (8).

Clearly, the optimal management of viral hepatitis infections faces numerous challenges. In this paper, we describe how biobanking of biological samples derived from viral hepatitis patients and healthy controls collected both in-hospital and during community outreach screenings provides a unique collection of samples that can be used to investigate unanswered questions on the pathogenesis of viral hepatitis, and to optimize management thereof. We report the quality metrics, organization, output variables, the unique logistics, planning and execution associated with biobanking for viral hepatitis research. Subsequently, we show an overview of how biobanking at the Antwerp University Hospital has resulted in novel insights relating to viral hepatitis infections over the last 5 years.

METHODS

General Considerations

Funding from the CMI program (Center for Medical Innovation) from the Flemish Government and existing biobanking infrastructure for oncology (Tumorbank@UZA part of the Belgian Virtual Tumorbank funded by the National Cancer Plan) allowed for the establishment of storage of biological samples for hepatotropic diseases, including samples collected for the study of viral hepatitis. As such, the established biobank is a disease-specific, hospital-integrated and community-based biobank.

The biological samples are managed by trained biobank personnel to ensure samples are handed, registered and stored according to an established biobank quality management system (QMS).

Important aspects of this QMS concerning sample maintenance include:

- Processing of samples by dedicated biobank personnel via Standard Operating Procedures (SOPs)
- Proper identification and traceability of samples via 2D barcode labeling of samples encoded in a sample management database (SLIMS, Genohm SA, Lausanne, Switzerland)
- Registration of important pre-analytical date/time stamps such as collection, reception, centrifugation, fractionation and storage in SLIMS
- Use of SPREC coding (9)
- Inclusion and exclusion criteria for uptake of samples in the biobank via fixed decision trees
- Regular checks of the defined critical dataset in SLIMS

The protocol was carried out in accordance with the recommendations of Good Clinical Practice, approved by the Antwerp University Hospital/University of Antwerp Ethical Committee (EC 15/21/227), with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The informed consent allows sampling and storage for blood, urine, fecal and liver materials from hepatology outpatient and inpatients clinics.

The established biobank comprises three different categories of samples: (1) prospectively collected samples during in-hospital based biobanking; (2) samples collected during outreach community screenings; (3) left-over serum samples from routine PCR analyses in the clinical laboratory and left-over liver tissue samples not needed for routine histological diagnosis at the department of pathology. Informed consent was obtained from all patients from whom samples of the first two categories were collected. For category 1, informed consent was obtained during outpatient or inpatient hospital care by attending medical staff. Presumed consent was applicable to samples of category 3. Presumed consent is based on Belgian law where it is stated that the use of leftover human materials is allowed for diagnostic and research purposes (19-12-2008, "Law pertaining the acquisition and use of human materials for medical use in humans or in scientific research"). These statues and reference to the applicable law are written in the patient admission information flyer.

Samples of category 1 and 2 are reserved for primary use within a predefined timeframe by the investigators mentioned in the ethical committee approval for biobanking. Upon termination of the timeframe for primary use, these samples can

be accessed by all researchers, including external researchers, upon approval of both the Ethical Committee and Biobank council. The concept of primary use does not apply to samples of Category three. These samples can be accessed immediately by all researchers upon ethical committee and biobank council approval.

During outreach community screenings, individuals were provided with Simplified Chinese and Traditional Chinese informed consent forms and information brochures, and additional translation support was provided on-site. These forms were approved by the Ethical Committee as part of the biobanking protocols. Prior to these community sessions, Q&A sessions were held to communicate the objectives of the study, the purpose of biobanking and the conditions of confidentiality/traceability (coding) of results and samples.

Setting Up an In-Hospital Biobank for Non-tumor Samples

Preparations for in-hospital biobanking consisted of the integration of an extra option in the blood analysis request forms, the creation of a workflow for the acquisition of informed consents as well as regular exchanges between all parties involved to discuss the optimal sample flow. Systematic biobanking was subsequently initiated in October 2014. Collected sample types include: (1) serum and plasma of all newly diagnosed HBV, HCV, HDV, and HEV positive patients; (2) left-over serum and plasma from all PCR analyses for HBV and HCV performed in the context of routine clinical care; (3) left-over liver tissue not needed for routine histological diagnosis after liver biopsy and (4) additional virus-specific, appropriate biological sample types using a scientific rationale-based approach. An overview of the sample flow and collected sample types per virus is depicted in **Figure 1**.

Biobanking of serum and plasma samples of newly diagnosed patients is requested by the physician through the electronic blood analysis request forms. Blood is then sampled by the nursing staff and sent to the central biobank for centrifugation, aliquotation and storage through an in-house pneumatic tube system. Collaboration with the clinical laboratory allowed for the collection of left-over serum and plasma samples of all HBV and HCV PCR analyses performed for routine clinical care purposes. Samples are temporarily stored at −20°C at the clinical laboratory and then transferred in batch to the central biobank.

Hepatitis viruses all infect human hepatocytes. Unraveling what happens at the site of infection, namely the liver, is of utmost importance to understand the complex interplay between virus and host. Left-over tissue not needed for routine clinical histological evaluation provides a highly valuable resource of samples in this regard. We therefore set up a routine flow to collect and store left-over material in a standardized way.

A thorough understanding of the immune responses against hepatitis viruses requires a close collaboration between clinicians, laboratory personnel and biobank. We optimized a workflow for both intrahepatic as peripheral lymphocyte flowcytometric analyses. In select cases a part of the left-over liver tissue not needed for routine histological diagnosis is put in cell culture medium. The latter is then transferred to the University Laboratory for isolation of intrahepatic lymphocytes using a Fluorescence Activation Cell Sorting (FACS) based approach. Isolated intrahepatic lymphocytes are stored at −150°C in the central biobank. In addition, peripheral blood mononuclear cells (PBMC) are collected. Sampling of heparinized blood is requested by scientific staff through the electronic blood analysis forms. Blood is taken by nursing staff and transferred to the University Laboratory for isolation of PBMC by Ficoll-Hypaque density centrifugation. Isolated samples are temporarily stored at −80°C at the Antwerp University laboratory before transfer to −150°C freezers at the central biobank. Temperature monitoring is part of a hospital-wide system.

When compared to HBV and HCV, a unique aspect of HEV infection is the fecal-oral transmission route, especially for genotype 1 infections. The virus is readily detectable in human stool and monitoring of viremia in stool has proven to be an important tool in the management of chronic HEV infections (8). To further characterize different aspects of Hepatitis E infections, collection and biobanking of a wide range of body fluids, including saliva, stool and urine in addition to blood samples, was initiated. Left-over samples of stool not needed for routine clinical monitoring of HEV viremia, are stored at −80°C. In addition, also PBMC, urine and saliva are collected through nursing staff.

Biobanking During Outreach Screening Projects

Asians have a higher seroprevalence of HBV infection, and presumably of HCV infection as well (10–12). This population is known to be difficult to reach, and epidemiological data in the Belgian-Asian/Chinese migrant population is lacking. While many screening studies have been performed in diaspora settings, biobanking has not. Biobanking during outreach community screening would constitute a unique "control" group: apart from disease-specific information in Hepatitis B surface Antigen (HBsAg) positive Asians, HBsAg negative persons in the same target population would provide an excellent control group with similar socio-demographic characteristics. These individuals have a high chance to be exposed to HBV but would not have been afflicted with HBV. This control group is typically lacking in previous biobanking efforts, where they are recruited from hospital or research environments, but not from the same community with high HBV prevalence.

Preparations for the community screenings and biobanking were executed simultaneously. This required a coordinated effort from screening staff (administrative, paramedic and medical), research laboratory staff, the hospital laboratory and the biobank itself. Additionally, community leaders and volunteers were crucial in providing preparatory, logistical and linguistic support during screening and biobanking. **Figure 2** provides an overview of the activities performed by these different entities. Central SLIMS labeling, as previously mentioned, was provided. To facilitate the logistics of biobanking, standardized sachets with

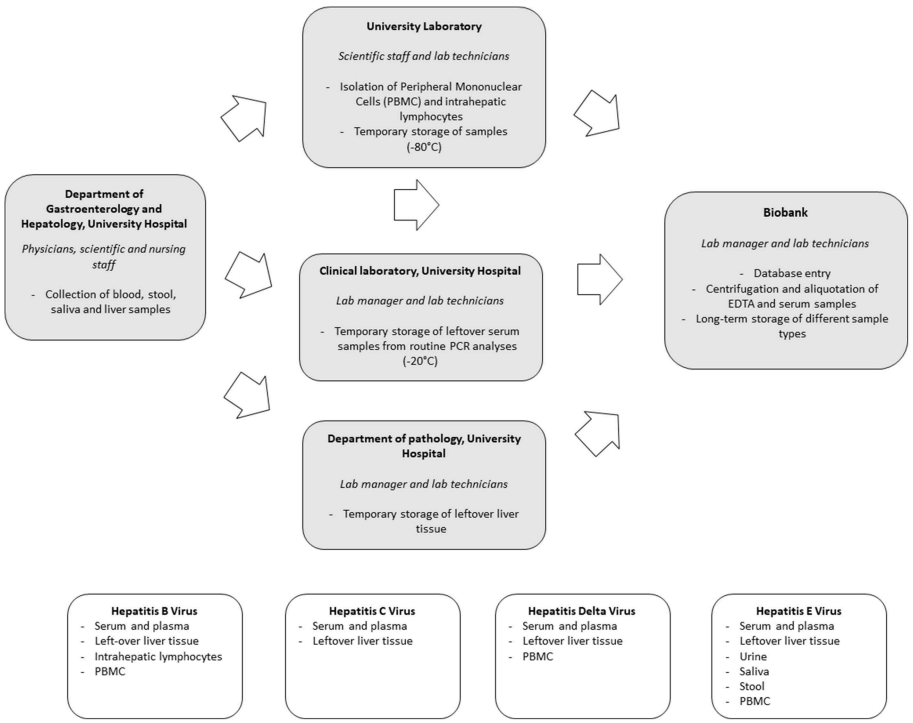

FIGURE 1 | Sample flow and types of samples collected during hospital-based biobanking.

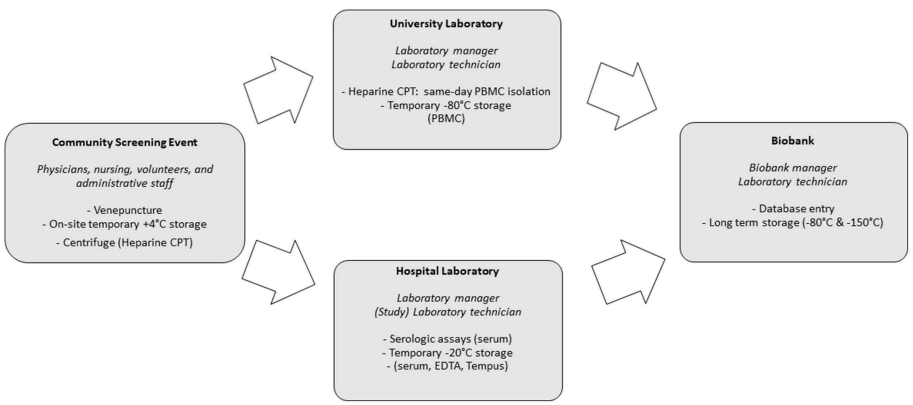

FIGURE 2 | Sample flow and roles in community screening and biobanking.

all required materials for screening and biobanking (including informed consent and request forms) were provided and labeled using the code registered in SLIMS.

Screenings were organized in three major Belgian cities: Antwerp, Brussels and Leuven between 9 a.m. and 5 p.m.

Serum, EDTA, Tempus Blood RNA (Applied Biosystems Tempus Blood RNA tube, 3 mL) and, for screenings organized in Antwerp, BD Vacutainer CPT (Mononuclear Cell Preparation Tubes, 4 mL) were collected during screening events. CPT tubes were centrifuged on-site for 15 min at 1,500 relative centrifugal force. CPT tubes were transported two or three times each session (depending on amount of samples and time) to allow for the University laboratory to perform the procedure to extract peripheral blood mononuclear cells (PBMCs) in a timely manner and prevent loss of cells by cell death. Serum, EDTA and Tempus were temporarily stored on-site at $+4^{\circ}$C (cooling elements) and after the end of each session, in the clinical laboratory, at -20°C. Within 2 days, serum, EDTA, Tempus and PBMCs were subsequently stored in the biobank at -150°C. Additionally, plasma left-overs from CPT tubes were also stored.

Venepuncture testing for HBsAg, anti-HBc and anti-HCV was performed at the Antwerp University Hospital

TABLE 1a | Hospital-based sampling (informed consent).

	2015		2016		2017		2018	
Sample type	**Stored**	**Retrieved**	**Stored**	**Retrieved**	**Stored**	**Retrieved**	**Stored**	**Retrieved**
Serum	110	0	875	23	1,552	8	1,280	1
EDTA plasma	53	0	561	1	900	0	542	0
EDTA buffy coat non-viable	18	0	216	0	370	0	221	0
EDTA red blood cell	16	0	220	0	365	0	0	0
PBMC	0	0	62	0	139	13	80	12
Stool	0	0	2	1	5	0	7	0
Saliva	0	0	0	0	0	0	40	0
Urine	0	0	0	0	0	0	2	0
Intrahepatic lymphocytes	0	0	0	0	0	0	42	0
Total	197	0	1,936	25	3,331	21	2,214	13
Patients: 616								

EDTA, Ethylenediaminetetraacetic acid tube; PBMC, peripheral blood mononuclear cells.

TABLE 1b | Hospital-based sampling (presumed consent/leftover samples).

	pre-2014		2014		2015		2016		2017		2018	
Sample type	**Stored**	**Retrieved**	**Stored**	**Retrieved**	**Stored**	**Retrieved**	**Stored**	**Retrieved**	**Stored**	**Retrieved**	**Stored**	**Retrieved**
Serum	151	0	287	0	785	0	105	6	183	0	480	0
Liver tissue	576	0	41	0	37	0	35	0	32	0	29	0
Total	727	0	328	0	822	0	140	6	215	0	509	0
Patients: 1,500												

laboratory (Elecsys HBsAg II, anti-HBc, anti-HCV, Roche Diagnostics GmbH, Mannheim, Germany). Additional funding for community screening and biobanking was obtained from grants (Roche Diagnostics, Gilead Life Sciences Inc., Bristol-Myers Squibb, Sandoz).

RESULTS

Number of Collected Samples

The number of samples stored in and retrieved from the biobank are shown in **Tables 1**, **2**: samples obtained using informed consent or using presumed consent (hospital based) and community screening are shown. Biobanking from hospital sources amounted to a total of 10,419 samples (2,116 patients), community sourced samples to a total of 4,136 (462 persons). Retrievals were used for study purposes, in accordance to subsequent, Ethics Committee approved protocols. Forty-seven non-conformities were logged from 2015 to 2018 (2, 7, 21 and 17 in 2015, 2016, 2017, and 2018, respectively). These storage failures were due to erroneous sample withdrawal, pre-storage Turn-Around Time (TAT) violation, insufficient data collection/wrong identification, insufficient sample volume or the incorrect use of sample recipients.

Cost Analysis

CMI structural funding for hepatotropic disease biobanking amounted to € 98,560. Personnel, operational, storage, database, QC and administrative costs were covered using these funds. Community sampling involved additional costs—these are shown in **Table 3**.

TABLE 2 | Community-based sampling.

	Stored	Retrieved					
Sample type	**Total**	**Total**	**At sampling**	**2015**	**2016**	**2017**	**2018**
Serum, prime	421	421	0	0	0	0	0
Serum, aliquots	1,654	46	0	0	8	38	0
EDTA	159	0	0	0	0	0	0
Tempus	458	8	0	0	8	0	0
PBMC	217	19	0	0	8	11	0
CPT (plasma leftover)	299	238	0	238	0	0	0
Saliva	467	426	426	0	0	0	0
Dried blood spots	461	420	420	0	0	0	0
Total	4,136	1,578	846	238	24	49	0
Patients: 462							

EDTA, Ethylenediaminetetraacetic acid tube; PBMC, peripheral blood mononuclear cells; Tempus, RNA blood collection tube; CPT, Cell Preparation Tube (for PBMC sampling).

DISCUSSION

Using a unique combination of outreach screening-based and hospital-based biobanking we were able to establish a

TABLE 3 | Community-based biobanking: costs (in euros).

Personnel	Nursing staff	3,634.0
	Administrative assistant	1,157.7
	Language services	160.0
	Study coordinator	1,719.8
	Physicians	4,366.8
	Total	11,038.3
Logistics	Blood tubes, venepuncture materials	4,737.2
	Event logistics (location rent, catering, etc.)	500.0
	Communication costs	1,211.0
	Total	6,448.2
Overall cost		17,486.5

TABLE 4 | Staff and tasks involved in the preparation and execution of biobanking during on-site screenings.

	Task	Preparatory/ support	On-site/ during screening
Administrative staff	Registration, on-site logistics	1	1
Paramedical staff	Venepuncture	1	2-3
Study coordinator		1	1
Medical staff	Informed consent, information	2	2
Volunteers	Translation, community coordination	4	5-10
Hospital laboratory	Serological testing, temporary storage	3	2
University laboratory	PBMC isolation and temporary storage	2	3-4
Biobank	Database, labeling, storage, sample processing and QA	3	1

continuously expanding collection of biological samples that enables a prompt answer to several relevant clinical and translational research questions in the field of viral hepatitis.

Numerous challenges arose during the execution of the project. Both types of biobanking required a different approach with inherently also different challenges. As for community outreach screening-based biobanking, despite the uniformity in data entry, labeling and storage, high personnel input from all participating entities is necessary to ensure success (**Table 4**). Additionally, a single coordinator is needed to ensure continuity and to remedy and track logistical and quality variance, for instance; traffic delayed CPT Heparine shipments from the screening locations to the university laboratory. This staff member had initially been planned to also perform screening, but personnel redundancy was quickly activated to ensure CPT Heparine transport could continue, whilst also being able to continue screening activities. By design, sample complexity was kept at a minimum, but the latter issue illustrates that PBMC collection in particular proved to be challenging during community outreach screening-based biobanking.

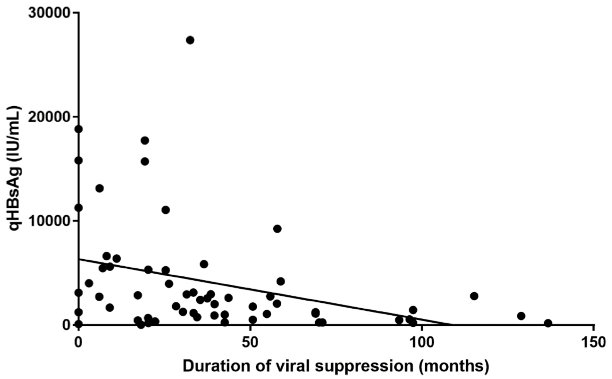

FIGURE 3 | HBsAg quantification compared to duration of treatment induced viral suppression (in months).

Community screenings typically scale from tens of samples to hundreds or even thousands (13). In our experience, ±100 is an upper limit that our clinical and research laboratories could handle for sample processing and testing within turnaround times (TAT) that fall within performance characteristics. Serum samples, e.g., which needed to be tested for HBV and HCV serology, arrived in bulk. The platform which was used (Roche Diagnostics Elecsys, Modular) was not designed to rapidly run such a large number of samples. Additionally, the samples arrived after the screening event had ended, plus, some events were held on weekends. Thus, staffing at the clinical laboratory was lower than during weekdays. Despite these challenges, analytical TATs were within 5 days.

Hospital based biobanking presented different challenges (14, 15). Staffing and logistics are less time and resource intensive, as systems to obtain, process and store samples are already in place on-site. However, samples from healthy controls (including those who are not necessarily negative for a specific disease) are not collected during hospital based biobanking as opposed to screening/community settings. Patient recruitment in a hospital setting is more specific, and dedicated study coordinators need to monitor, often complex, inclusion criteria.

Biobanked serum and plasma has so far been used for different purposes; one of which was the quantification of Hepatitis B surface Antigen levels in chronic hepatitis B patients. HBsAg quantification ("qHBsAg") provides extra information in terms of the natural history of a person chronically infected with HBV. In our center, we have observed that qHBsAg slowly declines when patients are treated with nucleoside/nucleotide analog antiviral therapy (**Figure 3**). Recent literature suggests that qHBsAg levels may guide physicians in their decision to interrupt long-term antiviral treatment for chronic hepatitis B (10).

DNA isolated from plasma samples derived from chronic HBV patients was used in an international multicentre study to investigate the prevalence of a Toll-Like Receptor 7-specific

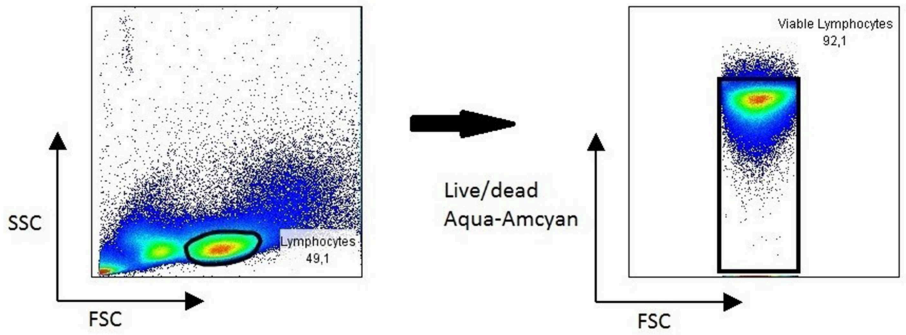

FIGURE 4 | Representative FACS plot showing viability of lymphocytes in PBMC 3 years after collection.

Single Nucleotide Polymorphism (SNP) rs 1790008 in a large group of chronic HBV infected patients ($n = 1{,}054$) and healthy individuals ($n = 231$). The SNP was almost exclusively detected in Caucasian subjects and was much more prevalent in healthy Caucasian females when compared to HBV infected Caucasian females, suggesting that the SNP might provide protection against chronic HBV infection in this population (11). Serum samples collected from chronic hepatitis C patients were used in an international clinical trial to assess the efficacy of an 8-week treatment regimen of ledipasvir/sofosbuvir in HCV genotype 4 infected patients. Among a total of 39 included patients, 6 (of whom 2 were patients at the Antwerp University Hospital) did not meet the primary study endpoint, being HCV RNA negative at 12 weeks after therapy. Retrospective phylogenetic analyses on biobanked samples revealed that 4/6 of these patients had been reinfected (12).

PBMC isolated from chronic HBV patients and healthy controls showed excellent viability 3 years after isolation (**Figure 4**), allowing for use for different immunological study purposes. In one study, paired serum and PBMC samples of chronic Hepatitis B patients were used to study Hepatitis B specific B cell responses. Results revealed a strong association of a potent Hepatitis B-core specific B cell response with clinical parameters in both treated and untreated patients (16). In another study, the global B cell transcriptome was profiled in chronic HBV infected patients and compared to healthy controls using a systems biology approach. Peripheral B cells of chronic HBV patients showed clinical phase dependent transcriptome alterations and proved to be very different from intrahepatic B cells on a transcriptome level (17).

Virus isolated from biobanked stool of a chronic hepatitis E patient was successfully used to establish a mouse model for Hepatitis E infections, allowing further HEV virology studies (18, 19). Interestingly, using biobanked urine samples, we discovered that HEV RNA can be detected in urine samples.

Of final note, the higher amount of retrieved samples from community biobanking is largely due to requirements in study protocols. Going forward, biobank procedures have been put in place to facilitate third-party use of samples. A biobank committee (with principal investigators of studies that collected samples in the biobank) will process and evaluate requests on scientific merit, logistical feasibility and ethical considerations.

CONCLUSION

In conclusion, in this chapter we described how the establishment of a biobank with samples collected both in-hospital and during community-outreach screening, resulted in a unique, continuously expanding collection of biological samples which provides an excellent platform for prompt answers to clinically and translationally relevant research questions. This information may guide other centers in setting up similar projects in possibly different contexts.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The protocol was carried out in accordance with the recommendations of Good Clinical Practice, approved by the Antwerp University Hospital/University of Antwerp Ethical Committee (EC 15/21/227), with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The informed consent allows sampling and storage for blood, urine, fecal and liver materials from hepatology outpatient and inpatients clinics.

AUTHOR CONTRIBUTIONS

EH and SV contributed equally to the work as shared-first authors. SG provided data and revisions of the text. PM and TV provided drafting and revisions of the text. All other authors listed (ES, MH, SF, and BD) have made also made substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

The authors declare that this study received funding from Roche Diagnostics, Gilead Life Sciences Inc., Bristol-Myers Squibb, Sandoz. These funders were not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication. Parts of this study was also performed with grants from the Centre for Medical Innovation—Authorities of the Region of Flanders. TV was supported by a Research Mandate of the Foundation Against Cancer Belgium, No. 2014-087.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

BRIEF RESEARCH REPORT
published: 06 September 2019
doi: 10.3389/fmed.2019.00198

Cardiogeneticsbank@UZA: A Collection of DNA, Tissues, and Cell Lines as a Translational Tool

*Maaike Alaerts[1], Gerarda van de Beek[1], Ilse Luyckx[1], Josephina Meester[1], Dorien Schepers[1], Aline Verstraeten[1], Johan Saenen[2], Emeline Van Craenenbroeck[2], Inge Goovaerts[1], Inez Rodrigus[3], Steven Laga[3], Jeroen Hendriks[4], Sofie Goethals[5], Annemieke De Wilde[5], Elke Smits[6], Philippe Jorens[6], Manon Huizing[5], Lut Van Laer[1] and Bart Loeys[1]**

[1] Center of Medical Genetics, Antwerp University Hospital, University of Antwerp, Antwerp, Belgium, [2] Department of Cardiology, Antwerp University Hospital, University of Antwerp, Antwerp, Belgium, [3] Department of Cardiac Surgery, Antwerp University Hospital, University of Antwerp, Antwerp, Belgium, [4] Department of Thoracic and Vascular Surgery, Antwerp University Hospital, University of Antwerp, Antwerp, Belgium, [5] Biobank, Antwerp University Hospital, University of Antwerp, Antwerp, Belgium, [6] Intensive Care Unit and Clinical Research Center, Antwerp University Hospital, University of Antwerp, Antwerp, Belgium

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Edited by:
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***Correspondence:**
Bart Loeys
bart.loeys@uantwerpen.be

Specialty section:
This article was submitted to Translational Medicine, a section of the journal Frontiers in Medicine

Received: 22 May 2019
Accepted: 23 August 2019
Published: 06 September 2019

Citation:
Alaerts M, van de Beek G, Luyckx I, Meester J, Schepers D, Verstraeten A, Saenen J, Van Craenenbroeck E, Goovaerts I, Rodrigus I, Laga S, Hendriks J, Goethals S, De Wilde A, Smits E, Jorens P, Huizing M, Van Laer L and Loeys B (2019) Cardiogeneticsbank@UZA: A Collection of DNA, Tissues, and Cell Lines as a Translational Tool. Front. Med. 6:198. doi: 10.3389/fmed.2019.00198

Cardiogeneticsbank@UZA is an academic hospital integrated biobank that collects aortic tissue, blood, cell lines (fibroblasts, vascular smooth muscle cells, peripheral blood mononuclear cells, and induced pluripotent stem cells), and DNA from patients with cardiogenetic disorders, for both diagnostic and research purposes. We adhere to a quality management system and have established standard protocols for the sampling and processing of all cardiogenetic patient related materials. Cardiogeneticsbank@UZA is embedded in the Biobanking and Biomolecular Resources Research Infrastructure Belgium (BBMRI.be) and samples from this biobank are available for commercial and academic researchers, through an established access procedure. Currently, the extremely valuable cardiogenetics collection consists of more than 8,700 DNA samples, 380 tissue samples, and 500 cell lines of 7,578 patients, and is linked with extensive clinical data. Some interesting potential research applications are discussed.

Keywords: cardiogenetics, biobank, sudden cardiac arrest, inherited cardiac arrhythmia, aortic aneurysm, cardiomyopathies

INTRODUCTION

In 2010 the Cardiogenetics Research Laboratory at the Center for Medical Genetics (CMG) of the University of Antwerp (UA) was founded by Profs. Van Laer and Loeys. This research group focuses on the genetics of thoracic aortic aneurysm and dissection (TAAD), inherited cardiac arrhythmias (Primary Electrical Disease, PED), cardiomyopathies (CM) and hereditary hypercholesterolemia (HC), and the molecular pathophysiological mechanisms underlying these disorders. Over the last years, dozens of genes have been identified as the molecular cause of these cardiogenetic diseases. The research laboratory is closely linked to the Molecular Diagnostic Unit of the CMG. Through a research-diagnostic collaboration, specific next-generation sequencing (NGS)-based molecular diagnostic gene panels for TAAD, PED, CM, and HC were designed and implemented (1, 2). In parallel, Prof. Dr. Loeys established a Cardiogenetics Clinic in collaboration with the Cardiology Department of the Antwerp University Hospital (UZA). With a multidisciplinary team

including a geneticist, cardiologists, a genetic counselor, a nurse, and a psychologist, more than 750 consultations of cardiogenetic patients and their family members are performed each year.

The aim of the cardiogeneticsbank@UZA is to systematically collect, store, and distribute different types of samples obtained from cardiogenetic patients or family members for diagnostic and/or research purposes. Initial diagnostic testing mainly involves the NGS-based TAAD, PED, CM, and HC gene panels and/or whole exome sequencing (WES) to identify causal genetic variants. This allows subsequent family testing and counseling, tailored patient management, and potential pre-implantation genetic diagnostics. The current cardiogenetics research projects aim to identify novel genes and genetic modifiers for TAAD, PED, and CM, and analyze the functional effects of the detected variants at molecular, cell, and organ level using cellular and animal models, such as mouse and zebrafish.

The specific disorders covered by the cardiogenetics@UZA database include Brugada syndrome, sick sinus syndrome, Long and Short QT syndrome, Arrhythmogenic Right Ventricular Cardiomyopathy, Catecholaminergic Polymorphic Ventricular Tachycardia, non-ischemic dilated, hypertrophic, non-compaction cardiomyopathy, Marfan syndrome, Loeys-Dietz syndrome, familial thoracic aortic aneurysm/dissection syndrome, bicuspid aortic valve associated aortopathy, vascular Ehlers-Danlos syndrome.

The Cardiogeneticsbank@UZA is part of the larger UZA biobank that was founded with the support of the Flemish initiative for biobanking (CMI) and is now included in the Biobanking and Biomolecular Resources Research Infrastructure network Belgium (BBMRI.be). In the Flemish initiative, different thematic fields were identified, including (auto)immune diseases, infectious diseases, cardiovascular diseases, metabolic diseases and diabetes, neurosciences, oncology, aging, reproductive medicine, and rare disorders. Within the cardiovascular theme, focus on sudden cardiac death (3) was defined and coordinated by the UZA. The sample types include aortic wall and aortic valve tissue, blood, DNA, RNA, skin and vascular fibroblasts, peripheral blood mononuclear cells (PBMCs), and induced pluripotent stem cells (iPSCs). All samples are collected and processed in a standardized way according to the "Standard Operating Procedure" (SOP) protocols stored in the Electronic Lab Notebook (ELN) account of the research group (E-Notebook 2014 Client, version 13.9.0.0, PerkinElmer) or in the document management system DocBase (Acanthis) of the UZA. These SOPs and any updates are validated by a senior scientist and receive a version number and date for correct referencing. All research group members (postdocs, PhD students and lab technicians) are properly trained to follow the correct SOPs and refer to the used protocols correctly in their personal ELNs.

Design and aim of the cardiogenetics@UZA biobank are comparable to other international biobanks focused on cardiovascular disease such as the Cardiovascular Biomedical Research Unit Biobank at the Royal Brompton & Harefield NHS Trust in London (https://www.hra.nhs.uk/planning-and-improving-research/application-summaries/research-summaries/cardiovascular-biomedical-research-unit-biobank/), the GENCOR (4) and CONCOR (5) databases in the Netherlands. Although the latter is mostly focused on structural congenital heart disease.

MATERIALS AND METHODS

Ethics—Informed Consent

For all research samples collected for the biobank, the informed consent corresponding to the correct research project is obtained from the patients, stored in folders at the CMG or electronically stored in the UZA Electronic Patient File (EPD). Every research project has been approved by the local UZA research ethics committee, including the information sheet and consent form for the patients. Patients that attend the Cardiogenetics clinic for diagnostic purposes are properly counseled and informed about potential inclusion in cardiogenetics research projects. They can then provide oral or written consent. If they don't consent, samples are only used for diagnostic purposes.

Database

All patient identification, clinical, and (genetic) diagnostic data are collected and stored in electronic patient records in the secured Hospital Information Database of the UZA (Joint Commission International (JCI) quality approval, 2017). To this purpose, every patient receives a unique UZA patient identification number (UZA ID). Patients and relatives belonging to the same family receive a unique family identifier and the pedigree is drawn using specific software (PASS). All relevant clinical and diagnostic patient data with corresponding UZA ID and family identifier are then transferred to the Cardiogenetics Research Database (Microsoft Access), stored on a secured UA server with automatic backup. The sample location, date of collection or storage and all research data, including genetic sequence data, functional experiment data and analysis results, are added to this database. Only the Principal Investigator and Medical Doctor Prof. Dr. Loeys can connect the UZA IDs with patient identification data. Hence, for all other research group members the data is anonymized. The necessary GDPR (General Data Protection Regulation) forms for the different data elements gathered in the Cardiogenetics Research Database have been completed and can be provided to the Privacy Commission of Belgium when asked for, in line with recommendation 06/2017 of June 14th 2017 of this commission. All requirements for the EU GDPR (2016/679) have been fulfilled.

Sample Collection

Blood samples are drawn at the UZA by trained hospital staff. Blood is collected in EDTA-tubes (5 ml) for DNA extraction or PBMC collection, or in PAXgene tubes (Qiagen) for RNA extraction. Aortic wall and valve tissue samples are collected at the UZA operating room and either snap-frozen and stored at −80°C at the UZA Pathology department within 30 min or transferred to the CMG in physiological solution. Skin biopsies are collected at the UZA dermatology department in sterile plastic tubes (Eppendorf) with physiological solution. These samples are transferred to the CMG at room temperature within 24 h after collection, where they are processed or stored at −80°C immediately upon arrival.

Sample Processing

DNA extractions from blood are performed on an automated nucleic acid extraction system (Perkin Elmer) with robotic liquid handling and DNA is stored at 4° or −20°C after measuring the concentration. RNA extractions are performed using the RNeasy Mini kit (QIAGEN) or the Quick-RNA MiniPrep kit (BaseClear) according to the manufacturer's instructions. RNA is stored in a −80° freezer after measuring the concentration. PBMCs are isolated from blood using a standard protocol based on Lymphocyte Separation Medium and centrifugation steps. The freshly isolated PBMCs are then cryopreserved in liquid nitrogen in a 10% DMSO solution (>3 million cells per cryotube) until further use. Fresh aortic wall or valve tissue samples or skin biopsies are cut in small pieces and digested with trypsin and collagenase, followed by standard culture in fibroblast medium (RPMI medium, Gibco) to obtain vascular, valvular, and dermal fibroblasts, respectively. After culture and expansion these fibroblasts are cryopreserved in liquid nitrogen in a 10% DMSO solution (>2 million cells per cryotube) until further use. Fresh aortic tissue samples are also frozen as a whole at −80°C. For selected patients, vascular smooth muscle cell (VSMC) lines are derived from the fresh aortic wall tissue before cryopreservation.

iPSC Generation

Both PBMCs and dermal fibroblasts are used to generate patient-specific iPSCs. Thawed PBMCs are cultured in StemSpan medium (STEMCELL Technologies) for 9 days to promote the expansion of hematopoietic cells. Thawed fibroblasts are cultured in regular fibroblast medium until they reach 90% confluency. Next, the Cytotune-iPS 2.0 Sendai reprogramming kit (ThermoFisher Scientific), containing the four Yamanaka transcription factors in non-integrating Sendai viral vectors, is used for the generation of iPSCs, following the manufacturer's instructions. After emergence of iPSC colonies, five rounds of manual picking are performed, followed by five rounds of enzymatic passaging and expansion. At least 12 different clones are selected for cryopreservation based on morphology and growth characteristics and frozen in liquid nitrogen in a 10% DMSO solution. Three of these clones are then fully validated by immunocytochemistry staining for pluripotency markers (Oct4, Nanog, Tra-1-60, and Tra-1-81) and by proving their trilineage differentiation potential using an embryoid body formation assay followed by qPCR assays for endodermal, mesodermal, and ectodermal markers.

Quality Assurance Measures

DNA and RNA extractions are quality controlled by spectrophotometry-based methods (NanoDrop or Qubit—Thermo Fisher Scientific). The temperature of fridges and freezers is continuously monitored and an alarm system will be activated if the temperature exceeds a specific threshold. The liquid nitrogen tanks are also equipped with an alarm system. All cell cultures are routinely checked to exclude Mycoplasma infection, and once more specifically before cryopreservation. Sustainability of the cardiogenetics@UZA biobank is guaranteed by its embedding within the Antwerp University Hospital and samples are stored for at least 30 years.

Specimen Types and Numbers

DNA samples: 8,700
RNA samples: 246
Blood samples (unprocessed): 450
Aortic tissue samples: 380
PBMC samples: 55
Fibroblast cell lines: 429—total of 1,860 cryotubes
VSMC lines: 64—total of 130 cryotubes
iPSC lines: 12—total of 610 cryotubes

These samples have been collected from 7,578 patients.

Access Procedures

Both academic and commercial researchers can be granted access to our collection of samples in the context of a specific collaboration. They will have to submit a Material Request Form in which they describe their project including study design, samples requested, project funding, and scientific relevance. Positive evaluation of this request by the Principal Investigator Prof. Dr. Loeys and the local UZA research ethical committee will lead to the signing of a human Material Transfer Agreement (MTA) and a contract agreeing on the costs to cover the consumables needed for collection, handling and storage of the samples. Samples and their associated coded data can then be released and according to the terms of the MTA the researches are committed to give feedback on sample quality and results and should acknowledge Cardiogeneticsbank@UZA in any scientific communication related to their findings.

APPLICATION POTENTIAL

The application potential of a cardiogenetics biobank is extremely diverse but some examples of current applications are discussed below. First, a large collection of DNA samples of patients with well-defined phenotyping can easily be used as a replication cohort for novel candidate genes of cardiogenetic disorders. For many subgroups of cardiogenetic diseases, e.g., dilated cardiomyopathy, Brugada syndrome or thoracic aortic aneurysm (6), the diagnostic mutation yield is far from complete and upon discovery of novel causal genes, validation of these genes can be obtained by resequencing of previously genetically unsolved DNA samples. Second, the collection of region-specific samples (e.g., Flanders) offers the opportunity to identify recurrent mutations in the same gene. Haplotyping can then be performed and identical haplotypes point to Flemish founder mutations. This can initiate larger cascade mutations screening efforts to identify region-specific *at-risk* individuals. At present, we have identified three novel founder mutations in our cardiogenetics biobank. Third, the identification of founder mutations sets the unique platform for the execution of modifier studies. Upon phenotypical characterization of all available founder mutation carriers for a specific gene and condition, one can take advantage of the shared genetic

background to identify differences between mutation carriers at the extreme ends of the phenotypical spectrum: e.g., old unaffected (completely asymptomatic) mutation carriers vs. very young affected, clearly symptomatic mutation carriers. Fourth, the collection of aortic wall and valve tissues offers opportunities to study expression patterns both at protein and mRNA level. Finally, the PBMC and fibroblast cultures allow the establishment of iPSC lines, which can be differentiated in cardiomyocytes or vascular smooth muscle cells. As such, these "adult" cell types can be generated from patients with well-defined cardiogenetic disorders for whom the collection of native cardiomyocytes or vascular smooth muscle cells is not feasible. The application potential of these iPSC-derived cell lines is tremendous, as they can be used both for pathomechanistic studies as well as for pharmacological research where they serve as a platform for testing of new drug compounds.

In conclusion, using well-documented standard operating procedures and quality control, combined with excellent and detailed clinical data from an extended network, the Cardiogeneticsbank@UZA provides an extremely valuable collection of patient samples that is used for both diagnostic and research purposes.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

MA, GB, LV, and BL drafted the paper. IL, JM, DS, AV, JS, EV, IG, IR, SL, JH, SG, AD, ES, PJ, and MH have revised the paper. All co-authors have contributed to the establishment and sample collection for the cardiogenetics@uza biobank.

FUNDING

This research was supported by funding from the University of Antwerp (GOA), the Fund for Scientific Research, Flanders (FWO, Belgium, G.0356.17), The Dutch Heart Foundation (2013T093), BL and EV are senior clinical investigators of the Fund for Scientific Research, Flanders and BL holds a consolidator grant from the European Research Council (Genomia—ERC-COG-2017-771945). AV and DS hold a postdoctoral fellowship from the Fund for Scientific Research, Flanders.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 17 October 2019
doi: 10.3389/fmed.2019.00225

Tumor Banks: A Quality Control Scheme Proposal

Ligia Craciun[1,2], Selim Alex Spinette[1,2], Marc Rassy[2], Roberto Salgado[2], Alexandre de Wind[2], Pieter Demetter[2], Laurine Verset[2], Maria Gomez-Galdon[2], Marie Chintinne[2], Nicolas Sirtaine[2], Nicolas de St Aubain[2], Ioanna Laios[2], Francoise Roy[2] and Denis Larsimont[1,2]*

[1] Tumor Bank, Institut Jules Bordet, Brussels, Belgium, [2] Department of Anatomical Pathology, Jules Bordet Institute, Brussels, Belgium

Introduction: Tumor banks make a considerable contribution to translational research. Using emerging molecular tests on frozen material facilitates the development of new diagnostic and therapeutic strategies, especially in rare cases. However, standard quality control schemes are lacking in the current literature.

Methods: In 2017, we have conducted a robust quality control test on 100 of 15,000 fresh frozen samples collected between 2000 and 2013 at the Jules Bordet Tumor Bank (Brussels). RNA and DNA extraction was done. The quality of RNA, DNA and proteins were evaluated, respectively by measuring RNA Integrity Number (RIN), by checking Electrophoretic Integrity (EI) and by performing Immunohistochemistry staining (IHC). A score, ranging from poor (1) to excellent (4), was attributed based on technical analysis.

Results: RNA purity was scored 4 in 97% of the cases, 3 in 2%, and 2 in 1%. RIN scores were similarly 4 in 89%, 3 in 10%, and 2 in 1% of the cases. DNA purity was scored 4 in 94% and 3 in 6%, EI was scored 4 in 100% of the cases. Despite morphology loss after freezing, HER2, ER, and Ki67 IHC stainings yielded a score of 4 in the majority of samples. Furthermore, participating in the ISBER Proficiency Testing helped us validate our techniques and the technician's work. Seven processing schemes were carried out, the scores obtained were very satisfactory (20/27) or satisfactory (7/27).

Conclusion: Tumor Banks can be precious for translational research. Nevertheless, firm quality controls should be applied to ensure high quality material delivery. Only then can biobanks contribute to diagnostics, biomarkers discovery and reliable molecular test development.

Keywords: biobank, electrophoretic integrity, quality control, DNA, RNA, morphology, quality scores

OPEN ACCESS

Edited by:
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Reviewed by:
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****Correspondence:***
Ligia Craciun
ligia.craciun@bordet.be

Specialty section:
This article was submitted to Translational Medicine, a section of the journal Frontiers in Medicine

Received: *21 June 2019*
Accepted: *30 September 2019*
Published: *17 October 2019*

Citation:
Craciun L, Spinette SA, Rassy M, Salgado R, de Wind A, Demetter P, Verset L, Gomez-Galdon M, Chintinne M, Sirtaine N, de St Aubain N, Laios I, Roy F and Larsimont D (2019) Tumor Banks: A Quality Control Scheme Proposal. Front. Med. 6:225. doi: 10.3389/fmed.2019.00225

INTRODUCTION

Collecting samples for research is an old concept among pathologists and researchers. Nevertheless, biobanking is only fairly recent (1), as well as legislation concerning collection of human tissue and data protection (EU Data Protection Directive—Directive 95/46/EC). Controlling the quality of collected material in a biobank is crucial before providing tissue specimens for research. Quality control procedures must be in place to evaluate the samples and the effects of long-term storage.

The lack of reproducibility in gene signatures is often associated with tissue heterogeneity due to, among other things, the lack of standardization of collection procedures (2). A good level of molecular integrity is essential to avoid variability in the results of research projects. The quality of nucleic acids is of major importance for several techniques used in genetic analysis.

Convenient quality control procedures must check the validity of final products (samples or derivatives) for different applications of end-use in research, irrespective of the used extraction method. Scores and cutoffs are to be adopted to determine the quality acceptance limits.

The first phase, termed pre-analytical phase, summarizes all steps from tissue sampling to the start of the desired end-use application. Each of these steps can affect the quality of the sample, the quality of the results, and their reproducibility (3, 4). Once the critical pre-analytical steps (medications, anesthesia, warm and cold ischemia time) for an application are known, researchers will only examine samples that meet the pre-analytical conditions previously defined. Quality control procedures (QC) are used to either confirm tissue quality from known pre-analytical conditions or investigate tissue quality from unknown pre-analytical condition(s). The ideal quality control "biomarkers" should be ubiquitous, measurable by accessible methods and leading to a dichotomous response to a specific pre-analytic variation.

Validation of clinically appropriate biomarkers should take into account the potential impact of pre-analytical variation on each of them. This validation process is the key to research using bio-resources (a requalified tissue sample for research and its associated data). Rapid stabilization of tissues by snap freezing immediately can reduce artifactually altered gene expression. Moreover, unlike FFPE tissue, the RNA and DNA from frozen tissue are of high molecular weight, lack cross-linking modifications and are therefore better candidates for the "next-generation" testing.

Good QC tools aim to test the molecular integrity and protein quality. They must also be compatible with genomic, epigenomic, transcriptomic, proteomic, and metabolomic tests.

Moreover, histological control of stored tissue is a crucial step. Generally, 10% of the frozen samples are unsuitable for the molecular analysis mainly because of insufficient quantity of malignant cells or necrosis (5).

The purpose of this work is to establish the quality limits of the tissues stored in tumor banks by independently evaluating the morphological (proteins) and molecular (DNA and RNA) characteristics on randomized selected frozen tumor samples.

In parallel, we have used the Biospecimen Proficiency Testing (PT) programme launched by IBBL, as an external quality assessment tool to verify the precision and accuracy of the in house biospecimens testing methods. Seven processing and testing schemes were performed: DNA Extraction from FFPE Material, DNA Extraction from Frozen Tissue, DNA Quantification and Purity, Total RNA Extraction from Frozen Tissue, RNA Integrity, RNA Quantification and Purity, and Tissue Histology.

MATERIALS AND METHODS

Ethical Approval

Ethical approval, concerning the biobank activities and objectives, was granted by the ethics committee of the Institut Jules Bordet (CE1891 and CE2897). Of note, and according to the Belgian laws (2008-12-19/44 and 2018-01-09/14), the ethics committee of the Institut Jules Bordet approved the study protocol and waived the requirement of patients written consent.

Biospecimens

One hundred biobank frozen samples (52 breasts, 13 thyroids, 12 lymph nodes, 9 endometrium, 3 ovaries, 2 sarcomas, 2 kidney, 2 colon, 1 prostate, 1 lung, 1 small intestine, 1 spleen, 1 uterus) originating from 100 patients were tested for DNA, RNA and protein quality. Selected samples dated from 2000 to 2013. Tumor samples were embedded in Tissue-Tek® O.C.T.™ Compound (by Sakura®) and frozen at −80°C. This method allows sectioning on a cryostat without residues during the staining procedure. Indeed, frozen sections were performed on a cryostat (by Leica Biosystems®). The first slide was stained by H&E (Hematoxylin and Eosin). Twenty serial sections were collected in RNase free Eppendorf tubes. Four additional sections were used for the IHC staining. Of note, all necessary material for sections handling was cooled on dry ice, to preserve the cold chain and avoid temperature fluctuations. The tumor morphology and cellularity were last reviewed by a pathologist.

DNA/RNA Extraction

DNA and RNA were extracted from frozen specimens using AllPrep DNA/RNA Micro Kit (Qiagen) according to the manufacturer's instructions. DNA and RNA were finally eluted in a volume of 20 μL.

DNA Quality Assessment

A good DNA quality is important for studies on genomic DNA and CGH analyses. Two parameters were evaluated: concentration and purity, measured by the OD and DNA integrity by electrophoresis gel. The ratio for pure DNA should be between 1.8 and 2.1: a lower ratio is indicative of protein contamination, while a higher ratio indicates a degradation of the DNA. This ratio is only an indication of purity of nucleic acids and does not necessarily reflect the integrity of the nucleic acids.

DNA Gel Analysis

A visual analysis on electrophoresis gel was done to estimate the sample integrity. The degree of DNA degradation was examined using electrophoresis in a 2% agarose gel (Reliant™ Gel System, 2% SeaKem® Gold Agarose, Lonza, USA). Intact genomic DNA appears as a compact, high-molecular-weight band with no scanty low-molecular-weight smears. 1 kb DNA ladder from Solis Biodyne was used as molecular marker. A quality score could be assigned (**Table 1**).

RNA Quality Assessment

The yield and purity of total DNA and RNA were determined using a spectrophotometer (Nanodrop TM ND-1000, Thermo

TABLE 1 | Electrophoresis integrity (EI) quality scores.

Quality	Ratio 260/280	Electrophoresis integrity (EI)	Score
Bad	1.2–1.4	Smear of 2 kb	1
Poor	1.4–1.6	Smear of 5 kb	2
Good	1.6–1.8	Smear of 10 kb	3
Very good	1.8–2.1	Single band of high molecular weight	4

TABLE 2 | RNA purity and integrity score attribution based on 260/280 OD Ratio and RIN.

Quality	260/280 OD Ratio	RIN	Score
Bad	1.2–1.4	1–4	1
Poor	1.4–1.6	1–4	2
Good	1.6–1.8	4.1–6.9	3
Very good	1.8–2.1	7.0–10.0	4

Fisher Scientific). A 260/280 OD ratio >1.8 was considered an indicator of acceptably pure RNA, relatively free of protein.

RNA Integrity Number

RNA was examined on the Agilent 2100 bioanalyzer, based on microfluidic capillary electrophoresis. RNA 6000 Nano LabChip kits were used. For each sample, 1 μL of extracted RNA was analyzed. RIN scores, ranging from 1 to 10, were retrieved. A RIN between 7 and 10 was associated with intact RNA.

RNA Purity and Integrity Score

Based on the 260/280 OD ratio and on the RIN, a score was assigned for each case, as shown in **Table 2**.

Immunohistochemistry Staining

Consecutive frozen tissue sections (4 μm) were immunohistochemically (IHC)-stained using a BenchMark XT IHC/ISH automated slide stainer (Ventana Mediated Systems, by Roche®). The following antibodies were used: anti-HER2/NEU (rabbit monoclonal antibody, clone 4B5, Roche® Ventana®); anti-Estrogen Receptor (ER) (rabbit monoclonal antibody, clone SP1, Roche® Ventana®); and anti-Ki-67 (mouse monoclonal antibody, clone MIB-1, Agilent®). Breast tumor samples ($n = 52$) were tested with anti-HER2/NEU and anti-ER antibodies. Non-breast tumor samples ($n = 48$) were tested with anti-Ki67 antibody.

Morphological and Proteins Quality

For this study, H&E staining allowed the evaluation of cellular integrity and morphology, while immunohistochemistry staining (Ki67, HER2, and ER) provided a practical evaluation of proteins quality control.

A quality score, based on visual evaluation of quality staining, was assigned. All scoring systems were based on two separate components: the specificity and the intensity of staining. Technical sensitivity and specificity cannot be accurately calculated when IHC is used as a qualitative test because it is merely a descriptive test. The relation between the staining and the protein availability isn't linear. Calibration controls aren't either available. Scoring was blindly done by two independent pathologists. While scoring, routine sections from FFPE (formalin fixed and paraffin embedded) blocks were used as reference (**Table 3**).

TABLE 3 | Visual evaluation of specificity and intensity of the IHC staining.

Quality	Visual evaluation	Score
Bad	Low specificity/Low intensity	1
Poor	Low specificity/Moderate intensity	2
Good	Moderate specificity/Moderate intensity	3
Very good	high specificity/High intensity	4

ISBER Proficiency Testing

DNA Extraction From FFPE Cells Scheme

The material used for this scheme was a Jurkat cell line. We received one tube containing 2 FFPE sections of 20 μm. We extracted the DNA following our usual routine silica membrane-based DNA extraction method. The extracted DNA sample was shipped back to the PT provider. The total DNA yield per 20 μm section, DNA purity, DNA integrity (DIN), DNA functionality and amplifiability (cross-linking assessment) and DNA quality (ENZO score) of all extracted DNA were assessed.

DNA Extraction From Frozen Tissue Scheme

The material used for this scheme was a pig (*Sus*) liver. We received one tube containing one CryoXtract core of 10 to 20 mg. We performed the DNA extraction following our usual routine silica membrane-based DNA extraction method. The extracted DNA sample was shipped back to the PT provider. The total DNA yield per mg of tissue, the DNA purity (A260/A280), the double-stranded DNA yield per mg of tissue, the DNA integrity (DIN) and the presence of PCR inhibitors using a SPUD assay were assessed by IBBL.

DNA Quantification and Purity Scheme

The DNA used for this scheme was extracted from whole blood. We received three different Test Items containing DNA at a different concentration and 260/280 ratio (i.e., Tube A, Tube B, and Tube C). For each Test Item (Tube A, Tube B, and Tube C), we measured the DNA concentration (μg/ml) and 260/280 ratio by spectrophotometry.

RNA Extraction From Frozen Tissue Scheme

The material used for this scheme was a pig (*Sus*) liver. In this scheme, we received one single "Processing Item" (one tube containing one CryoXtract core of 10 to 20 mg). The RNA was extracted following our usual routine silica membrane-based RNA extraction method. The extracted RNA sample was shipped back to the PT provider. The total RNA yield per mg of tissue, the RNA purity (A260/A280) and the RNA integrity (RIN) were assessed by IBBL.

RNA Integrity Scheme

The RNA used for this scheme was extracted from a Jurkat cell line by a silica-based method. Three different Test Items containing RNA at a different level of integrity (i.e., Tube A, Tube B, and Tube C) were received. For each Test Item (Tube A, Tube B, and Tube C), we measured the RNA Integrity (RIN) on the Agilent® 2100 Bioanalyzer System.

RNA Quantification and Purity Scheme

The RNA used for this scheme was extracted from a Jurkat cell line by a silica-based method. Three different Test Items containing RNA at a different concentration and 260/280 ratio (i.e., Tube A, Tube B, and Tube C) were received. For each Test Item (Tube A, Tube B, and Tube C), we measured the RNA concentration (μg/ml) and 260/280 ratio by spectrophotometry.

Tissue Histology Scheme

The Test Items were pictures of human colon adenocarcinoma (Test Items A) and human breast adenocarcinoma (Test Item B, Test Item C, Test Item D, and Test Item E). The tissue characterization/mapping was done through the assessment of the percentage of uninvolved tissue areas (Test Item A, Test Item B, and Test Item C) and of viable tumor areas (Test Item D and Test Item E).

For each test, the scoring system was based on deviation from the assigned value. A consensus score was established as follow: below 1 standard deviation: 0 (very satisfactory); below 2 standard deviations: 1 (satisfactory); above 2 standard deviations: 2 (questionable); and above 3 standard deviations: 3 (requiring action). The results were reported through the website http://biospecimenpt.ibbl.lu.

RESULTS

Total DNA Quality Control

The morphology was successfully determined in the majority of samples. Two samples were tumor free and one has been totally consumed through sectioning.

Based on 260/280 ratio, the majority of tested samples were evaluated with a score of 4 (94%) (**Figure 1**), and 6 samples were scored with 3. The 260/230 ratio were used as a secondary measure of nucleic acid purity. The data is available but doesn't provide any supplementary information. No contamination by salt or organic compounds was noted. The EI was estimated at score 4 for all the tested samples.

Total RNA Quality Control

Upon optical density (OD) measurement of extracted RNA, most samples were evaluated with a score of 4 (97%), two samples were scored with 3 and one sample was unusable due to insufficient RNA amount. In the latter, the tissue fragment was mainly fibrotic on microscopic examination. RIN values (**Figure 2**) were classified as of sufficient quality: score of 4 (89%) and score of 3 (10%); the sample characterized by a score of 2 (1%) was considered inadequate for further analysis.

Assessment of Morphological and Proteins Quality

All tested samples were characterized by a good histologic quality control. The percentage of area of the tissue involved with tumor was considered acceptable despite the presence of freezing artifacts in almost all cases (**Figure 3**). The majority of screened samples were scored with 3 or 4 (**Table 4**).

Proficiency Testing Report

DNA Extraction From FFPE Cells

Our results (16,990 ng/20 μm slice) were compared to all the results' average (7,453.22 ng/20 μm slice) and silica membrane-based (8,141.92 ng/ 20 μm slice). They have been designated as "accurate" or "very satisfactory," consensus score "0." The ratio 260/280 (1.95) has been designated as "accurate" or "very satisfactory," consensus score "0." The DIN (5.40) was also designated as "accurate" or "very satisfactory," score 0, compared to all results average (4.49). The ENZO score was qualified as good-excellent and the level of PCR inhibitors were qualified as compatible with CGH assay.

DNA Extraction From Frozen Tissue Scheme

Our results (1,733.60 ng/mg tissue) were compared to all results average (1,865.18 ng/mg tissue) and silica membrane-based (2,045.98 ng/mg tissue). They have been designated as "accurate" or "very satisfactory," consensus score "0." The double-stranded DNA yield per mg tissue were 1,134.60 ng/mg tissue. It was considered "very satisfactory" when compared with the mean of all results (955.83 ng/mg tissue). The ratio 260/280 (1.90) has been designated as "accurate" or "very satisfactory," consensus score "0." The DIN (6.30) was also designated as "accurate" or "very satisfactory," score 0, compared to all results average (5.67).

DNA Quantification and Purity

The accuracy of our measurements was qualified as "very satisfactory," consensus score "0" when compared with mean values: Tubes A/B/C, 246.1/117.6/ 31.5 vs. 248.5/119.5/32.7 μg/ml. DNA purity was evaluated as "very satisfactory," consensus score "0": obtained values were 1.71/1.30/1.83 compared with expected ratio values: 1.72/1.32/1.92.

RNA Extraction From Frozen Tissue Scheme

The average of all expected results was 1,843.68 ng/mg tissue, our result was 572.9 ng/mg tissue, designated "acceptable" or "satisfactory," consensus score "1." RNA purity was evaluated as "very satisfactory," consensus score "0": obtained ratio value was 2.1 compared with expected ratio value 2.03. The obtained RIN value was 6.8 instead 6.55 mean all values considered "very satisfactory," consensus score "0."

RNA Integrity Scheme

The three obtained values (2.73/4.33/9.47) were slightly different from expected values (2.55/4.74/9.43), yielding a consensus score of "0"/"1"/"0."

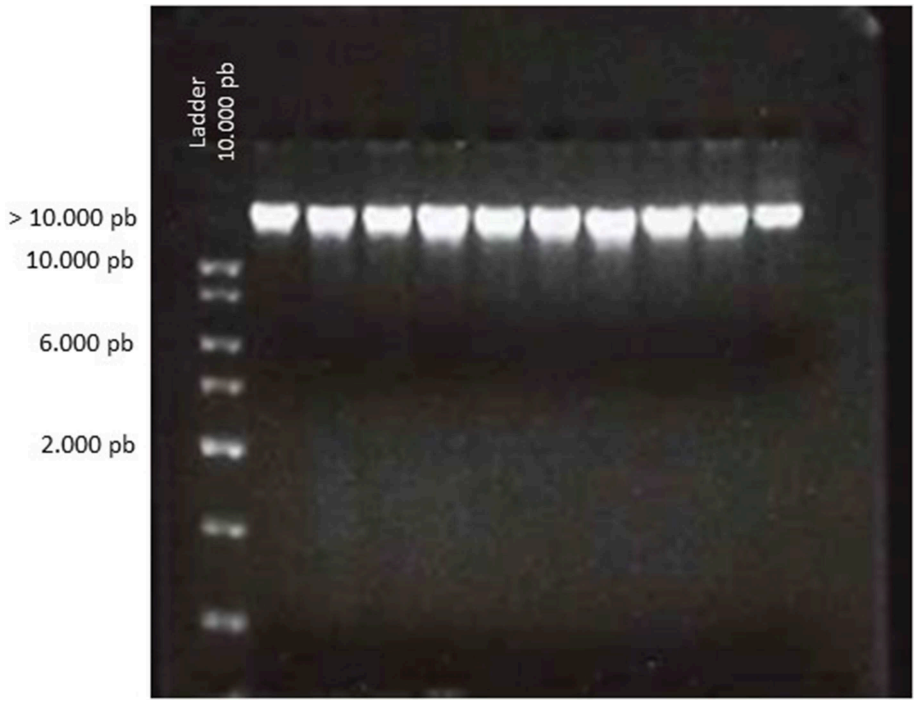

FIGURE 1 | Electrophoretic analysis of genomic DNA from biobanked frozen tumor samples. DNA (5 μL) was loaded on a 2% agarose gel and visualized by ethidium bromide staining. The gel shows the result of 10 representative samples. Compact bands of DNA were observed for all samples at a high molecular weight according to the ladder. The absence of smearing favors the absence of DNA degradation.

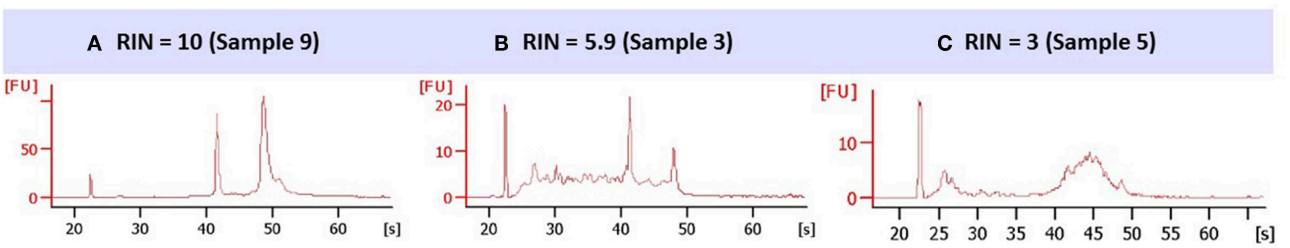

FIGURE 2 | Representative electropherogram for different RIN classes. 1 μL of sample RNA was charged in the microfabricated chips. **(A)** RIN = 10, from one representative sample classified as score 4; the different regions (pre-, 5S-, fast-, inter-, precursor-, post-region) and peaks (marker, 18S, 28S) are correctly presented. **(B)** RIN = 5.9, from one representative sample classified as score 3; intermediate peaks appear on the zone 5S and fast-regions, pointing to RNA degradation. **(C)** RIN = 3, from one representative sample classified as score 2; peaks of ribosomal subunits, 18S and 28S, are absent.

RNA Quantification and Purity Scheme

The three obtained values (93.6/60.1/33.4 μg/ml) were slightly different from expected values (90.5/60.1/33.4), yielding a consensus score of "0"/"0"/"1." RNA purity was evaluated as "satisfactory" and "very satisfactory," data not shown.

Tissue Histology Scheme

Regarding tissue histology, our consensus score required revision for the slide A, colon adenocarcinoma, and was satisfactory or very satisfactory for slides B and C, breast carcinoma. Evaluating viable tumor tissue was satisfactory or very satisfactory for slides D and E.

DISCUSSION

The Institut Jules Bordet tumor bank is completely integrated in the Pathological Department. The pathologist and technician pathologist are critical to identify the presence and type of tumor lesion and are responsible for ensuring diagnostic use prior to releasing tissue for research. The proximity of the operating room allows specimens quick handling reducing pre-analytical biases. Some samples stored in our biobank are already more than 20 years old.

Medical research projects are dependent on biobanked tissue of high quality because the gene expression analysis is affected by the quality of extracted RNA and DNA (6). Different factors

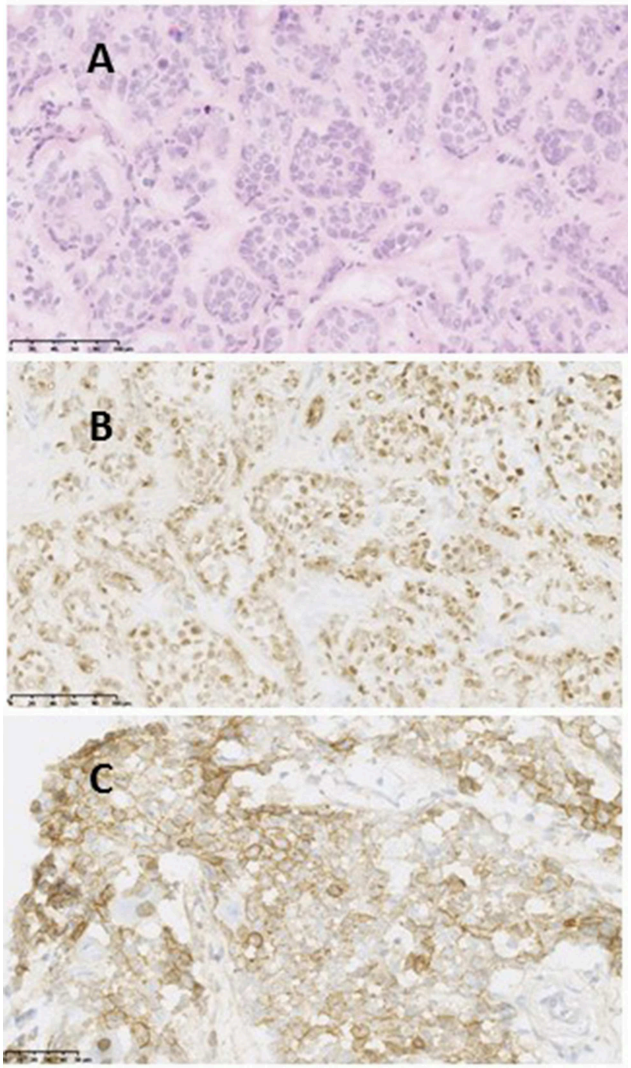

FIGURE 3 | Frozen breast tumor sample. **(A)** Hematoxylin/eosin staining (30x); **(B)** ER IHC staining; **(C)** Her2 IHC staining. The tumor was characterized as ductal carcinoma, ER/Her2 positive, by the pathologist in 2001 and confirmed on the 16-year-old frozen sample. The pathologist evaluation is based on four criteria: the intensity of staining, the percentage of positive cells, background, and the localization of hybridized antibody (membrane, cytoplasm).

TABLE 4 | Summary of the IHC scores assigned to samples of the study cohort based on the visual evaluation.

IHC staining	Score 4 (%)	Score 3 (%)	Score 2 (%)	Score 1 (%)
Her2	64	31	5	0
ER	57	33	10	0
Ki67	82	16	0	2

TABLE 5 | Suggested molecular biology applications based on the value of the RIN.

RIN	Suggested application
1–4	PCR Amplification of small fragments
4.1–6.9	qRT-PCR applications
7.0–10.0	Any application evaluating gene expression

influence the quality of nucleic acids and proteins including pre-analytical variables, transport, duration of processing at ambient temperature, necrosis, temperature and freezing products, size and number of aliquots and storage duration. The long-term storage temperature could impact the tissue quality. We currently use the OCT embedding medium on cryovials, followed by −80°C storage temperature for the solid tumors. The OCT embedding medium acts as cryoprotector from the freeze-thaw effects and gives the possibility to verify the histology after frozen sectioning and H&E staining.

Histologic quality control must be performed on biological samples. Different percentage cut-offs of tumor nuclei are required for each downstream use of the sample. The percentage of viable tumor cells is very important to perform NGS. It can range from 2% (7) to 80% (8). In our opinion, it is crucial to inform the researcher on the histologic quality before performing sensitive and expensive techniques.

RNA preservation is particularly important for gene expression analysis. RNA is known to be quickly degraded by ubiquitous RNase enzymes. OD reading is useful for determining the amount and purity of nucleic acids. Ribosomal RNA integrity is often used to reflect all RNAs physical integrity. In our study, RIN values were evaluated at score 4, even for the oldest samples. In addition to rRNA which represent 80% of total RNA, the messenger RNA (mRNA) and microRNA (miRNA) which constitute a small class of coding and, respectively, noncoding cellular RNA are the most interesting target for research. The stability of mRNA is better despite complete degradation of rRNA (9). Once again, the researcher together with biobank staff has to establish the tissue quality requirements before starting the research project. If the rare tumors are concerned by the research, too stringent criteria must be revised.

We have determined the suggested applications based on the value of the RIN (**Table 5**) (10).

At our institute, optical density and gel electrophoresis are commonly adopted for quick evaluation of extracted DNA purity and integrity. On a 2% agarose gel, intact genomic DNA appears as a compact, high-molecular-weight band with no low-molecular-weight smears. The scores assigned to our 2017 QC were mostly of 4, demonstrating an excellent quality of stored frozen tissues. Amplifying a specific sequence by PCR could give an information about usability of DNA for downstream molecular applications. Low amounts of PCR products can be attributed to poor quality DNA or poor quality tissues.

Investigation of different surface proteins can yield useful information on pathological pathways or biomarkers related to a particular disease. Specific immunohistochemical stains can be performed to evaluate specific antigens. This technique is routinely used on FFPE diagnostic blocs.

Assessing protein integrity is important since the freezing process may result in proteolysis and protein degradation (11, 12). Nevertheless, an accessible comprehensive way to assess protein quality is not available for frozen samples. Histologic evaluation of the tissue by the pathologist can provide a preliminary screening of degraded tissues. Our method fits with our lab equipment. Evaluating proteins quality by IHC of frozen tissue is really challenging because of cell structure freezing-related modifications. Training or experience is required for the pathologist scoring the stained slides. Mass spectrometry has become a crucial technique for almost all proteomics experiments, it should be considered for further analysis. It represents, indeed, the gold standard technique to test the protein quality, this technique is judicious when available. Using one or other technique is depending on the laboratory equipment and possibilities.

External quality tests, such as the ISBER Biorepository proficiency testing (13), allow both validation and improvement of protocols. Every failed QC item is deeply analyzed and corrected. If necessary, a dialogue with the external partner is established for additional information. Data can be exchanged regarding the test performance or the technician's work. The protocol deviation is then registered and corrective action adopted.

CONCLUSION

In conclusion, we proposed in this paper an easy quality control schema of biobank stored frozen samples with different ages, different tissue types and different types of morphology. Quality control for RNA, DNA and proteins might be performed periodically on a subset of samples in a biobank. The quality of our tumor samples was very satisfactory and adapted to a large panel of "next-generation" technologies. Our methods and techniques were validated by the external ISBER Proficiency testing program. Based on easy scoring procedures, the biobanks can give indications for downstream molecular biology application (14).

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

LC conceived and designed the experiment. SS performed the techniques. LC, SS, MR, RS, AW, PD, LV, MG-G, MC, NS, NSA, and DL analyzed the data. IL and FR quality management supervisor. LC and MR wrote the paper. DL oversaw the project.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

BRIEF RESEARCH REPORT
published: 18 October 2019
doi: 10.3389/fmed.2019.00230

Inflammatory Bowel Disease (IBD)—A Textbook Case for Multi-Centric Banking of Human Biological Materials

*Isabelle Cleynen[1], Loes Linsen[2], Sare Verstockt[1], Bram Verstockt[3], Vera Ballet[4], Eline Vandeput[4], Gert Van Assche[4], Marc Ferrante[3,4], Kristel Van Landuyt[2], Séverine Vermeire[3,4] and Nadine Ectors[2]**

[1] Laboratory for Complex Genetics, Department of Human Genetics, KU Leuven, Leuven, Belgium, [2] Activity Center Biobanking, University Hospitals Leuven, Leuven, Belgium, [3] Department of Chronic Diseases, Metabolism and Ageing (CHROMETA), Translational Research Center for Gastrointestinal Disorders (TARGID), KU Leuven, Leuven, Belgium, [4] Department of Gastroenterology and Hepatology, University Hospitals Leuven, KU Leuven, Leuven, Belgium

OPEN ACCESS

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Johns Hopkins Medicine, United States

***Correspondence:**
Nadine Ectors
nadine.ectors@uzleuven.be

Specialty section:
This article was submitted to Translational Medicine, a section of the journal Frontiers in Medicine

Received: 24 May 2019
Accepted: 03 October 2019
Published: 18 October 2019

Citation:
Cleynen I, Linsen L, Verstockt S, Verstockt B, Ballet V, Vandeput E, Van Assche G, Ferrante M, Van Landuyt K, Vermeire S and Ectors N (2019) Inflammatory Bowel Disease (IBD)—A Textbook Case for Multi-Centric Banking of Human Biological Materials. Front. Med. 6:230. doi: 10.3389/fmed.2019.00230

Inflammatory bowel disease (IBD) is a chronic relapsing inflammatory condition affecting mainly the gastro-intestinal tract with two main entities: Crohn's disease (CD) and ulcerative colitis (UC). Although the exact mechanisms underlying the initial development of IBD are not fully understood, it is believed that an abnormal immune response is elicited against the intestinal microbiota in genetically predisposed individuals. Crucial elements of the etiopathogenesis have been elucidated by research using human biological materials. The estimated prevalence of IBD is 0.5% in the Western world. Although incidence rates are increasing, both conditions are not "common" in general terms mandating a multicentric approach. Biological material from numerous Belgian patients have been collected over time in a number of university hospitals in Belgium (UH Ghent: 800 CD patients, 350 UC patients, 600 normal controls; UH Leuven: 2,600 CD patients, 1,380 UC patients, 98 IC/IBDU patients, 6,260 normal controls). Within the setting of the Flemish Center Medical Innovation (CMI) initiative and later on the Flemish biobank network a prospective study was set-up across three Belgian IBD centers (University Hospitals Brussels, Ghent, and Leuven). Human biological materials and data have been collected prospectively from newly diagnosed CD and UC patients. The analyses hereof have generated new insights which have been published in the most renowned journals. The approach of well-thought off, multi-centric, structured, and systematic biobanking has proven to be a success-story and thus a textbook case for multi-centric banking of human biological materials. This story is being told in this article.

Keywords: inflammatory bowel disease, biobank, BBMRI.be, PSI, BBMRI.nl, Crohn's disease, ulcerative colitis

INTRODUCTION

A limited number of European biobanking initiatives relate to Inflammatory Bowel Diseases (IBD). These initiatives have shown to be highly effective in output, output as quantified by numbers of publications, grants obtained, multicentric collaborations. Unfortunately, they also confirmed the greatest fear and risk for biobanks i.e., concerning sustainability.

Inflammatory Bowel Diseases in a Nutshell

Inflammatory bowel disease (IBD) is a chronic inflammatory condition mainly—but not solely—affecting the gastro-intestinal tract. The two main subtypes are Crohn's disease (CD) and ulcerative colitis (UC). These two main forms of IBD have both overlapping and distinct clinical pathological features. About 11.2 million people are affected with IBD as of 2015 (1). Incidence and prevalence are also increasing since the 1950s (2, 3). Each year it newly occurs in 1–20 per 100,000 people, and 5–500 per 100,000 individuals are affected (2, 3). The disease is more common in North America and Europe than other regions, although incidence is also increasing in previously considered low risk groups (3). Often it begins in people aged 15–30 years or among those over 60[1]. Males and females appear to be affected in equal proportions (2). For both conditions, the etiopathogenesis is multifactorial (environmental and genetic). Numerous environmental risk factors have been identified by means of epidemiological studies: smoking, appendectomy, infections, antibiotics, diet and lifestyle, … (4). The genetic background of IBD has been extensively studied, with the identification of circa 240 genetic loci associated with IBD thus far (5–7). More recently, the microbiome—the bacterial content—of the gut has been demonstrated to play a crucial role (8). The clinical presentation (symptoms, onset) as well as the course and outcome of the disease are variable. Treatment options are medical and surgical. Standard treatment depends on the extent of involvement and disease severity. The goal is to induce remission initially, followed by prevention of relapse as long as possible. IBD can be treated with a number of medications including biologics and more recently bacterial recolonization. For some, the disease has a mild course, while for others surgery is necessary. The current classification system based on symptoms does not always predict which path the patient will take. Genetic data also shows the same uncertainty. Researchers have calculated the genetic risk scores of 30,000 IBD patients based on 160 loci that determine the predisposition to IBD (9). They discovered that Crohn's disease in the small intestine and Crohn's disease in the colon differed genetically as much as Crohn's disease in the colon compared to ulcerative colitis.

BIOBANKING

Belgium—University Hospitals

The University hospitals of Leuven (UZ Leuven) have a tradition of clinical care and research in IBD spreading over several generations. At the forefront of the internal medicine—gastroenterology G. Vantrappen, P. Rutgeerts, and S. Vermeire have carried on the tradition. Obtaining and investigating human body materials (HBM) have naturally always been part of these processes.

The creation of the VLECC—biobank (VLAAMS ERFELIJKHEIDSONDERZOEK CROHN EN COLITIS ULCEROSA; Flemish inheritance study of Crohn's and Ulcerative colitis) in 1997 was a turning point. The main aim was to collect serum, DNA, and clinical characteristics of IBD patients. The biobank collected serial serum samples of patients with IBD and healthy controls that gave informed consent to participate in this study. From then onwards, collection of HBM and associated data has been performed prospectively in a structured manner, and now also includes tissue (biopsies) and fecal samples. The biobank started as a monocentric initiative. Numerous projects on different topics have arisen from hereon: genetic studies; investigations on treatment with biologics; the issue of anti-TNF antibodies; effectiveness; and safety of biologics, role of genetics on the response to biologics, investigations on environmental factors, and the role of bacteria in the bowel, research on serologic markers … to name only a few. These projects in turn generated numerous multicenter projects, and has led to many publications in high-ranked international journals (Lancet, Nature, Nature Genetics, Annals of Internal Medicine, Gastroenterology, Gut…) (9–14). The UZ KU Leuven Biobank operates its activities according to a quality management system, based on ISO 9001 for quality management systems, complemented with the biobank specific ISO 20387 standard and the ISBER Best Practices for biorepositories.

The Leuven biobank now contains DNA and serum of >4,000 IBD patients, >3,000 unaffected relatives, and 1,300 healthy controls, and a unique set of 60 multiple-affected IBD families. This large biobank with patient material has put the Leuven IBD group at the forefront of translational research in the field of IBD, not in the last in the IBD genetics field. Together with the IBD centers of Liège, Ghent, and Brussels, the Leuven IBD group conducted a Belgian genome-wide association study in 2007 (15), and co-founded the International IBD genetics consortium in the second half of the years 2000. They joined the combined analysis of GWAS across different countries (meta-analysis), leading to the identification of up to 99 confirmed loci in 2010 (16–18). In 2013, the largest international endeavor so far was initiated with the combined analysis of over 75,000 samples, including a few thousand samples from Belgium (Leuven, Ghent, Liège, and Brussels) (5) and culminating in the identification of over 200 loci associated with the risk to develop CD (19). Currently, the newest technologies are applied on these datasets (next-generation sequencing), and will undoubtedly lead to important new discoveries to further disentangle the genetic architecture of IBD and insights in disease pathogenesis.

Belgium—Center Medical Innovation—CMI

The White Paper of FlandersBio in 2006, the VRW advice 120 regarding translational biomedical research in 2008, and the Technopolis business plan for the CMI (then the CTBI) in 2009, presented proposals for a translational biomedical research initiative in Flanders[2] (20). The development of a strong center for translational biomedical research is also in accordance with the Flemish policy as an important part of the ViA Doorbraak "Flanders Innovation Centre," the development of Flanders' Care, 2 and the policy plans of the Flemish Minister of Innovation, Ingrid Lieten[3]. The aim of the CMI was to ensure high quality

[1]NIDDK. (2014). Archived from the original on 28 July 2016.

[2]White Paper of FlandersBio 2006 www.flanders.bio.

[3]Policy memorandum, Science and Innovation, Policy Priorities 2011–2012, Minister Lieten, 26 October 2011.

translational biomedical research at an international level, based on cooperation between the Clinical Research Centers (CRCs) in Flanders and their partners, and the development and exploitation of the Flemish Biobank in this context. The growth of translational research in Flanders aims at contributing to better health care for the patient, and related to this, to economic and societal added value for the region and beyond.

The CMI has achieved important milestones in its preparatory task, in particular the preparation of the Flemish Biobank. Cooperation between the University Institutes and their CRCs is important for the prospective collection of biobank samples because the broadest possible population is covered in this way to collect as many samples as possible. On the basis of the inventory of the existing biobanks and the needs of translational biomedical research, "Focus Biobanks" were set up. High quality samples in the Focus Biobanks will be included in the Flemish Biobank with a central ICT backbone. The FFEU funds made it possible to establish the infrastructure for a good quality biobank in all the 4 affiliated CRCs, for CRC Leuven in cooperation with UHasselt.

For the CRC Leuven, the IBD initiative was an obvious choice as "Focus Biobank." Intermediary analyses related to the performance of the focus biobank demonstrated clear-cut correlations with use of HBM and scientific output (**Figures 1A,B**).

At the time of the final evaluation, data (minimum data sets—MDS) from this collected HBM were uploaded on the central ICT backbone of the CMI. By the end of 2017, the data of 70,347 samples of IBDs were visible. These collections were obtained prospectively in the course of time in the context of multiple specific research projects. The uploaded cases fit with the participation in the initiated projects within the CMI (focus biobank/research platforms lead Leuven).

Within the CMI setting different projects were started, a retrospective project (University Hospitals Leuven, Ghent) led by Ghent and a prospective multicenter HBM collection (University Hospitals Leuven, Ghent, Brussels) ["BIB" (biobank IBD) project] led by Leuven.

In the meantime the initial CMI initiative has been ended. After an initial funding provided by the Flemish Government (8 mio € for the set-up of 4 CRCs and a centralized IT backbone) the CMI stopped to exist because of lack of funding at the end of 2017.

However, the "BIB" (biobank IBD) project is still running and has been renamed to "PANTHER" ("Prognostic factors in patients with early Crohn's or colitis" study). The PANTHER study is aimed at characterizing newly diagnosed IBD patients and their disease progression. The design consists of a multicenter, standardized longitudinal follow-up, which not only includes phenomics, but also resampling at specified time points. End 2015, the IBD centers at the University Hospitals Leuven, Ghent and Brussels started to prospectively collect DNA, stool, serum, and endoscopy-derived intestinal biopsies (inflamed and uninflamed) of patients newly diagnosed with IBD (max. 6 months), naïve to biologic and immunosuppressive therapy, and no previous surgery related to the disease. Corticosteroid or 5-ASA use at diagnosis is noted in 8–9% of the cohort, and is considered as a covariate for downstream analyses. The included patients are followed longitudinally; clinical information (disease characteristics including standard biological measurements, medication use, demographics) is gathered; and different sample types are collected according to agreed SOPs and at predefined time points or when there is a marked change in disease characteristics or treatment policy (see **Figure 2** for details). So far we included 234 patients (150 CD, 84 UC), with maximal follow-up at this moment of 3.6 years. Patient inclusion and follow-up has thus been ongoing since end 2015, a ratio of 80 inclusions per year. Inclusion rates and clinical characteristics are as expected, and is a continuous effort.

The previous years, the PANTHER cohort was predominantly used for diagnostic purposes, and thus to identify signatures that can separate patients from controls. Results from these analyses have been presented at (inter)national meetings and are being prepared for full paper submission. They are part of an ongoing PhD and master thesis project conducted at KU Leuven under the supervision of Prof. I. Cleynen and Prof. S. Vermeire. With continued inclusion and follow-up, the next stage of the project is to better understand disease heterogeneity to facilitate biomarker development and patient stratification. A new project proposal has been submitted to now identify biomarkers for disease progression by multi-dimensional holistic analyses of mucosal tissue and peripheral samples based on genetics (single cell), transcriptomics, and serology. With these unique datasets, we work toward a precision medicine approach aiming to tailor treatment of individual patients instead of a one-size-fits-all approach. The data gathered in this project will also allow data sharing with similar international consortia (see below Parelsnoer), and thus enable *ad hoc* international collaboration, data sharing, and access to larger sample sizes.

The Netherlands—Parelsnoer Institute—PSI

The Parelsnoer Institute (PSI), established in 2007 by the Dutch Federation of University Medical Centers (NFU), offers researchers within the eight University Medical Centers and external researchers an infrastructure and standard procedures for the establishment, expansion and optimization of clinical biobanks for scientific research (21). By collecting and storing clinical data, images and human biomaterial together in a uniform manner from carefully documented patients suffering the same illness, large cohorts are established (the so-called "Pearls") that enable broader scientific research.

To this aim, the prospective Dutch IBD Biobank was created. Gastroenterologists who specialized in treating patients with IBD in all eight Dutch university medical centers (UMC), together with a team of information architects and laboratory experts, built up the Dutch IBD Biobank. The main objective of the biobank is to facilitate the discovery of predictors (both epidemiological risk factors and biomarkers) for individual disease course and treatment response, by: providing full clinical records of patients describing their individual disease course over a prolonged period of time; providing high-quality biomaterials; standardizing patient data collection; and questionnaires during outpatient clinic visits and thereby improving clinical care (22).

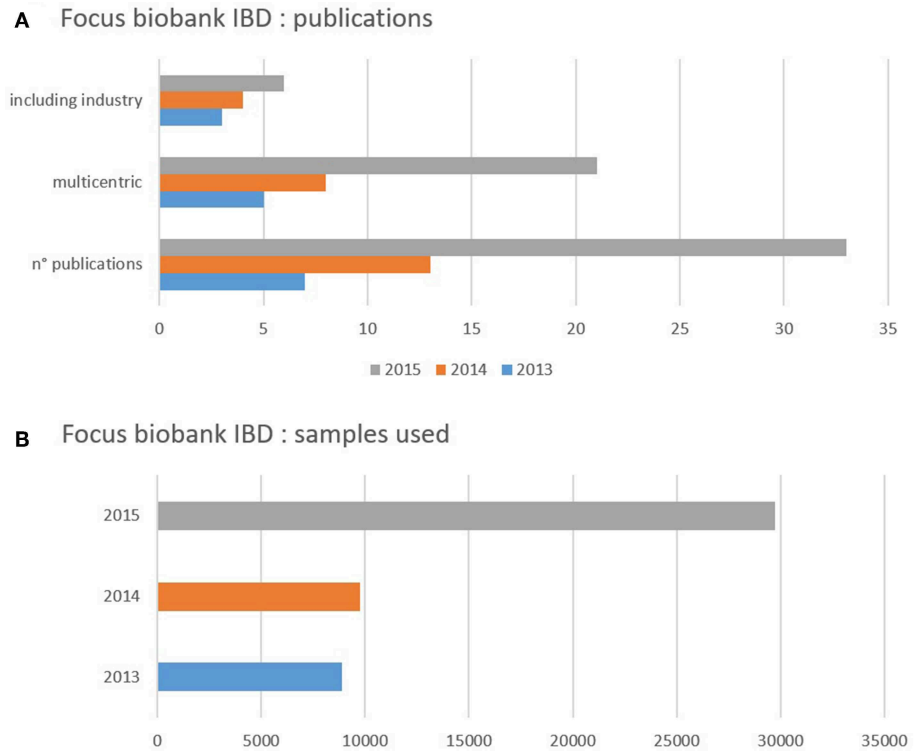

FIGURE 1 | (A,B) Impact of biobanking initiative on samples used in research projects (in function of time) and initiating publication. **(A)** Shows the number of samples of human biological material used during 1 year (2013 blue, 2014 orange, 2015 gray). The figure demonstrates a clear increase in the use of samples over time which correlates with the activities in the biobank but also and more importantly with the output in publications **(B)**. **(B)** Shows the number of publications (*n*° publications) reporting on results achieved using the samples of human biological material during 1 year (2013 blue, 2014 orange, 2015 gray). The figure demonstrates an overall increase in number of publications. The total number of publications was split up highlighting two distinct categories: most of the studies were multicentric with an increasing number of the years. This highlights one of the needs/advantages in relatively rare diseases. The other highlight is the proportional increase of studies whereby the industry was one the scientific partners (participation not limited to sponsoring) which demonstrates one of the strengths of Biobanking.

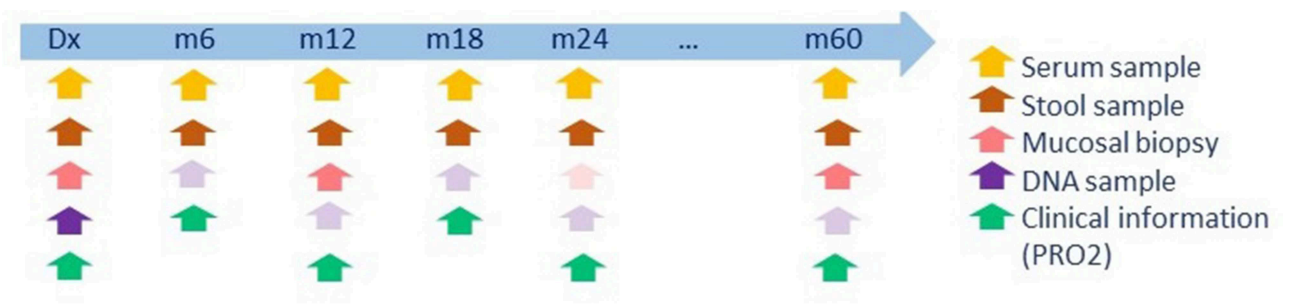

FIGURE 2 | Time points and sample collection of the PANTHER cohort. Biopsies are only collected when a colonoscopy is required for clinical follow-up. If macroscopic inflammation is present, a biopsy is taken both at an inflamed site, and a macroscopically inactive site. Bright arrow colors indicate required samples; faint colors indicate optional samples. Dx, diagnosis; m, month.

In their article Spekhorst et al. refer to 3,388 patients with IBD enrolled in June 2014, IBD: 2,118 Crohn's disease (62.5%), 1,190 ulcerative colitis (35.1%), 74 IBD-unclassified (2.2%), and 6 IBD-indeterminate (0.2%) (22). Besides samples of HBM the Dutch IBD Biobank prospectively collects 225 standardized data items on various topics, including patient demographics, family history, diagnosis, disease activity, disease localization, results of physical examinations, radiographic imaging results, laboratory and endoscopy results, previous and current treatment, as well as a wide array of disease and treatment complications.

Similarly to the CMI project, after a large initial grant provided by the Dutch government to the Netherlands Federation of University Medical Centers facilitating the establishment of the Dutch IBD Biobank and seven similar biobanks ended in 2011, the Dutch UMCs had to fund the continuation of the Dutch IBD Biobank themselves, meaning a reduction of staff that assisted

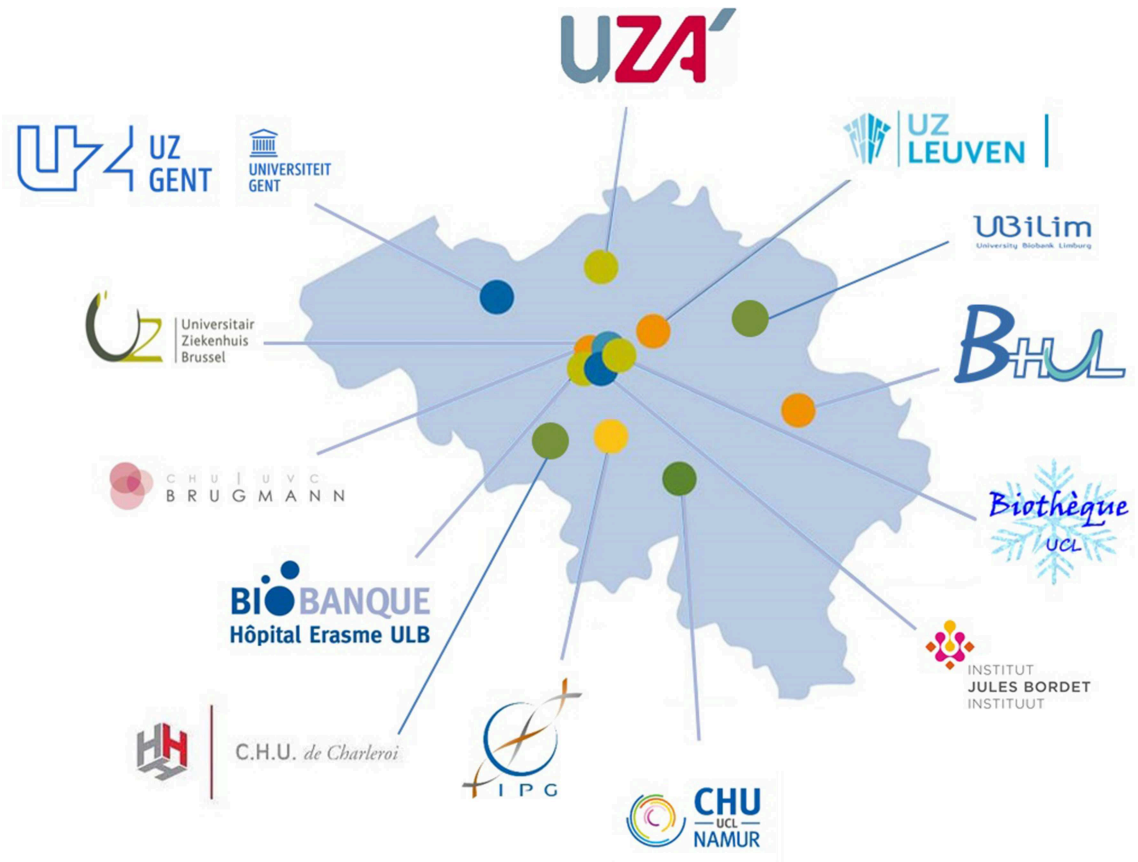

FIGURE 3 | Overview of the Belgian biobanks currently connected to the BBMRI.be network. The BBMRI.be network connects 13 biobanks that are linked to public institutions such as hospitals, universities, and research centers.

in patient inclusion in some centers. As a consequence, the enrolment of patients has slowed down in these centers (22). Here too the project kept on going.

Europe—BBMRI—EU

The European Strategy Forum on Research Infrastructures (ESFRI) produced its first roadmap in October 2006 (23). Biobanking and BioMolecular resources Research Infrastructure (BBMRI) was one of the proposals, it is the largest infrastructure launched in Europe in health research. The ambitious mission of the BBMRI was to sustainably secure access to biological resources and data required for health-related research in Europe. The 7th European Union Framework program funded a 3-year BBMRI preparatory phase project (5 million Euros). Over time, a catalog from existing major population-based and clinical or disease-orientated biobanks was created with overall 20 million human biological samples (24). The members of BBMRI-ERIC were the European countries and intergovernmental organizations that have signed the BBMRI-ERIC Statutes. Founding Member States at that time were Austria, Belgium, Estonia, Finland, France, Germany, Greece, Italy, Malta, the Netherlands, and Sweden. BBMRI-ERIC primarily aims at establishing, operating, and developing a pan-European distributed research infrastructure of biobanks and biomolecular resources. This will facilitate the access to biological resources as well as biomedical facilities and support high-quality biomolecular and medical research. By nature it is a distributed infrastructure, in which biological samples and data are hosted by the European Member States biobanks.

BBMRI.be was set up in order to support the ever-increasing need of research with regard to quality control, access, transparency, and interconnectedness of biobanks. The scientific participation of Belgium in BBMRI-ERIC is exerted by a national node that was initiated by uniting the three existing Belgian network biobank initiatives i.e., Belgian Virtual Tumourbank project assigned to the Belgian Cancer Registry (BVT-BCR), Biothèque de la Fédération Wallonie-Bruxelles (BWB), and the Flemish Biobank Network (CMI). This network connects 13 biobanks that are linked to public institutions such as hospitals, universities and research centers and is included in the Directory of BBMRI-ERIC (**Figure 3**). BBMRI.be has matured into a solid partner network on biobanks in Belgium and has proven to reach out to a broader community beyond the founding partners. Data from the CMI focus biobank on IBD have been listed in the BBMRI-ERIC catalog as a clinical/disease-orientated biobank.

TABLE 1 | Based on a data search in the BBBMRI-ERIC directory (performed on the 29th April 2019) 8 biobanks were found on https://directory.bbmri-eric.eu/menu/main/app-molgenis-app-biobank-explorer/biobankexplorer?diagnosis_available=K50,K51 (21).

	Collection	Type	Materials	#Samples
Nl	Academic medical center biobank			
	Collection types: Longitudinal, disease specific			
	Juridical person: AMC			
	Low countries Vedolizumab in Ulcerative Colitis study	Longitudinal	Serum, tissue (paraffin preserved)	100–1,000
	Low countries Vedolizumab in Crohn's disease study	Longitudinal	Serum, tissue (paraffin preserved)	100–1,000
	Predictive biomarkers and the role of the microbiome on treatment for inflammatory bowel disease	Disease specific, longitudinal	Feces, tissue (frozen), whole blood	1,000–10,000
B	Bimetra Biobank @ UZ Gent			
	Collection types: Longitudinal, disease specific, hospital			
	Juridical person: University Hospital Ghent			
	CRC Focus Collections @ Bimetra	Longitudinal, disease specific	DNA, plasma, serum, feces, other, whole blood, tissue (frozen)	19,848
	Inflammatory Bowel disease focus collection	Longitudinal, disease specific, hospital	Serum, plasma, DNA, RNA, tissue (frozen), feces, tissue (paraffin preserved)	2,617
Nl	BioBank Maastricht UMC			
	Collection types: Cohort, disease specific, longitudinal, population-based			
	Juridical person: Maastricht UMC+ (MUMC)			
	Inflammatory Bowel Disease Zuid Limburg Biobank	Cohort, disease specific, longitudinal, population-based	DNA, feces, other, plasma, RNA, serum, tissue (paraffin preserved)	1,000–10,000
B	Biobank-University Hospitals Leuven			
	Collection types: Disease specific			
	Juridical person: University Hospitals Leuven (B0383)			
	Inflammatory Bowel Disease (IBD)	Disease specific	DNA, plasma, serum, urine, saliva, feces, other, RNA, tissue (frozen)	100,000–1,000,000
Nl	CDDSS Knowledge base			
	Collection types: Cohort, disease specific, hospital			
	Juridical person: No information			
	Clinical Diagnostic Decision Support System on Anemia	Cohort, disease specific, hospital	Pathogen, plasma, whole blood	100–1,000
It	Cell line and DNA Biobank from patients affected by Genetic Diseases			
	Collection types: Case-Control, disease specific			
	Juridical person: Istituto Giannina Gaslini			
	Collection all Samples	Case-control, disease specific	Cell lines, DNA, other, plasma, serum, urine, RNA, whole blood	12,430
Nl	Parelsnoer			
	Collection types: Disease specific			
	Juridical person: No information			
	Parel Inflammatory bowel disease	Disease specific	DNA, feces, serum, tissue (frozen), tissue (paraffin preserved)	1,000–10,000
B	University Biobank Limburg			
	Collection types: Disease specific			
	Juridical person: University Hasselt/Jessa Hospital			
	University Biobank Limburg	Disease specific	DNA, other, plasma, RNA, serum, tissue (paraffin preserved), urine, whole blood, tissue (frozen), feces	14,431

PSI and the Dutch IBD Biobank participate in the BBBMRI-ERIC project too as are part of the Biobanking and Biomolecular Resources Research Infrastructure of the Netherlands (BBMRI-NL). This is the Dutch national node of BBMRI-ERIC, the largest research infrastructure project in Europe (25). The BBMRI-NL biobank catalog is a searchable database, containing information on several Dutch bio- and databanks. To date, there are over 200 bio- and data collections listed.

Based on a data search in the BBBMRI-ERIC directory (performed on the 29th April 2019) 8 biobanks were found (as shown in **Table 1**) https://directory.bbmri-eric.eu/menu/

TABLE 2 | Analysis of strengths, weaknesses, opportunities, and threats of a biobank.

Strenghts	Weaknesses
Large number of samples Better quality of samples Presence of associated data Presence of access procedures	Sustainability vs. Non-profit setting "Ownership" Public trust Social acceptability Difficulty in implementing longitudinal sampling strategies Communication/marketing
Opportunities	**Threats**
Higher scientific output Development of innovative projects, new clinical trials, new diagnostics tested Integration of data Increased service provision Scientific collaboration with different partners	Lack of contingency plans Integration of big data incl. imaging data Accreditation requirements may increase cost structures

main/app-molgenis-app-biobank-explorer/biobankexplorer?diagnosis_available=K50,K51 (26).

LESSONS LEARNED—SWOT ANALYSIS

SWOT analysis (syn. SWOT matrix) is a strategic planning tool used to help a person or organization identify strengths, weaknesses, opportunities, and threats related to business competition or project planning[4]. It is intended to identify the internal and external factors that are favorable and unfavorable to achieving those objectives.

The strengths of a biobank are obvious and numerous (**Table 2**). They are related to the number and especially the quality of the samples of HBM and specific features, associated data and procedures for access to samples. Quality is based on and identified as adherence to standard quality principles and procedures, certification, accreditation … Numerous systems do exist (e.g., ISBER, OECD, WHO, …). The Organization for Standardization (ISO) has set in 2018 specific requirements for bioresources for research i.e., Biotechnology—Biobanking—General requirements for biobanking (ISO 20387). Specific features may relate to the clinical origin, detailed information on the pre-analytical procedures, … which will determine the uniqueness or rarity of the HBM. Opportunities relate directly or indirectly to scientific outcome and examples of threats are disasters and the lack of contingency plans. The most commonly recognized "weakness" for biobank is sustainability, the difficulty of covering the total cost of the initiative independently of the economic model adopted. The primary support for biobanks is nearly evenly divided among grant support, public (government), and private funding at nearly 30% each (27, 28). Both the CMI initiative and the Dutch IBD Biobank went through a difficult time when external (public/governmental) funding stopped. In times of tight economic realities in research the need to discuss with stakeholders and reappraise financial models for biobanking is mandatory. Especially since biobanking has finally attained recognition as a key infrastructure for scientific research and clinical care.

[4]SWOT Analysis: Discover New Opportunities, Manage and Eliminate Threats. www.mindtools.com.

CONCLUSIONS

A biobank collects, stores, processes, and distributes HBM and related health data for use in both fundamental research and clinical studies. The biobanking field has changed greatly over the last three decades, in general and in particular as demonstrated in this case study, starting with a university-based collection developed for the needs of particular project e.g., the VLECC—biobank. It then gradually evolved to an initiative supporting different projects, generating multicentric collaboration and an exponential increase in scientific output both in volume and in quality (impact factor and citation index). As described in this paper on IBD, biobank research does provide novel insights into amongst others the genetic component of disease, ultimately leading to a more personalized approach to healthcare.

As described in this article, long-term sustainability of biobanks remains a major concern. Literature review demonstrates that total cost-recovery strategies are not the best approach to reach and maintain sustainability. Biobanks will always require support by long-term investment and commitment, preferentially from public and governmental sources.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

AUTHOR CONTRIBUTIONS

The authors as well as the acknowledged participants participated in the biobanking project and the realization of initiative. IC, LL, and NE wrote the article.

FUNDING

In 2006, the Flanders White book Life Sciences recommended Translational Medicine as focus for a Public-Private Partnership. The Flemish government funded a regional biobanking initiative with FFEU resources (Debt Reduction Financing Fund and One-off Investment Expenditure). Funding was obtained via the Institute for Science and Technology (IWT) and the Ministry for economy, science and Innovation (EWI). The University Hospitals Leuven invested the money in the automation of sample storage. In the meantime the CMI—center medical innovation initiative has been aborted because of lack of funding.

ACKNOWLEDGMENTS

The authors acknowledge the participation of the following persons in the CMI initiative: for the University

Hospital—University Ghent, Department of Gastroenterology: Elien Glorieus, Pieter Hindryckx, Martine De Vos, and Debby Laukens (VIB Center for Inflammation Research). For the University Hospital—University Brussels Department of Gastroenterology: Murielle De Decker and Fazia Mana. The authors thank Ilse Debroek for administrative support.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

PERSPECTIVE
published: 22 October 2019
doi: 10.3389/fmed.2019.00224

Raising to the Challenge: Building a Federated Biobank to Accelerate Translational Research—The University Biobank Limburg

Loes Linsen[1,2,3†], *Kimberly Vanhees*[1,2,3†], *Evi Vanoppen*[1,3], *Kim Ulenaers*[1,4], *Suzanne Driessens*[1,5], *Joris Penders*[1,2,5], *Veerle Somers*[1,4], *Piet Stinissen*[1,4] *and Jean-Luc Rummens*[1,2,3*]

[1] University Biobank Limburg (UBiLim), Hasselt, Belgium, [2] Faculty of Medicine and Life Sciences, Limburg Clinical Research Center, Hasselt University, Diepenbeek, Belgium, [3] Clinical Laboratory, Jessa Hospital, Hasselt, Belgium, [4] Faculty of Medicine and Life Sciences, Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium, [5] Clinical Laboratory, Hospital East-Limburg (ZOL), Genk, Belgium

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Integrated BioBank of Luxembourg, Luxembourg

***Correspondence:**
Jean-Luc Rummens
jean-luc.rummens@jessazh.be

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Translational Medicine, a section of the journal Frontiers in Medicine

Received: 24 May 2019
Accepted: 30 September 2019
Published: 22 October 2019

Citation:
Linsen L, Vanhees K, Vanoppen E, Ulenaers K, Driessens S, Penders J, Somers V, Stinissen P and Rummens J-L (2019) Raising to the Challenge: Building a Federated Biobank to Accelerate Translational Research—The University Biobank Limburg. Front. Med. 6:224. doi: 10.3389/fmed.2019.00224

Irreproducibility of research results is one of the major contributing factors to the failure of translating basic research results into tangible bedside progress. To address this, the University Biobank Limburg (UBiLim) was founded by a collaboration between Hasselt University, the Hospital East-Limburg, and the Jessa Hospital. This paper describes the evolution of this process and the barriers encountered on the way. UBiLim evolved from an archival collection over a single-site biobank into a federated structure, supporting translational research at the founding institutions. Currently, UBiLim is a federated biobank, with an established organizational structure and processing, and storage facilities at each of the three sites. All activities are integrated in an ISO15189-accredited Quality Management System and based on (inter)national biobank guidelines. Common methods for processing and storage of a plethora of sample types, suitable for state-of-the-art applications, were validated and implemented. Because the biobank is embedded in two hospitals, the request of researchers to include certain sample types or enroll specific patient groups can quickly be met. Funding has been a major challenge in each step of its evolution and remains the biggest issue for long-term biobank sustainability. To a lesser extent, the Belgian legislation and the operational cost of information management system are also concerns for smooth biobank operations. Nonetheless, UBiLim serves as a facilitator and accelerator for translational research in the Limburg area of Belgium that, given the fields of research, may have an impact on international patient care.

Keywords: UBiLim, quality, biobank, multi-disciplinary, translational research

INTRODUCTION

Despite major advances in life sciences and medical technologies, there often is a large gap between basic science outcomes and their translation into the clinic (1). A major contributing factor is the irreproducibility of preclinical research results, with one of the primary causes being the quality of the biological reagents and reference

materials used (2). Consequently, many researchers now question the validity of their previous findings because of concerns about the quality of the biospecimens (3). In the last decades, these observations led to the development of modern biobanking, with a focus on improved biospecimen quality through a more professional operational development (4). Furthermore, biobank networks were established to facilitate the acquisition of a higher number of biospecimens in a shorter amount of time to meet the increasing scale and complexity of research studies (5). Scandinavia has a headstart in establishing these networks because of their long tradition of large-scale biobanking combined with comprehensive, population-based health data registries linkable to unique personal identifiers, enabling follow-up studies spanning many decades (6). In 2004, these Nordic biobanks partnered together in the "Cancer control using population-based registries and biobanks (CCPRB)" project. The goal of this project was to facilitate and improve cancer research by combining biobank samples and registry data and to establish Good Biobanking Practices (7). The partner "Limburg Cancer Registry (LIKAR)" was incorporated into the network because of its pioneering and state-of-the-art cancer registration practices in the Belgian province of Limburg (8, 9). For the hematology data of the registry, it relied on a close collaboration with the Virga Jessa Hospital (Hasselt, Belgium), which routinely stored bone marrow (BM) smears used for the registration. In 2006, the CCPRB consortium decided to transform the ongoing hematology collection into an actual biobank. This was the founding step for the creation of the translational research supporting University Biobank Limburg (UBiLim), a collaboration between two regional hospitals and a university. This paper describes the evolution of an archival collection into a professional, federated biobank structure that successfully supports multi-domain translational research through provision of qualitative sample processing, storage, and distribution activities. It also highlights the barriers that were overcome at each stage.

RESULTS AND DISCUSSION

From Archive to Local Biobank: Starting Small but Aiming High

The systematic storage of stained BM smears in the clinical laboratory of the Virga Jesse Hospital had started in the mid-nineties. The archive was built to contain samples from all BM punctures routinely performed at or sent to the hospital. The scientific potential of this archive for hematological diseases was anticipated, also because of the presence of precursor stages of malignant diseases and the possibility to capture potential aggressive transformation events (10). Within the context of the CCPRB project, this archival collection was transformed into a biobank [defined here as a structured facility that receives (processes), stores, and distributes biospecimens coupled to associated (clinical) data and with all aspects (including personnel, infrastructure, etc.) managed according to professional and quality standards]. To this end, the collection was expanded from 2007 onwards to contain additional sample types such as plasma and white blood cell pellets from peripheral blood and BM, stored at −80°C. Additionally, the biobank processes were developed in accordance to the ISO 15189 accreditation of the laboratory to already ensure a high standard of all aspects of work (also including document/record management, training, etc.). Furthermore, sample processing methods were validated prior to use to provide fit-for-purpose samples (11). Finally, an in-house built sample management system was used to capture a limited set of donor and sample data on the samples. This multifaceted approach provided a clear added value to the quality and integrity of the sample, allowing for future research with more demanding needs.

The biggest challenge in the transformation to a local biobank was the availability of the necessary financial resources. All of the above was initially in the hands of one operational biobank manager, supported by the clinical lab director, and eventually supported by trained lab technicians for the actual sample processing. Generally, only 37% of costs were funded by the CCPRB project, while 63% of the cost was taken by the hospital. Personnel cost had the highest impact, with a 2:1 ratio to other costs (consumables, equipment, and miscellaneous). In this initial phase, the average operational cost was about €100.000, about half of what has been reported for biobanks of similar (budget) size and time in operation (12). This difference can probably be explained by the embedded nature of the biobank in the clinical laboratory, allowing the use of common facilities.

The sample management system built in-house proved to be an additional challenge. Initially, it had been set up by the local IT department to accommodate the processing and storage phase of the sample. However, it quickly became obvious that coverage of the pre-collection and post-storage phase (sample distribution, assign studies and projects, informed consent management, etc.) was also required to support the complete set of biobank activities. This required additional configuration, which was not budgeted for, and increased the dependency on the IT department imposing a higher risk of failure (13, 14). Given the selection of a commercial system within the CCPRB project, the in-house system was not developed further in attendance of implementation of the commercial system.

By 2009, at the end of the CCPRB project, a local biobank focusing on a single disease domain had successfully been established from an operational point of view. In addition to 39,060 bone marrow smears, 3,313 samples were available for research, representing twenty different hematological diagnoses. **Figure 1** shows the number of samples gathered in this collection yearly until December 2018. Initially, a sharp increase in sample number of the newly added sample types can be observed, as can be expected from process optimization to improve collection rates. A fairly stable number of samples were collected afterwards, with a trend of increased numbers showing for the last 4 years. This results from expanding the collection to incorporate paired blood samples and by introducing a smaller container type in 2017. Currently, the collection holds 60,897 samples in total and their fitness-for-purpose for omics-technologies has been demonstrated (15).

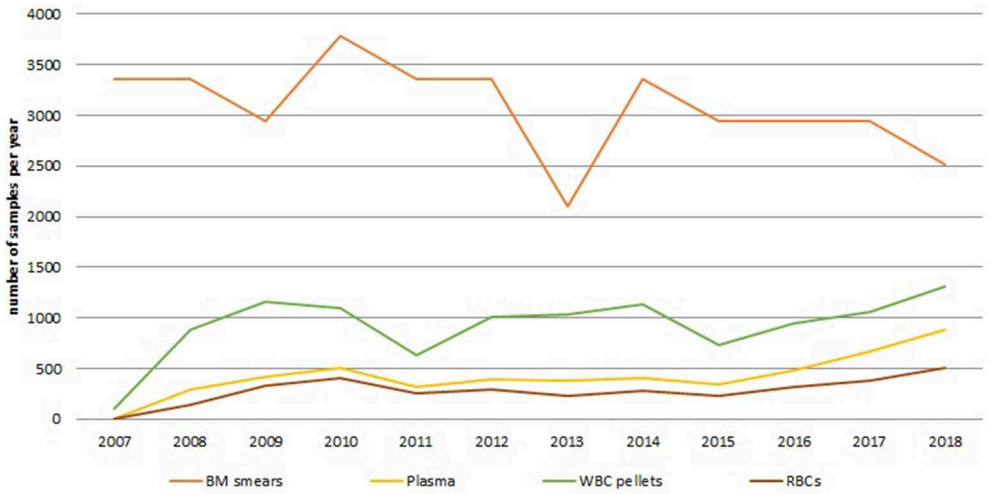

FIGURE 1 | Annual number of samples for the different sample types collected within the hematology collection since the start of the biobank to December 2018. The orange line shows the number of BM smear samples, the yellow line shows the number of plasma samples, the green line shows the number of white blood cell pellets, the brown line shows the number of red blood cell samples. BM, bone marrow; RBCs, red blood cells; WBC, white blood cell.

From Local Biobank to Federated Biobank: The Governmental Phase

Mid-2008, different biobank funding schemes were launched in Belgium: the National Cancer Plan and the Center for Medical Innovation organized by the federal and regional government, respectively. To increase the chances of obtaining financing for the biobank activities, a collaboration was set up between the two biggest regional hospitals of Limburg: the Virga Jesse Hospital, by then renamed to the Jessa Hospital, and the Hospital of East-Limburg, together with Hasselt University to form the University Biobank Limburg (UBiLim). Given the different physical locations, it was decided to construct a federated biobank with processing and storage facilities at the three sites. Its structure and processes were centrally controlled but allowed federated sample management according to harmonized procedures. However, despite the prominent and qualitative nature of the biobank, no direct funding for UBiLim could be obtained from the National Cancer Plan. A two-phase funding was granted for 4 years (2009–2013) by the local government to strengthen the collaboration by setting up a common framework and evolve to a federated biobank. The setup was built from the biobank platform already present at the Jessa Hospital, integrating the "external" activities into one structure according to the requirements of the existing biobank quality management system (i.e., training, document management, etc.). The activities were expanded further to include method validation for new sample processing procedures (16). Additionally, the performance of these procedures was assessed by participation in biobank-specific proficiency testing schemes since 2011 (17).

The available funding covered 80% of total costs of UBiLim (**Figure 2**). Fifty-six percent of the available budget was used to maintain the biobank personnel at the Jessa hospital and to gradually expand it with dedicated biobank technicians at the newly added sites of Hasselt University and the Hospital East-Limburg. Twenty-two percent of funding was invested in additional storage capacity, equipment for sample processing and quality control, and the local acquisition of a commercial biobank information management system accessible from the three sites. Operational costs averaged at 12% of total costs. Annual total costs tripled compared to the earlier phase of the biobank, due to increase of activities and associated needs for personnel and equipment. These numbers are comparable to those recently reported for international biobanks of similar size and time in operation (12). This study also indicated that on average up to 20% of biobank costs is covered by institutional funding. It should be noted, however, that the majority of biobanks in this study was US based, where dependency on publicly funded clinical research is higher compared to the other international respondents. In Belgium, however, the biobanking activities have not been incorporated in the national health program, generating a situation similar to the US. Nevertheless, the potential cost and availability of funding source aspects should be taken into consideration when starting up a biobank and demonstrate the need for support by the institution housing to achieve operational biobank success.

During this 4-years period, UBiLim's in-house built sample management system was replaced by the biobank information management system Labvantage–Sapphire, configured to meet the common biobank needs within the CCPRB project. Although this decision came with a large upfront cost, several arguments supported this conclusion: the completeness of the system regarding biobank processes, the ready-to-deploy state, the modularity to maintain flexibility, the stability of the system to contain several thousands of data, and the web application allowing external access. Typically, the first two items are decisive

FIGURE 2 | Annual cost distribution in the federated biobank phase (June 2009 to December 2013). Costs are subdivided in the six main accountancy cost categories: Personnel (green bar), IT infrastructure (yellow bar), Operations (brown bar), Promotion (olive bar), Equipment (orange bar), and Overhead (dark green bar). Percentage of cost coverage by public funding is displayed on the secondary *Y*-axis (orange line).

to choose for commercial solutions (13). The system's installation and additional customization costs attributed to 7.5% of total costs of biobank operations during this 4-years period (**Figure 2**). Actual implementation and validation at the biobank required additional configuration by a trained biobank super-user. This super-user commitment takes on average about 10% of personnel time for the overall period, similar to findings reported elsewhere (12). While training a dedicated biobank employee to super-user level requires additional investment, it reduces long-term running costs by avoiding expensive programmer hours for small adaptations. Furthermore, the inter-institutional access to this system, while allowing enough flexibility to cover site-specific needs, accelerated the harmonized approach ensuring the same quality standard across the three sites.

Consistent with best practices in the field, a governance structure was established at UBiLim, consisting of a steering committee, a scientific review board, and a management group, membered by representatives of the three institutes (18–20). The challenge in the composition of the scientific review board was to represent all relevant disciplines, while maintaining a manageable group size and institutional representation. This was achieved by a preselection of members by the management group, proposed to and approved by the institutional directors, which allowed immediate buy-in from all researchers and is a reported shared success factor for biobanks globally (21, 22). An access policy was set up, allowing access to internal and external researchers of both academic and commercial affiliation upon approval of (ethical review board approved) projects by the scientific review board based on the evaluation of scientific validity, sample prioritization, and funding. To protect the interest of the original sample collector, up to 5 years of exclusive access can be granted. Custody of the samples however remains at UBiLim. This approach is in line with the ethical and scientific consensus regarding access policies for biobanks improving/facilitating sample and data sharing for global health and again accentuates the progressive role UBiLim played in the Belgian landscape (23–28). In parallel, a national law was released in 2008 stating the conditions regarding the collection and use of human bodily material for therapeutic or research use (29). While primary and secondary use of samples for further research requires consent from the donor, an exception is made for leftover samples from clinical practices where an opt-out system is put in place. Although the biobank aspect of this law did not come into effect until 2018, the necessary processes were already put in place to accommodate these requirements.

From Federated Biobank to Translational Research Supporting Facility: Bridging the Gap

The next step in the evolution of UBiLim was to use its federated biobank approach to facilitate translational research. To accelerate the translation of innovations in health care into practical applications, the Flemish government invested in the establishment of the Flemish Biobank in 2009, by the foundation of the Center of Medical Innovation (CMI, now the Flemish Biobank Network). The primary approach was to set up professional biobank facilities in four Clinical Research Centers and centralizing the data in a virtual Flemish biobank catalog to allow increased visibility, use, and sharing of biospecimens. Five focus domains were identified, with Hasselt University heading

the activities of the Rheumatology focus group (the others being Sudden death, Hepatotropic viruses, Diabetes, and Inflammatory bowel disease) and UBiLim acting as Hasselt University's central biobank. Each CMI affiliate collected samples according to agreed harmonized quality guidelines and procedures within the CMI network, while samples remained under control of the collecting institution. This approach was similar to the Dutch String of Pearls Initiative, which has proven to be a successful method in addressing translational research challenges (30, 31). With Hasselt University/UBiLim not acknowledged as a full-blown center within the CMI project, only partial funding could be obtained for operational activities and participation in the development of the centralized catalog. Nevertheless, UBiLim passed a peer-review audit, set up within the CMI to verify the quality status of the affiliated biobanks, with flying colors despite being the only complex federated biobank in the project. Unfortunately, even though all key deliverables of the project were realized, funding for the CMI was discontinued in 2015 due to the changing political landscape, resulting in the abolition of the project. However, the rheumatology focus group for instance continues to collaborate through sample sharing to this day at their own expense, albeit at a slower pace, highlighting the strength of the project, and the resilience of the affiliated members (32).

In parallel to the CMI project, the three institutes that conceived UBiLim also set up the Limburg Clinical Research Program (LCRP) in 2010. Funding for this project was obtained from the Flemish and local provincial governments to enhance local innovation, health care, and education. Five research domains were defined in the LCRP program as key focus areas for project-based research (cardiology, oncology, anesthesia/neurology, gynecology/fertility, and infection diseases/immunity). Several of the research projects within the LCRP domains were in need of biobank support for their activities. Given its unique position, UBiLim could act as a facilitator for these projects. Furthermore, with the expertise gained, it also contributed to the further improvement of study quality from a biospecimen perspective. From 2014 onwards, part of the LCRP funding was hence invested in the UBiLim operations to accommodate for the heightened activities and secure the number of staff as set out for the federated approach. Not all LCRP projects require biospecimens for their research; however, when human bodily material is collected within the LCRP projects, it is processed and/or stored in the UBiLim facility. As a result, some key collections for translational research are present in the biobank (33–36). **Figure 3A** shows the current representation of samples for all research domains currently present in the inventory (including LCRP and CMI domains). About half of the samples are related to autoimmune diseases (48%), followed by oncology (25%), and rehabilitation sciences (12%). This is not unexpected given the long-standing focus on auto-immune diseases of Hasselt University and its CMI-related incorporation in UBiLim. The oncology collection mainly consists of the hematology collection forming the foundation of UBiLim. Different types of samples are being collected, with plasma samples making up 53% of collected samples (51% heparin plasma, 20% EDTA plasma, and 29%

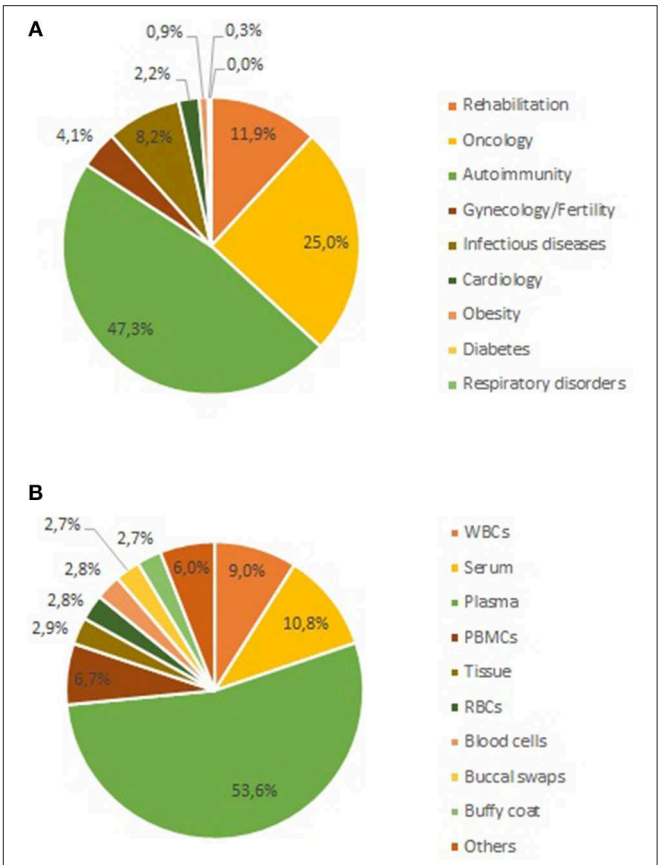

FIGURE 3 | Current sample content of the UBiLim catalog in December 2018. **(A)** Shows the proportional distribution of stored samples per research domain, and **(B)** shows the distribution of stored samples per sample type. PBMCs, peripheral blood mononuclear cells; RBCs, red blood cells; WBC, white blood cell.

either citrate or oxalate plasma), serum 11%, and white blood cell and PBMC fractions 9 and 6%, respectively (**Figure 3B**). Tissue samples represent only 3% of the biospecimens present, resulting from the biobank originally embedded in a clinical laboratory, not a pathology department. Finally, UBiLim also stores some rare sample types, such as skin tapes to strip the stratum corneum of low-level laser-treated cancer patients to investigate inflammation pathways.

Already by the end of 2013, UBiLim was effectively supporting the translational research community's biobank needs, as evidenced in **Figure 4A**. Since 2007, on average 11,900 samples are stored and about 1,400 samples were distributed annually. The number of studies (collections starting) and projects (use of samples) shows on average an upward trend (**Figure 4B**). The observed average utilization rate of 1.5% is lower than that reported for classic biobanks (37). Since most collections are still in their "exclusivity" period, the majority of samples are distributed to members linked to UBiLim, which also temporarily affects the use rate. However, it is to be expected that this rate will increase in the near future, as many projects for which samples are stored are still in either collection phase or have

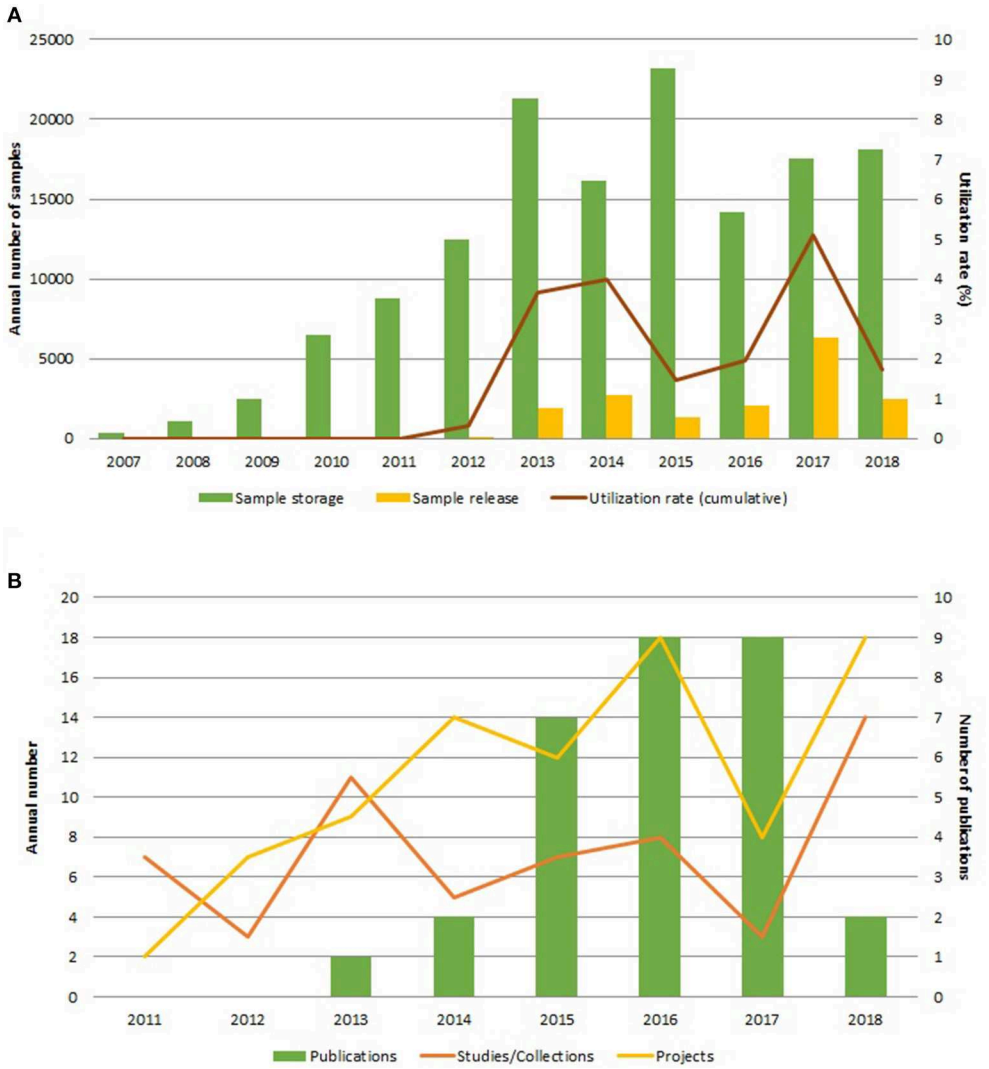

FIGURE 4 | Overview of the sample-based activities, cumulated for all sample collections of UBiLim. **(A)** Annual number of samples stored (green bar) and released (yellow bar) and annual utilization rate of samples (brown line, displayed on secondary *Y*-axis). **(B)** Annual number of studies/collections setup (orange line) and projects for sample use started (yellow line) by UBiLim and annual number of publications using samples derived from UBiLim (green bars, displayed on the secondary *Y*-axis).

not started analyses yet. Additionally, most of the collections in the UBiLim inventory are project-based and less at risk of "biohoarding" (38). Nonetheless, UBiLim's support has resulted in 29 publications based on samples stored in its facilities, a key performance indicator demonstrating operational sustainability of the biobank and demonstrating UBiLim's added value for translational research (39).

The Future of UBiLim: and the Beat Goes on…

In November 2018, the Belgian biobank law eventually came into effect imposing that any human body material used for scientific research has to be obtained from a notified biobank (40). This evolution has increased the need for the UBiLim infrastructure, extending to other research ongoing in the three founding institutes and external parties in the Limburg area. As a result, many researchers that currently control their own active and historic sample collections request to be incorporated into the UBiLim biobank. Compared to the first quarter of 2018, an increase of 60% of collections/studies was observed in the first quarter of 2019. Although the legislation comes with its own set of challenges, it appears to induce the transition of researchers no longer setting up individually managed collections, but starting them within the context of already established biobanks. While this is a positive trend with respect to sample quality and harmonization, its impact on accelerating translational research will only become clearer in time.

With the public funding for the LCRP ending, Hasselt University, the Hospital East-Limburg, and the Jessa Hospital have each dedicated funding to transform it into the Limburg Clinical Research Center, in order to sustain the ongoing projects, among which UBiLim. The funding available to the biobank,

however, would only cover 56% of the expenditure compared to the total costs of the biobank in 2018. Additionally, given the increased number of collections expected due to the changed legislation, the total cost in 2019 will be higher than that of 2018, potentially resulting in a higher deficit. Up to now, UBiLim has never charged fees for the services and/or samples delivered, mainly because of common funding sources, but also because of the limited budget available to researchers. However, it is clear that the sustainability of UBiLim needs to be addressed. The business aspect of biobanking, including sustainability, had recently received a lot of attention in the biobank community (41–44). However, while cost recovery appears to be an obvious solution, it has been reported to not contribute significantly to sustainability on its own (45). This can be partially overcome by providing a catalog of samples and improved "marketing" of the biobank (46, 47), but public funding is reported to be a critical component of an overall business plan (12, 48). UBiLim is currently investigating cost recovery as a model to at least partially cover operational costs, based on calculation tools available elsewhere (49, 50). Additionally, it is actively pursuing visibility of its infrastructure and its resources by displaying its catalog metadata on its website and in the publicly available BBMRI-ERIC directory (51).

CONCLUSION

UBiLim, as it stands today, is a federated biobank, with processing and storage facilities at each of the three sites. Common procedures, corresponding to the medical laboratory quality standard and biobanking guidelines, are used to harmonize the activities and ensure comparable, qualitative samples, independent of the originating site. Funding has been a major challenge in each step of its evolution and remains the biggest issue for long-term biobank sustainability. To a lesser extent, the Belgian legislation and the operational cost of information management system are also concerns for smooth biobank operations. Nonetheless, the need for UBiLim's infrastructure is still apparent and increased growth is to be expected. Efforts are ongoing to improve the utilization rate as well as the sustainability of the biobank to ensure its long-term development. Several publications have arisen from the use of the samples, which may result in improved care and/or therapy of the patients involved. Nonetheless, through provision of professional biobank services, UBiLim serves as a facilitator for translational research in the Limburg area of Belgium that, given the fields of research, may have an impact on international patient care.

MATERIALS AND METHODS

Data on sample numbers, types, and diagnoses were acquired by running dedicated queries in the Biobank information management system Labvantage. Utilization rate was calculated as the percentage of the number of samples released annually vs. the cumulated number of samples in storage that year. The publications counted were those that effectively used samples that were processed and/or stored by UBiLim. Publications without biomedical content were excluded. Financial data were acquired from the accounting departments and divided into six main categories (personnel, operations, equipment, IT infrastructure, promotion/marketing, and overhead). Data were analyzed and visualized using Microsoft Excel 2016.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the manuscript/supplementary files.

AUTHOR CONTRIBUTIONS

LL, KV, EV, KU, SD, JP, VS, PS, and J-LR conceived of the presented idea and critically reviewed the manuscript. LL and KV designed the project, performed the data analysis, and co-wrote the paper.

FUNDING

This work was funded by the CCPRB project (EU FP6 program grant agreement ID: 503465) and the LSM projects N-LSM-85 and N-LSM-203, in addition to financial support of LSM and the Governments of Flanders and the province of Limburg in the context of the SALK-plan.

ACKNOWLEDGMENTS

The authors wish to thank all technicians that have contributed to the establishment of UBiLim. Note: LL's current affiliation is Biobank University Hospitals Leuven, Leuven Belgium.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 09 January 2020
doi: 10.3389/fmed.2019.00309

Automated Sample Storage in Biobanking to Enhance Translational Research: The Bumpy Road to Implementation

*Loes Linsen, Kristel Van Landuyt and Nadine Ectors**

AC Biobanking, University Hospitals Leuven, Leuven, Belgium

The low reproducibility of biomarker research is a major holdback for the translation of research results to the bedside. Sample integrity has been identified as a key factor that contributes to improved reproducibility. The key mission of biobanks is to ensure that all activities and materials are managed according to standardized procedures and best practices to ensure and preserve sample integrity. When handling large numbers of biospecimens automation of sample handling and storage is often the method of choice to maintain and improve sample integrity. In December 2013, the centralized Biobank of the University Hospitals and the Catholic University of Leuven (UZ KU Leuven) decided to implement automated systems for sample storage and retrieval, one for storage at −20°C and one for storage at −80°C. Here we describe the extensive process of installation, acceptance, validation, and implementation of these two systems. Overall it took about 4 years to effectively take the systems into production. Multiple issues resulted in the delayed implementation, with labware change, quality of the initial installation, and misunderstanding of biobank concerns being the most impacting. Significant effort in terms of time and resources from both the automated store supplier as well as the biobank itself was needed to achieve a successful implementation. Within 15 months of actual integration in the biobank workflow, over 63 k samples were placed into the systems. Actual hands-on sample handling and retrieval times were substantially reduced, although this implied the shift of dedicated personnel time from the researchers' laboratories to the biobank. With the successful implementation of automated frozen sample storage systems, the centralized UZ KU Leuven Biobank is now also able to efficiently support large-scale translational research.

Keywords: biobank, automation, sample storage, quality, temperature mapping, qualification, translational research

OPEN ACCESS

Edited by:
Elena Bravo,
Istituto Superiore di Sanità (ISS), Italy

Reviewed by:
Jing He,
Guangzhou Women and Children's Medical Center, China
Tiziana Franchin,
Bambino Gesù Children Hospital (IRCCS), Italy

****Correspondence:***
Nadine Ectors
nadine.ectors@uzleuven.be

Specialty section:
This article was submitted to Translational Medicine, a section of the journal Frontiers in Medicine

Received: *13 May 2019*
Accepted: *06 December 2019*
Published: *09 January 2020*

Citation:
Linsen L, Van Landuyt K and Ectors N (2020) Automated Sample Storage in Biobanking to Enhance Translational Research: The Bumpy Road to Implementation. Front. Med. 6:309. doi: 10.3389/fmed.2019.00309

INTRODUCTION

The low reproducibility of biomarker research is a major holdback for the translation of research results to the bedside (1, 2). The integrity and quality of the biospecimens used for research, has been identified as one of the key factors that contribute to improved reproducibility (3). Biobanks play a vital role there, because they are the custodians of the biospecimens required for research (4). As such, it has been recognized that the biobanks are a cornerstone of precision medicine (5).

The key mission of biobanks is to ensure that all activities and samples are managed according to standardized procedures and evidence-based best practices to ensure and preserve biospecimen integrity (6). Several initiatives of biobank harmonization and standardization have ultimately cumulated into the publication of the biobank standard ISO 20387:2018, intended to ensure quality, fitness-for-purpose, and reproducibility in biobanking to facilitate translational research progress (7).

It is a well-known fact that up to 70% of the analytical errors are due to variations in the pre-analytical phase in the clinical laboratory environment (8). In the context of biospecimens and biobanking, the pre-analytical phase covers all processes between the collection of the sample until it is removed from storage for analysis (9). When handling large numbers of biospecimens for processing or analysis, automation of tasks is often the logical next step to reduce labor costs and increase sample throughput. There is a consensus in bioanalytical laboratories that automation also shortens method development time and improves biosafety and quality of data and samples (10). Automation of non-analytic functions has even greater importance, since it is recognized that non-analytic errors are more significant than analytic errors in terms of general laboratory quality (11). Tasks that are repetitive and monotonous, such as sample storage and retrieval, are the most prone to human errors but are also more easily automatable (11). Furthermore, biospecimen retrieval from frozen storage often is performed manually, thereby frequently exposing samples to large temperature fluctuations which can introduce variation in the sample and may have a detrimental effect on its quality (12). Automating the biospecimen storage and retrieval process reduces the exposure of samples to temperature variation and is therefore an efficient option to preserve the sample quality for sustainable translational research.

The Biobank of the University Hospitals and the Catholic University of Leuven (UZ KU Leuven) was founded in 2008 in response to changing national regulation. Its current main objective is to centralize the storage of human bodily material for scientific purposes and make it available for intra- and extramural research projects. The biospecimens are collected and processed by different partners of the biobank, such as the laboratories for pathology, clinical chemistry, and molecular diagnostics, but also by various research groups belonging to the University and University Hospital. As a result, a plethora of sample types and associated containers have to be accommodated at different conservation conditions. In an effort to optimize the storage of frozen liquid biospecimens such as serum, urine and plasma, it was decided in December 2013 to implement two automated systems for sample storage and retrieval, one for storage at −20°C and one for storage at −80°C.

Regulatory standards such as those from the biobanking ISO 20387:2018 require a formal qualification and validation of an automated frozen storage system before taking it into production, as well as frequent reassessment of its performance of both the automation and temperature component (13). However, the validation requirement is subjective and leaves the implementing biobank without specific criteria to tackle this process. Additionally, our extensive literature searches revealed no information regarding guidelines for automated storage system validation and qualification. Furthermore, no procedures for customer validation were available from the manufacturer. We therefore developed a validation/qualification method for automated frozen sample storage systems, based on the implementation of two systems (one at −20°C and one at −80°C) at the UZ KU Leuven Biobank. Furthermore, we describe the challenges encountered and the solutions created in the process of implementation, allowing others to learn from our experience.

MATERIALS AND METHODS

Automated Sample Store Structure

The Sample Store I (−20°C) and the Biostore (−80°C) (Brooks Life Sciences, Manchester, UK) have a similar main design which is described in the **Supplementary Material**.

Automation Operational and Performance Testing

The system qualification and assessment test (SQAT) was designed to assess the operational functionality of the system used in daily routine and used in the operation qualification of the system: input, scanning, placement in storage, reformat of samples from standard density to high density trays, picking of samples from high density to standard density trays, output. The SQAT routine was devised by the UZ Leuven super-user, based on previous experience of IQ/OQ/PQ validations of analytical equipment and standard practice thereof, as no information was available in literature or from the manufacturer for validation of automated storage systems. The SQAT was performed as follows: one standard tube rack with 96 tubes filled with 900 µl saline was put on a standard density tray together with five empty tube racks and put in the input/output module for input. Correct identification of tray, rack and sample barcodes by scanning was verified using the user software interface. Upon successful input, an order for sample reformat was created. Tray loading and sample reformat in and tray unloading from the cherry picker, as well as pulling trays from and putting trays back in storage were observed. Storage locations were registered. Pick retry and place retry rates were recorded before and after reformat for continuous monitoring and should remain below 10% for the Biostore and below 5% for the Sample Store I. Upon successful reformat, an order to pick 50–100% of samples from the input was generated, where the same parameters as described for the reformat order were registered. Upon completion of the pick order, an order was generated to output and scan the tray holding the picked samples. Barcodes of outputted trays, racks and samples were verified. The SQAT takes 15 and 20 min to complete for the Sample Store I and the Biostore, respectively. To measure reformat and picking times, the relevant parts of the SQAT were used and adapted to contain the number of samples required for the tests (1–10–100–1,000 tubes). Time was calculated from start time of the order to end time of the order as defined in the user interface.

Formal performance qualification was performed through empty runs mimicking the first customers' sample flow.

Based on current practices at the time of the test, it was estimated that 125 frozen samples would be inputted in one batch daily and about 25 samples would be outputted in one batch weekly. During a 2-week period these movements were simulated with saline-filled tubes for both the Sample Store I and the Biostore and recorded using the SQAT document. Formal qualification criteria were: no user-intervention required to complete the test apart from the default use. Identification of tubes entering and exiting the automated stores concordant with independent identification (FluidX Perception scanner), pick and place retry ratio's remained below 5 and 10% for the Sample Store I and Biostore, respectively.

Temperature Homogeneity Testing

Temperature mapping was performed using a protocol modified from the Energy Star Program Requirements for Laboratory Grade Ultra-low Temperature Freezers (14). Fifteen PT 100 air temperature monitoring devices (TMD) (Testo 184 T4 data logger, Testo, Lenzkirch, Germany) were placed on high density trays and inputted into the automated stores according to positions for environments with a storage volume between 2 and 20 m^3 as stipulated in the NF X 15-140 standard. The TMDs are distributed in three planes, one located at the highest possible tray location accessible for samples, one at the geometric center and one at the lowest possible tray location accessible for samples. For the upper and lower planes, the TMDs are positioned in each corner and at the center. For the geometric center plane, TMDs are positioned at the four midpoints between all corners and at the center. TMDs were left to acclimatize for at least 5 h before starting the test. Temperature was measured each minute for 24 h. During the 24 h test period, door opening and closing impact was performed by opening the inner doors between the input/output module and the store for 45 s (followed by opening of the tile wall for 10 s in the Biostore) and repeating this schedule 3 times once per hour for a period of six consecutive hours. The average store temperature was calculated from all TMDs for the 24 h measurement period. The peak variance was calculated as the difference between the maximum and minimum temperatures measured across all TMDs over the 24 h measurement period. The stability was determined as the difference between the maximum and the minimum temperature measured by an individual TMD over the 24 h test period. The uniformity was calculated as the difference between the maximum and minimum temperature measured inside of the unit at any given time. The impact of door opening and closing was assessed by calculating stability and uniformity for a 3 h period starting with the first door opening exercise and for a 3 h period, starting 3 h after the last closing of the door. Warm spot and cold spot are defined by the TMDs showing on average the highest or the lowest temperature over the 24 h measurement period, respectively. The data were exported to comma separated files using the Testo Comfort Software (Testo, Lenzkirch, Germany). All calculations were done using Microsoft Excel 2016 (Redmond, Washington, U.S.).

TABLE 1 | Overview of causes for automation failure of Biostore.

		Tests ($n = 43$)		Total activity ($n = 414$)	
Element	**Cause**	***n***	**%**	***n***	**%**
Robot	Minicrash	3	7.0	4	1.0
Imaging	Tube not detected	2	4.7	21	5.1
Imaging	Failed image integrity	1	2.3	5	1.2
Picker	Picker calibration	4	9.3	11	2.7
Picker	Tube on tube error	2	4.7	3	0.7
IT	Software crash	0	0.0	1	0.2
Other		1	2.3	11	2.7
Totals		13	30.2	56	13.5

RESULTS

On-Site Installation of Automated Sample Stores and Site-Acceptance Testing

In 2013, the Sample Store I (−20°C sample storage) and Biostore I (−80°C sample storage) from Brooks were selected for installation at a dedicated site of the UZ KU Leuven Biobank. About 1.5 years after purchase, the two systems were ready for a site-acceptance test (SAT). The 13-point SAT was drafted by the manufacturer and was a high level assessment of the systems' functionality. The Biobanks' expectations/criterion to pass the SAT was that all elements would be cleared consecutively on the first attempt. While the Sample Store SAT passed without problems, the Biostore SAT was only passed by repeatedly attempting the individual elements and making modifications to the system in between at that time. As a result, the Biostore SAT was not signed off by the UZ KU Leuven Biobank.

Following the initial SAT, the Biostore system presented persistent problems regarding automation and cooling performance afterwards. In an effort to address these issues, the Biobank decided to appoint an internal dedicated super-user whose main focus was to obtain a deeper understanding of the system and design a structured approach for improvement. To chart the robotics issues, a system qualification and assessment test (SQAT) was devised by the Biobank, assessing the four main components of routine use: sample input and scanning, sample reformat from standard density to high density trays, sample picking from high density to standard density trays and sample output and scanning. **Table 1** shows that over a period of 7 months, 30.2% of these tests failed ($n = 196$), with a 13.5% failure rate observed for overall activities, identifying the SQAT as a simple but effective tool to assess the systems' performance. The main causes for failure were due to imaging issues, tube picking problems and mechanical crashes. Failures occurred irregularly and the type of failure was unpredictable.

In addition to the automation issues, the Biostore cooling system also did not show the expected robustness, evidenced by one of the redundant cooling units failing at weekly switch-over due to recurrent high-pressure problems (32 times across 6 months). Furthermore, minor incompletions of the installation

resulted in an increased frost build up, leading to imaging problems explaining some of the issues summarized in **Table 1**.

Overall, these observations suggested a suboptimal quality of the original installation. Corrective actions were taken by the manufacturer upon increasing pressure by the Biobank, requiring significant efforts from both the manufacturer and the biobank to rectify the situation over a period of 2 months. After completion of the works in December 2016, a second SAT was performed which was passed on first attempt, without any additional modifications needed, declaring the automated stores ready for validation by the Biobank. This was confirmed by a post-SAT2 SQAT failure rate of <1% (data not shown).

Sample Storage Tube Integrity Testing

At the same time as the implementation of the corrective measures, the labware showed clear fractures in the wall of the tubes when these were filled with the allowed amount of water and frozen in either the Biostore or the Sample Store I (brand 1, 1 ml high density tubes, 2D barcoded bottom, internal threaded caps). Experiments were conducted to assess the extent and cause of the problem. Similar tubes obtained from two additional manufacturers were subjected to the same conditions (brand 2, brand 3). Maximum fill volumes as defined by the manufacturers was ≤920 µl. The tubes were filled with 900–970–1,000 µl of distilled water, saline, serum or a suspension of cells in 10% serum and subsequently frozen in either the Sample Store or the Biostore. Tubes were deliberately overfilled (970–1,000 µl) to simulate pipetting mistakes made by customers providing their pre-filled sample tubes to the biobank.

Fractured tubes were only observed when tubes were filled with distilled water. As shown in **Table 2**, all brands tested showed this behavior when the tubes were frozen in the high-density tray at −80°C, even when the fill volume was within the manufacturers' range, except for brand 1. However, this brand also showed fractures when frozen in the Sample Store at −20°C while the other brands did not show any fractures in that condition. Clearly, the different tubes behaved differently to different conditions, which might be explained by the composition of the tube plastic/polypropylene. As the cherry picker module of the installed stores was specifically configured to the tube type selected by the UZ KU Leuven Biobank (brand 1), these findings were presented to the manufacturer. Based on the manufacturers' test results, a different tube type was suggested (brand 1, 1 ml high density tubes, 2D barcoded bottom, external threaded caps), with the same tube diameter albeit 6.6 mm shorter, but still compatible with the existing cherry picker module given some minor modifications to the pick head height. These tubes were subjected to the same tests as described above and indeed showed no formation of fractures in any of the tested conditions. When overfilled, the caps were ejected from some of the tubes due to increased internal tube pressure upon ice formation (75% for 970 µl; 100% for 1,000 µl). This caused problems with the reformat/picking functionality of the cherry picking module. To prevent this problem, a volume assessment routine at sample intake was implemented. Furthermore, the routine input approach for non-frozen samples into the automated stores was set to freeze the samples overnight in the standard density racks before reformatting into the HD tray. This also allowed a visual check of cap presence to occur before any reformat/picking action was started. Although this resulted in a reduction of the volume stored, it was decided to accept the new tube type. The necessary modifications to the cherry picker module were executed in parallel with the adjustments required to improve the overall installation. The performance of the modification was qualified during the second SAT.

TABLE 2 | Proportion of fractured tubes upon freezing in Biostore and Sample Store I at different fill volumes of distilled water.

	Fill volume (µl)	Brand 1 (HD)	Brand 2 (HD)	Brand 3 (HD)	Brand 1 (SD)	Brand 1 (HD—external)
Biostore	900	0.0%	66.7%	83.3%	8.3%	0.0%
	970	70.8%	33.3%	50.0%	45.8%	0.0%
	1,000	100.0%	66.7%	83.3%	95.8%	0.0%
Sample Store	900	37.5%	0.0%	0.0%	0.0%	0.0%
	970	16.7%	0.0%	0.0%	0.0%	0.0%
	1,000	41.7%	0.0%	0.0%	100.0%	0.0%

HD, samples frozen seated in high density tray; SD, samples frozen seated in standard density SBS racks.

System Qualification and Validation

Upon formal acceptance of the installation, an additional set of assessments was performed to qualify the automated stores for the intended use in the UZ KU Leuven Biobank. The automation aspect was tested on several levels with the main criterion to pass being the absence of critical errors requiring user intervention: reformat and pick actions for 1–10–100–1,000 tubes filled with 900 µl saline to time the duration of each action; stress tests picking/reformatting over 3,000 tubes per run overnight without close user monitoring; scan performance for input of frozen and non-frozen samples; successful closure of activities during a power interruption (Universal Power Supply test Biostore). No critical errors were observed during any of the tests performed for both the Biostore and the Sample Store. **Table 3** shows the time needed to reformat or pick samples by the two automated stores. Generally, reformatting samples takes more time than picking samples (1.2 and 1.3 times quicker for 1,000 samples for Sample Store and Biostore, respectively). As the reformat action moves the tubes from the standard density SBS racks to the high density racks with tightly fitting aperture, the picker module settings have been optimized to maintain accuracy while compromising on speed. Furthermore, the Sample Store (−20°C) handles the tubes about 3 times faster than the Biostore, which again is due to optimized picker module settings for the Biostore to accommodate changes in tray size due to exposure to different temperatures (sample storage at −80°C, sample picking/reformatting at −20°C). No difference in scan performance was found when inputting frozen or non-frozen tubes and overnight runs finalized without any issues.

Additional testing was performed to assess the automated store behavior when exposed to unintended use: generating pick

TABLE 3 | Reformat and pick times per store per number of tubes.

	Sample Store I		Biostore I	
	Reformat time*	Pick time*	Reformat time*	Pick time*
1 tube	4	2	5	4
10 tubes	4	2	7	4
100 tubes	7	6	14	9
1,000 tubes	40	33	125	91

**Time in minutes.*

orders for tubes that are not in the store or for duplicated tube barcodes; input Biostore sample trays into the Sample Store and vice versa; input sample racks without barcodes; input sample trays backwards. No criterion for acceptance was set, the resulting observations were used to define the procedure to handle the systems by the users. Apart from the backward tray input, all events were handled to satisfaction by the stores to prevent unintended misuse of the system: orders for tubes not physically present in the store were executed for the tubes where available and remained in "waiting" state until the missing tube was inputted (which successfully finalized the order) or until closure of the order by the user. Duplicated tube requests within one order where ignored and sample racks without barcodes were taken into the store for storage at temperature, but were marked as "problematic" and could only be handled upon intervention by the user. Biostore sample trays could not be accepted into the Sample Store and vice versa. Upon backward input of the sample tray, the tray was lifted and dropped in the input/output module, leading to samples jumping out of the tray and falling into the unit, risking sample loss on the one hand and mechanical obstruction of moving parts on the other. A backward placed tray cannot be detected by the system and is a user-dependent event. It was therefore incorporated as an important part of the internal user training to prevent the event from occurring. Finally, a 2-week period of empty runs mimicking first customers' sample flow was used as formal performance qualification. All acceptance criteria were passed, and no issues were encountered.

With the temperature and cooling performance of the automated store being critical to the Biobank requirements, these aspects were also qualified through additional assessments. Temperature homogeneity was assessed based on the Energy Star Program Requirements and the French standard NF X 15-140 using 15 temperature monitoring devices. Results are displayed in **Table 4** and **Figure 1**. The average temperature over the 24 h measurement period was −22.19 and −79.84°C for the Sample Store and the Biostore, respectively. The increased temperature stability of the Sample Store vs. the Biostore (4.67 vs. 1.13°C) is most likely due to the defrost cycling of the Sample Store I occurring every 12 h. Because the Biostore is purged with ultra-dry pressurized air, no frost build-up occurs, omitting the need for defrost cycles which results in a higher temperature stability at a specific location. However, the temperature uniformity within the store at any given time was 2.32°C for the Sample Store I and 8.07°C for the Biostore, indicating a better temperature homogeneity in the Sample Store compared to the Biostore.

TABLE 4 | Temperature homogeneity for sample store and biostore.

	Sample Store	Biostore
Average temperature (24 h)	−22.19 ± 0.77	−79.84 ± 1.99
Max temperature (24 h)	−17.19	−74.90
Min temperature (24 h)	−25.03	−84.10
Peak variance (24 h)	7.84	9.20
Stability (24 h)	4.67	1.13
Uniformity (24 h)	2.32	8.07
Stability (DOC1)	1.69	0.87
Uniformity (DOC1)	2.29	8.07
Stability (DOC2)	1.78	0.28
Uniformity (DOC2)	2.02	8.06
Warm spot	Row 3, column 1, front	Row 5, column 1, front
Cold spot	Row 38, column 30, mid	Row 5, column 13, mid

DOC, door opening an closing, calculated for a 3 h period starting at the first door opening exercise (DOC1) and calculated for a 3 h period starting 3 h after the last door closing event (DOC2).

Warm spots and cold spots were also determined, the locations of which are in line with the layout of the store and the type of cooling and distribution of cold air. Door opening and closing sessions did not measurably impact the stores' temperature stability and uniformity, as evidenced by similar or even lower stability and uniformity values during and after the exercise (**Table 4**).

In addition to the temperature homogeneity, alarm connections to the building monitoring system were successfully tested for all the critical and warning alarms generated by the two automated stores. Furthermore, sustenance of cooling was also determined while the cooling units were running on city water to simulate the backup procedure in case of failure of the chilled water system and during switch to emergency power to simulate power interruptions. In both cases, cooling was sustained as evidenced by normal cycling behavior of the cooling units and stability of the systems' temperature (data not shown). The system qualification and validation phase took about 1 year for 0.5 full-time equivalent of an internal dedicated super-user to complete. These data show that both the cooling and automation aspect of the automated stores meet the requirements of the UZ KU Leuven Biobank qualifying the Sample Store and the Biostore for routine use.

Implementation

The first actual samples were inputted into the automated stores in February 2018. Performance of the systems is continuously monitored through the evolution of the pick retry and place retry ratios. Typically, these should be below 10% for the Biostore and below 5% for the Sample Store and these thresholds have not been exceeded since first actual input (data not shown). Additionally, the SQAT is used to assess and qualify overall functionality and is performed pre and post preventive maintenance, after unplanned intervention by the user or the technician or as evaluation of the store functionality in case of doubt. Apart from a dysfunctional tray consistently causing reformatting issues, no systematic issues were detected. Errors that could not be addressed by the

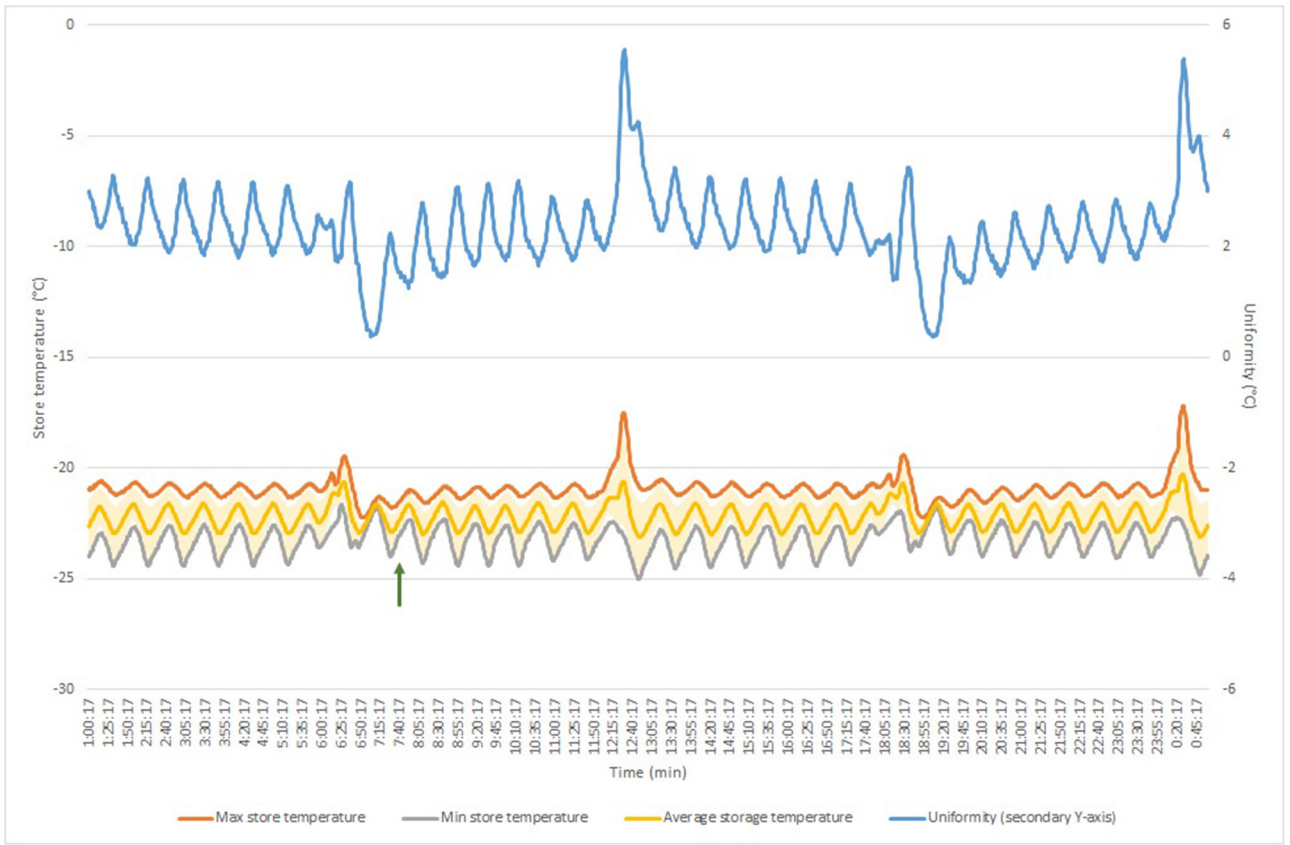

FIGURE 1 | Temperature homogeneity measurement in Sample Store over 24 h measurement period. Green arrows indicate door opening session start. Blue line shows temperature uniformity (displayed on secondary axis); yellow line displays average store temperature (with standard deviation indicated in light-yellow error bars); orange line shows maximum store temperature; gray line shows minimum store temperature.

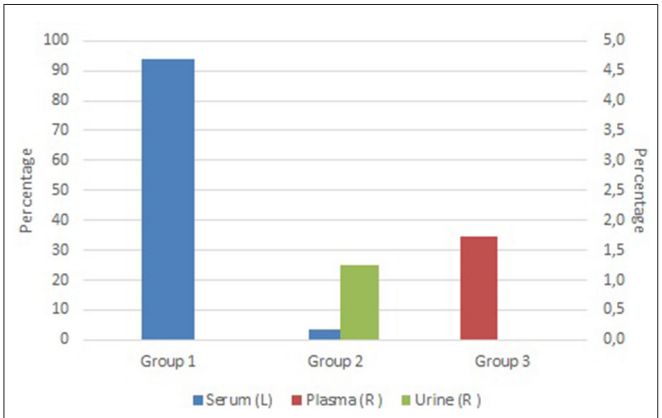

FIGURE 2 | Overview of current sample type distribution stored in the Biostore, clustered by provider. Blue bars show the percentage of serum samples (Y-axis), red bars show the percentage of plasma samples (right Y-axis) and green bars show the percentage of urine samples (right Y-axis).

Biobanks' superuser were swiftly addressed by either remote intervention or on-site visit. Overall, the manufacturer has shown increased attention to the needs of customers within a regulatory environment compared to the earlier stages of the installation. However, the Biobank still needs to keep a close eye to maintain quality of service.

At the time of writing, the stores hold about 63,000 samples provided by three different research groups. The grand majority of samples are serum samples (97%), followed by plasma (1.7%), and urine samples (1.3%) as shown in **Figure 2**. This can be explained by the biobanking approach of the different groups. Group 1 has already systematically been collecting and processing various biospecimens from a large patient group at different moments during their treatment since before the year 2000. Group 1 switched to the automated store workflow immediately after final qualification of the equipment. Group 2 and 3 only started to collect and process samples when the automated stores were already in production and target a smaller patient group (Group 2) or focus on small scale projects (Group 3). Transition to automation has decreased actual hands-on sample picking and retrieval time at the expense of the Biobank, but has increased the cost for the research group due to the use of automation-friendly, more expensive tubes. Overall, the transfer from a manual to the automated retrieval process was positively received by the end-users. Some difficulty was encountered for Group 2 and 3 users because of the transfer to tubes only identified by 2D barcodes.

TABLE 5 | Overview of the validation approach for the automated stores of the UZ KU Leuven Biobank.

Phase (duration @ UZ KU Leuven Biobank)	Content	Who
Installation qualification (3 years)	Installation by manufacturer and Site Acceptance Test	Manufacturer in presence of user
Operational qualification (1 year)	Intended use: – Develop System Qualification and Acceptance Test – Time to place/pick 1–1000 samples – Time to freeze 1–100 samples – Scan performance of frozen and non-frozen tubes – Stress test by placing/picking >3,000 tubes – Universal Power Supply test during operation Labware verification: – Determine maximum fill volume and impact on system Unintended use: – Create pick order for duplicate tube ID – Create pick order for tube absent from store – Insert labware with incorrect orientation Cooling: – Determine Temperature homogeneity – Emergency power test and impact on cooling operations – City water backup test and impact on cooling operations Alarm connection Test	User
Performance qualification (1 month)	Simulation of intended routine use of the equipment (2 week repeat of estimated sample submissions and requests)	User
Implementation	Routine operation, daily monitoring of PIR and PLR, use of SQAT before/after PM and planned interventions, SQAT after unplanned intervention	User

PIR, Pick Retry Ratio; PLR, Place Retry Ratio; PM, Preventive maintenance; SQAT, System Qualification and Assessment Test.

However, modification of the research groups' workflow to incorporate scan-based confirmation steps overcame this issue. Step-wise expansion of the number of research groups using the automated stores is ongoing, with another three groups currently in transition. Integration of the automated storage of the Biobank in the clinical laboratories' workflow is planned to start spring 2020. The current utilization rate of the samples is 3.2% which is expected to increase over time when sample follow-up (and significance) increases.

DISCUSSION

The low reproducibility of biomarker research is a major holdback for the translation of research results to the bedside. Sample integrity has been identified as a key factor that contributes to improved reproducibility. One way of preserving the sample integrity is through automation of processes, thereby significantly reducing the introduction of variation. When handling large numbers of biospecimens in the biobank setting, automation of sample handling and storage is often the method of choice to save labor and improve turnaround times and quality. In this paper, we described the lessons learned during the implementation of two automated frozen sample storage systems at the UZ KU Leuven Biobank (**Table 5**).

Regulatory standards require a formal qualification and validation of an automated frozen storage system before taking it into production, as well as frequent reassessment of its performance of both the automation as temperature component (13). However, actual method specifications are not provided, nor available in literature for these kinds of systems. The UZ KU Leuven Biobank devised a SQAT test to assess performance of the automated systems and set up a method for temperature homogeneity testing. Although these are tailored to our systems, the underlying concept is applicable to other automated storage systems as most of them share a similar basic concept regarding sample management and storage at a specific temperature (15–17). Our temperature mapping results similar to those reported elsewhere for standard (ultra-low temperature) freezers, even though the storage volume is about 10 times greater (18). Furthermore, door opening and closing actions did not have significant impact to the temperature of the store, whereas this is an important effect in standard freezers, potentially affecting the sample integrity (18). These findings demonstrate the added value of automation in reducing unwanted variation in biospecimens, of major importance for translational research.

The lengthy installation and approval process indicates that automated storage systems are not off-the-shelf products but require substantial adaptations to accommodate site-specific requirements and facilities. Additionally, it involves significant resources from the customer on top of the initial purchase of the equipment, such as assistance from the technical department. It also has to be appreciated that an intense relation has to be set up and maintained between the manufacturer and the biobank during the life-cycle

of the product. Our experience and those of others also underline that these appliances will not run out of the box or deliver for the life of the system without ongoing investment (19). Remarkably, the issues we encountered are not site-specific, nor manufacturer-specific: others within the biobanking community have experienced similar difficulties upon implementation of automated storage systems (Europe Biobank Week 2019 pre-conference Automation workshop and GCM & KB, personal communication). Although several reasons underlie these problems, the common ground are mismatched expectations between both manufacturer and client, which require adequate customer service and technical support to be overcome (20). In our experience, appointing one of the biobank personnel as a dedicated super-user was essential to successfully complete the installation and to keep the automated stores operating at optimal performance in collaboration with the manufacturer. Additionally, the implementation of a more structured customer-oriented approach by the manufacturer also contributed significantly to resolving ongoing issues at the UZ KU Leuven Biobank. A similar approach has been reported for large-scale automated compound management systems which emphasized the internal support structure as major factor to maximize return on investment and increase the systems' life-cycle (19).

In conclusion, the centralized UZ KU Leuven Biobank succeeded in the implementation of two automated frozen sample storage systems, which currently hold over 63,000 samples. The majority of ongoing issues have been satisfactorily resolved by the manufacturer in a constructive collaboration with the Biobank. Moreover, additional investments have been made to expand the Sample Store I with an additional module to incorporate additional tube types into the system. As a result, the UZ KU Leuven Biobank is now also able to efficiently support large-scale sample storage for translational research.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

LL, KV, and NE devised the conceptual idea. LL executed the work and wrote the manuscript. KV and NE critically reviewed the manuscript.

FUNDING

In 2006 the Flanders White book Life Sciences recommended Translational Medicine as focus for a Public-Private Partnership. The Flemish government funded a regional biobanking initiative with FFEU resources (Debt Reduction Financing Fund and One-off Investment Expenditure). Funding was obtained via the Institute for Science and Technology (IWT) and the Ministry for economy, science and Innovation (EWI). The University Hospitals Leuven invested the money in the automation of sample storage. In the mean-time the CMI—centre medical innovation initiative has been aborted because of lack of funding.

ACKNOWLEDGMENTS

The authors wish to thank Chris Connolly, Justin Fallon, Graham Jones, and Christopher Glenn for substantial contribution to the successful implementation of the automated storage systems.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2019.00309/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 11 February 2020
doi: 10.3389/fmed.2020.00033

Sharing of Clinical Trial Data and Samples: The Cancer Patient Perspective

Stefanie Broes[1,2], *Ciska Verbaanderd*[1,3]*, *Minne Casteels*[1], *Denis Lacombe*[2] and *Isabelle Huys*[1]

[1] Clinical Pharmacology and Pharmacotherapy, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Leuven, Belgium, [2] European Organisation for Research and Treatment of Cancer, Brussels, Belgium, [3] Anticancer Fund, Strombeek-Bever, Belgium

Introduction: Today, many initiatives and papers are devoted to clinical trial data (and to a lesser extent sample) sharing. Journal editors, pharmaceutical companies, funding agencies, governmental organizations, regulators, and clinical investigators have been debating the legal, ethical, and social implications of clinical data and sample sharing for several years. However, only little research has been conducted to unveil the patient perspective.

Aim: To substantiate the current debate, we aimed to explore the attitudes of patients toward the re-use of clinical trial samples and data and to determine how they would prefer to be involved in this process.

Materials and Methods: Sixteen in-depth interviews were conducted with cancer patients currently participating in a clinical trial.

Results: This study indicates a general willingness of cancer patients participating in a clinical trial to allow re-use of their clinical trial data and/or samples by the original research team, and a generally open approach to share data and/or samples with other research teams, but some would like to be informed in this case. Despite divergent opinions about how patients prefer to be engaged, ranging from passive donors up to those explicitly wanting more control, participants expressed positive opinions toward technical solutions that allow indicating their preferences.

Conclusion: Patients were open to sharing and re-use of data and samples to advance medical research but opinions varied on the level of patient involvement and the need for re-consent. A stratified approach for consent that allows individualization of data and sample sharing preferences may be useful, yet the implementation of such an approach warrants further research.

Keywords: data sharing, sample sharing, patient perspective, ethical and legal implications, neoplasms, e-consent

OPEN ACCESS

Edited by:
Michel Goldman, Institute for Interdisciplinary Innovation in healthcare (I3h), Belgium

Reviewed by:
Sung Won Choi, University of Michigan, United States
Annelies Debucquoy, Belgian Cancer Registry, Belgium

***Correspondence:**
Ciska Verbaanderd
ciska.verbaanderd@kuleuven.be

Specialty section:
This article was submitted to Translational Medicine, a section of the journal Frontiers in Medicine

Received: *12 April 2019*
Accepted: *22 January 2020*
Published: *11 February 2020*

Citation:
Broes S, Verbaanderd C, Casteels M, Lacombe D and Huys I (2020) Sharing of Clinical Trial Data and Samples: The Cancer Patient Perspective. Front. Med. 7:33. doi: 10.3389/fmed.2020.00033

INTRODUCTION

Interventional clinical research leads to a change in the clinical management of the patient (e.g., by the experimental intervention). Therefore, informing patients about the nature, significance, implications, and risks of the research they will participate in and obtaining their subsequent consent are established procedures embedded in current ethical and legal frameworks [i.e., Declaration of Helsinki (1), EU Clinical Trial Directive (2), and upcoming Clinical Trial Regulation (3)]. In addition, most ethical frameworks stipulate–in line with the data protection and biobanking legislative frameworks–that it is required to re-inform people about the (further) processing of their personal data and human samples, unless impossible to do so (1). Even though the mere further processing of data or samples ("secondary use") does not lead to a new intervention, it may still lead to discussions about ethical and moral values, for instance where patients have not consented or have not been informed about such further use (4). Respecting one's consent is important since trust in the participant-researcher relationship is maintained insofar as there is proper use of the donated items in accordance with what was agreed (4).

Privacy consequences in case of data breach can be substantial. Disclosure of sensitive, personal data may lead to embarrassment, stigmatization, discrimination for loans or insurances, unwillingly unveiling biological ties, loss of employment, etc. In this respect, anonymizing the data may be a welcomed solution, since this is not subject to legal EU data protection requirements (5, 6). However, anonymizing data is not always advisable, desirable or even possible and even if data are anonymized at one point in time, safeguarding against re-identification can be challenging. For example, Lin Z and colleagues demonstrated that as little as 30–80 single nucleotide polymorphisms (SNPs) from a single person can uniquely identify that person (7), and other examples exist (8, 9). Such examples stir up social concerns and can potentially undermine research participants' trust in research. Public concerns are further fueled by the extraordinary pace of technological developments and public communications about potential misuse of medical data, for instance by pharmaceutical companies (10, 11).

At present, the many initiatives and papers devoted to the topic of clinical trial data (and to a lesser extent sample) sharing illustrate the increasing attention that is being paid to this subject (12, 13). In a previous study, we identified the pros and cons of increased clinical trial data and sample sharing (14). The legal, ethical, and social implications of clinical data and sample sharing are largely being debated by journal editors, pharmaceutical companies, funding agencies, governmental organization, regulators, clinical investigators, etc. (15–20). Many uphold a moral obligation vis-à-vis study participants (i.e., "research participants *want* their data to be used for further research") as the number one motivation for increased sharing efforts. Yet, it is unclear how the assumptions drawn by these stakeholders reflect the views of research participants, as only little empirical evidence is available when it comes to patient and research participant perspectives on the sharing of clinical trial research data and human samples. Moreover, only a small body of evidence is available on new tools to give patients a voice to express their opinion, and contribute to a transparent system where data are shared and re-used in accordance with the donors' preferences (21).

Some evidence exists from patient preference studies about the access and sharing of medical data captured in electronic health records (EHRs) (22, 23). Although not completely similar to the re-use of clinical trial data (and samples), since in the context of re-use of EHRs for research it constitutes a situation of re-purposing (i.e., from care to research) and data are not collected on the basis of informed consent [but on the basis of art. 8(3) EU Data Protection Directive (6), namely for the purposes of preventive medicine, medical diagnosis, the provision of care or treatment or the management of health-care services], interesting parallels can be drawn. A systematic literature review on this topic shows that the public has little knowledge on how their EHRs are shared and used for research purposes, and that a lack of transparency and engagement can undermine public trust (24). Furthermore, focus group participants expressed concerns about data sharing for commercial gain and the potential misuse of information (24). In view of these concerns, people may be more willing to share their medical data for research by public organizations (24). However, the United Kingdom government's care.data initiative, a program that enabled sharing anonymized EHRs with researchers outside the National Health Service (NHS), received widespread criticism and was stopped eventually in 2016 due to a lack of public trust (25). In addition, a survey with 1,011 respondents from 2014 indicated that a majority of the U.S. public had little trust in an integrated health data sharing system (26).

A patient at the European Patients' Forum stated the following: "*We, as patients, are increasingly aware of the value and importance of sharing our data. From the patients' perspective, use of health and genetic data is vital to advancing health research*" (27). At the risk of singling out opinions from (potentially) active and engaged patients, additional research is needed to understand the patient perspective on data and sample sharing. In 2016, Jones et al. conducted a survey on the topic of clinical trial data sharing with 799 (general) patients who entered the emergency department in a United States (US) hospital (28). Of these patients, 16% had previously participated in a trial. Eighty-five percent of the total group strongly favored clinical trial data sharing, and only 9% were against or strongly against it. Further, they report that approximately 85% of the survey respondents indicate that upfront disclosing a fully detailed data sharing plan is important since it increases transparency. These results provide guidance. However, the "patient" group was not specifically targeted toward clinical trial participants; but rather represents a broad category of people, which may obfuscate certain patient-specific attitudes. For the purpose of this study, we focused on a patient population participating in a trial in a particular domain, namely cancer. Further, we diversified between "re-use by the original research team" and "re-use by a new research team." We propose that this might influence patients' viewpoint since they originally consented to use by one research team in specific, and not yet to an unknown group. We also inquired whether

patients' opinions varied between sharing with either academic or pharmaceutical company researchers. Because a number of more dynamic and interactive consent approaches are being proposed to increase patient involvement (21), attention was paid as to how patients would like to exert control over sharing their samples and data.

MATERIALS AND METHODS

Interviewees

Recruitment of cancer patients currently participating in a cancer clinical trial was undertaken at the gastroenterological or oncogynecological day hospital of the University hospital in Leuven, Belgium, through purposive sampling. All contacted participants took part in the study ($n = 16$). All participants were provided with an oral explanation of the study and a patient information sheet describing the study. Next, they were asked to sign an informed consent form (ICF) before the start of the interview. All patients had reached the age of majority. Patients with either gynecological or gastroenterological cancer were invited for the interview. The patients were at the UZ Leuven for their treatment at the time of the interview, so they did not have to make extra time for the interview.

Interview Guide

An interview guide was developed based on available literature and was optimized by a team of experts active in the research field (**Supplementary Material 1**). The interview guide was piloted with non-cancer patients ($n = 5$) to ensure questions were drafted in lay language. The interview questions related to the following topics: (i) demographics; (ii) (re-)use of data and/or samples, (iii) use of data and/or samples by academia or industry, (iv) approval by ethics committee, (v) e-consent platform.

Data Collection

The interviews ($n = 16$) were conducted face-to-face by three interviewers using the same interview guide in February 2017 and lasted about 30 min each. Recruitment ceased once data saturation was established. All interviews were conducted in Dutch. Written informed consent was obtained prior to the interview. The interviews were audio-recorded and transcribed *ad-verbatim*.

Data Analysis

Interviews were pseudonymized and analyzed deductively via a content analysis by three researchers, based on the QUAGOL method (29). Interviews were coded and analyzed in Dutch. All concepts and codes were collected in writing and discussed orally amongst involved researchers. On such basis, consensus could be reached in all cases. The final text was translated in English after analysis.

RESULTS

Of the 16 participants, 9 (56%) were women. Ages ranged from 35 to 79 (mean 62, median 64). With the exception of one Polish woman, all participants described themselves as being Belgian. Participants had following cancer types: colorectal cancer ($n = 4$), ovarian cancer ($n = 3$), gastric and lung cancer ($n = 1$), colorectal and lung cancer ($n = 1$), pancreas cancer ($n = 2$), gastric cancer ($n = 2$), cholangiocarcinoma ($n = 1$), unreported ($n = 2$). Of the 16 participants, 10 participants reported to have followed higher education, of which six participants had completed college or university studies, and six participants did not enroll in any higher education.

Sharing Data vs. Sharing Samples

Interviewed patients were aware of certain types of samples (e.g., blood, tumor tissue…) and data that are being collected. However, the majority of these patients did not seem to make a distinction in the sharing and re-use of their data vs. their samples. Moreover, interviewed patients reported only little interest about the purposes for which their data and samples are being used. They trust the clinicians to use the data and samples correctly in the scope of the research related to their disease. One stated for example:

> "*I think they took a biopsy but I do not know much about it actually… but if it is in the context of the study, yes then I think it is normal that you give away these pieces." (patient #6)*

And another:

> "*We got a document stating what would happen (to our data and samples), which we approved without reading it in detail." (patient #9)*

Re-Use by the Same or a Different Research Team

All participants hypothetically allowed that their data and samples would be used by the original research team for further research, as long as, according to one participant, the research "*stays within the oncology research area*" (patient #2), again highlighting the level of trust in the initial research team. None of the participants found it necessary to be asked to re-consent in such case.

Participants appreciated medical research, and encouraged data re-use out of altruistic reasons, i.e., to help other or future patients as much as possible. Two patients even found it their duty to contribute to science, and expressed strong hopes that the maximal potential of their data and samples would be extracted:

> "*It is only rarely that they find sufficient people to participate, so I feel that if you are eligible (for a study), that in some way it is your duty… because for instance, in my study now, we are only with seven patients." (patient #2)*
> "*If you can help other people, you have to help other people (…) so it would be better if everything would be more open and used." (patient #6)*

Less than half of the interviewed patients indicated that they would like to be informed of any further use, "*if this would be possible*" one patient continued (patient #7). Of this group, some expressed a sense of curiosity, whereas others find it important

that patients are informed about such further use because of transparency reasons:

> "*I would like to be informed, definitely. In principle, I do not object to such further use, but I would like to have as much information as possible, so that at least I know what the research is about. Absolutely." (patient #8)*

The majority of participants, however, did not find it necessary to be informed. One in particular described concerns about an abundance of unnecessary e-mails, which he perceived as annoying. Rather, he encouraged full "open use" of his donated data and/or samples (patient #12).

The desire for control seems to be greater in case of secondary use by a research team other than the original study team. At the one extreme, one patient favors complete open data re-use, thereby renouncing any form of control, on the condition however, that the secondary purposes are limited to research:

> "*Everything is allowed by me. I would make data fully accessible. Of course, not for other purposes like advertisement; no, no, only for research purposes." (patient #13)*

In contrast, two patients tended to distrust these unknown researchers and expressed concerns relating to misuse and security of their data.

> "*I prefer to be asked (…), otherwise (researchers) can give away everything without informing anyone." (patient #3)*

Overall, participants acknowledged the scientific value of re-use of their data and/or samples by another research team. Yet, in this case, some expressed a wish to be informed, again mostly out of curiosity reasons, i.e., to know in which studies their data are being used, by whom and to know to what they contributed. Patients would also like to be informed because this provides them with some form of verification on who is using their data; and thus, to ensure that there is no misuse of their data and samples.

> "*... I would like to know what they would… What their plans are or... just out of curiosity" (patient #8)*

It should also be noted that two participants explicitly specified that the information provided to other research groups would be anonymized or coded, illustrating a wish to protect their privacy. If this can be secured, only little risk was perceived and thus the willingness to share increased.

> "*Apparently everything happens coded, and as long as that is the case, I don't have anything against it." (patient #5)*

The Role of Ethics Committees

Further, we asked participants to consider the idea of an independent ethics committee (EC) that would decide about the re-use of samples and data for further research projects on their behalf. All but two interviewed patients liked this idea, stating that they have trust in the fact that these people will have a good level of expertise to make appropriate decisions, "*as long as there is just some form of control*" (patient #2). Some even felt more comfortable with an independently appointed body making such decisions for them, since such a body is more knowledgeable to do this.

> "*Yes they can because with their education and everything, they will know what to do. … by the way... my education does not have any link with these things... so... what can my opinion contribute to what is happening? I understand very little of all of this… why should I even want to….." (patient #8)*

However, even if the EC makes the decision in their place, a number of patients very much insisted to be informed about the further research purposes:

> "*I would trust a body like an ethics committee, but I insist: I would like to be informed, logically (…)" (patient #8)*

Two participants held contrary views on the intermediary of an independent ethics committee. They indicated to find such control unnecessary, favoring open use of their data and/or samples (patient #12 and #13).

Opinion on For-Profit and Not-For-Profit Research

Subsequently, participants were asked whether their opinion about re-use would be different when it constitutes academic or pharmaceutical industry research. Two patients preferred their data to be shared and used by academic researchers rather than by pharmaceutical companies, with the simple reason that pharma companies have commercial interests.

> "*Yes, this is different for me. I would prefer it to be a university, maybe because they are independent. Of course you can say "but you also entered a study, and it is a commercial study," but yes you look after yourself, which is logic, but ideally it would be better if this would be a university, the research centers that are independent vis-à-vis such studies" (patient #7)*

Interestingly, the majority of patients did not make this distinction. Even if the goal of companies is to make profit, in the end, they achieve this by bringing treatments to the market and therefore, patient data and samples should be shared as much as possible.

> "*No, I am not selective on this point, no. This is the same as... these are all people working for the same goal. Pharmaceutical companies are involved in research, because they make the medicines…" (patient #5)*
> "*It all boils down to the same thing; for the company of course there is money involved but in the end it is for the patients" (patient #13)*

However, patients did deplore a lack of sharing of data and/or samples because of commercial reasons and some expressed that this protective attitude should not be allowed. Some interviewed patients expressed great hopes that researchers share

and collaborate to exploit the full potential of the participants' data and samples.

> "*They should bring together all these data, and aim to achieve goals together since in the end everybody is doing research for the same purpose (…). If you invent a coffee pot, I can understand that you want to protect your invention, but this is about human lives, the wellbeing of people." (patient #2)*

While the majority expressed the view that scientific advances and medical research should be the greatest motivation, some understood that pharmaceutical companies are protective over the sharing of data and/or samples:

> "*I can understand from a company's perspective that you want to protect those things, but if it could benefit other people… it would be better if they would open up the data." (patient #6)*

One participant even clearly stated that the donated material belongs to the study sponsor, since they invested a lot of money in collecting it (patient #11). Therefore, this patient found it appropriate that it is up to the study sponsor to decide with whom he shares the data and/or material.

> "*I can relate to that, the pharmaceutical sponsors have put a lot of capital in that, and you also have patents and so on… I think it is good that this (material and data) belongs to them and that they can determine either yes or no. In the end, this is their material and data*" *(patient #11)*

Interactive, Electronic Tools for Increased Patient Control

Some participants have a desire for greater involvement and/or greater need for information. This was reflected when we introduced the idea of a more interactive consent tool where they could individualize their preferences toward their data and sample management. Interviewed patients were positive about the use of an electronic platform that provides opportunities to enable greater control over their consent. Participants highlighted that today's consent practices do not allow to indicate what can happen with the donated data/samples or how they would like to be informed about any further use or to get research results communicated back to them. Although the majority of interviewed patients mentioned that they would share their data openly without any further limitations, consent practices incorporating such preferences were found useful.

> "*That would be really easy as a matter of fact. This does not exist yet and it would be really interesting for patients*" *(patient #6)*

Even though many interviewed patients indicated that it would not be of relevance to them (since they were not actively working with multi-media devices), they recognized the importance for other, more IT-minded people. Especially, some acknowledged such tools to be beneficial for those putting more emphasis on their privacy or their individual preferences. As a condition for use, however, the privacy and security of those systems should be guaranteed. Two participants clearly expressed concerns about multi-media devices replacing the personal doctor-patient contact, which is perceived as very valuable. Despite the potential benefits, one participant (patient #8) expressed his distrust against new, electronic systems. Although it was explained during the interview that such tools would not replace (but rather support) the personal doctor-patient contact, he feared electronic tools to become alternatives of the traditional care provision and treatment.

DISCUSSION

The current study presents the opinion of cancer patients participating in a clinical trial on a number of themes that may affect the willingness to share data and samples. These themes (the re-use by the same or a different research team, the role of independent ethics committees, the opinion on for-profit and not-for-profit research and the value of interactive, electronic tools for increased patient control) were introduced to the participants during face-to-face interviews. A number of key findings can be derived from our study that should be taken into account when designing patient-approved data/sample sharing frameworks in clinical research.

First, most of the cancer patients interviewed in this study have the view that their data and/or samples can and should be re-used to stimulate medical research in their disease domain. Participants felt that it is their duty to contribute to science, almost as if it is their social responsibility to do so. In this respect, the current results echo those by Jones et al. (28). However, our results indicate even more liberal attitudes toward data sharing. One reason might be that where Jones et al. targeted a broad patient population, we specifically targeted oncology patients participating in a clinical trial in the University Hospital of Leuven (Belgium). Considering their disease status and participation in a trial, it may be that our target group is more open toward sharing and re-using with the ultimate aim to support research; whereas a number of patients included in the study of Jones et al. are slightly more risk averse. The question may arise whether patients with cancer place a greater premium on the public benefits of medical research, and less on their individual rights to privacy. This is important, since overemphasizing such individual rights could present challenges to the conduct of activities performed for public rather than for individual benefit, for instance medical research. Or as Selinger puts it: "*Total autonomy of one individual can have a negative effect on autonomy of other individuals*" since one could approve data use for his own treatment, but hamper it to improve care for others (30).

Second, even though interviewed patients clearly want to contribute to advances in medical research, they showed little interest in the specific purposes for which their data and samples are being used. This finding is in line with the results from Mello et al. that showed that the willingness to share data is

not really affected by the purpose for which the data would be used (31). It suggests a form of institutional trust in the hospital as well as in the clinicians, but this also raises questions about how well research participants read and understand ICFs. Moreover, this study indicates that trial participants view data and samples as similar resources, while from a legal perspective they are not considered the same, which complicates their re-use and/or sharing.

Third, although participants support re-use by the initial and other research teams, divergent opinions exist as to the level of control and patient involvement, which is in line with the results from two quantitative surveys by Shah et al. (32, 33). A small group of participants favored completely open use of their donated data and/or samples, thereby renouncing any form of control. These patients are comfortable as being "passive observers" of the whole research project. Considering the myriad of initiatives initiated to increase "patient empowerment," "patient centricity," and "patient engagement" the last few years (34), it is important not to obfuscate this finding: we should not overdue patient involvement or put an undue burden on patients to actively manage their care process where this is not desired. The majority of participants favored easy re-use but valued a higher degree of control/engagement in this process. However, it was recognized that the lack of opportunities for greater involvement complicates this. Lastly, another small group of patients strongly felt the need for being actively involved (i.e., by re-consenting) when data is shared with initially unknown research groups. Although these patients did not object to such sharing, they expressed concerns about security and a lack of trust with respect to potential recipients. Trust and transparency about data and sample sharing arrangements is of utmost importance in medical research since experience of inappropriate disclosure could negatively impact on participants' willingness to share information, or at worst, avoid future participation (4).

Fourth, all but two patients expressed their trust in ethics committees taking up the task of intermediary decision maker. In a previous quantitative study with 2,005 patients with rare diseases, about half of the respondents indicated that they would allow an ethics committee to decide on their behalf (35). Our finding reflects the practices as prescribed by ethical recommendations such as the Helsinki Declaration, although not echoed in all legal frameworks since the EU data protection framework does not stipulate any intermediary form of control for secondary re-use of sensitive data. In general, confusion exists among researchers about whether or not informed consent is needed for re-use of data for further research. The General Data Protection Regulation (GDPR) stipulates different legal grounds for processing of personal data. Aside from explicit consent from the participant [Art. 6(a)], public interest [Art.6(e)] may also be considered. The GDPR leaves it up to member states to define what constitutes "public interest". Belgian law does not mention scientific research as a type of public interest. Therefore, consent for research may remain the important legal basis for re-use of personal data in Belgian context.

Fifth, patients in this study expressed only few concerns about the for-profit/not-for-profit nature of organizations, explaining that even if pharmaceutical companies are driven by profit, their profit is made by developing products that benefit patients, thus ultimately all medical research serves the same purpose. This finding is somewhat contrary to the results from previously published quantitative studies, which indicated that research participants and rare diseases patients were more likely or comfortable to allow their data to be shared with not-for-profit stakeholders (e.g., academic researchers, health care professionals, non-profit and patient organizations) than with researchers in for-profit companies or insurance companies (31, 32, 35, 36). Yet, most participants in our study did mention that they deplore a lack of collaboration and sharing between researchers because of commercial reasons.

Finally, digitalization has opened up new possibilities for patients to be engaged in research. However, beyond the current popular rhetoric of patient empowerment, this study aims to clarify patients' attitudes concerning the use of new tools to consent and to enable greater control over data and/or sample management. Participants were mostly positive about the use of such tools, and valued, besides increased control and transparency, the possibility for the provision of feedback from research results. Some patients explicitly recognized that even if privacy was less important to them, individualized consent methods could be valuable to others paying more attention to their privacy. However, there is an important issue to consider when thinking of implementation of e-consent tools. One should carefully consider the consequences when conducting research based on data from "information altruists," especially the potential selection bias. Previous research reports that, from the general public, those with higher educational qualifications are more likely to share their EHRs (37). Further, it was recognized by almost all participants that in practice, such system might not yet be of direct benefit to them (which can be linked to the high age of the participants). However, they acknowledged such an approach to be more important for younger people or in the future. Nonetheless, technological (e.g., security), operational (e.g., ease of use), and legal concerns (e.g., privacy) were expressed. Importantly, interviewed patients highly valued personal contacts with their treating physician, emphasizing that in the existence of such system, this should not replace these face-to-face discussions.

Although, the current qualitative study provides some interesting new insights into different aspects that may affect a patient's (un)willingness to share his or her data and samples, it is exploratory in nature and has some important limitations. First, this is a single center study with a small sample size and a homogenous cohort (i.e., gastroenterological/oncogynecologic diseases only). Consequently, the study results are not generalizable to other patient groups or countries. Patients with a chronic or terminal illness might be more willing to share data in comparison to patients with better health outcomes, lower impact, or higher stigmatization. In addition, other factors influencing a patient's willingness to share could include culture, educational level or sociodemographic factors. Second, this study applied qualitative research methods only (i.e., in-depth interviews), so our results do not allow us to quantify the

patients' perspectives and we cannot draw any conclusions about potential links between demographic parameters (i.e., disease stage, educational level, age, and sex) and the willingness to donate data and/or samples. A follow-up quantitative study, through surveys for example, could be useful to investigate this in more detail. Of note, a number of large-scale, quantitative studies investigating similar topics regarding data and/or sample sharing in various study populations have been published in recent years and should be taken into consideration when designing future research projects (31–33, 35, 36, 38). Third, the questions in Part III and IV of the interview guide refer to hypothetical situations, meaning that patients' answers may differ from real-life decisions. However, the patients in this study were in fact participating in a clinical trial so they could relate well to these situations. Fourth, only adult patients currently participating in a clinical trial were included. As a result, this study cannot draw conclusions about the need for re-consent to use samples and data of pediatric cancer patients once they become adults who can consent on their own behalf, which would be an interesting topic for additional research. Finally, additional themes were brought up by patients during the interviews, such as reciprocity (e.g., the need to communicate back research results). However, further research is needed to better understand these topics, which is why they were not discussed in more detail in this study.

CONCLUSION

Discussions about clinical trial data sharing have largely taken place among experts. This study indicates a willingness of cancer patients participating in a trial to re-use their trial data and/or samples by the same research team, and a generally open approach to share these with other research teams albeit with the provision of information. Although the majority of interviewed patients had not thought much about sharing their data and/or samples in advance, they regretted the current lack of re-use and expressed wishes for (both for-profit and not-for-profit) organizations to collaborate in the future, to ensure the optimized use of their data and/or samples to achieve therapeutic improvements for fellow patients. Divergent opinions exist about how patients prefer to be engaged, ranging from passive donors to more actively involved patients, up to those explicitly wanting more control. To respect all attitudes, a stratified approach may be useful, in which those patients who want to have more say in the potential re-use of their donated data and/or samples can do so, for instance by e-consent approaches allowing individualization of preferences. However, the implementation of such an approach warrants further research and goes hand in hand with fully informing research participants about how their donations may be broadcasted and used by others. Educating and informing the patients sufficiently about the risks and the benefits of increased sharing is a sine qua non for participating more actively in the process.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article can be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

This study protocol was approved by the Ethics Committee of UZ Leuven, Belgium (reference: S59829). All subjects gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

IH, DL, MC, and SB developed the idea for and were involved in the design of this study. SB and CV reviewed available data sources and drafted the manuscript. IH, DL, and MC critically revised the manuscript. All authors read and approved the final manuscript.

FUNDING

SB's work as a Fellow at EORTC Headquarters was supported by a grant fromthe EORTC Cancer Research Fund. CV's work as a PhD researcher at the KU Leuven was supported by a grant from the Anticancer Fund. This publication was supported by a donation from Kom op tegen Kanker from Belgium.

ACKNOWLEDGMENTS

The authors would like to thank Virginie Stroobants, Nathalie Wellens, and Jolien Musseeuw for conducting the interviews in the context of their Master's thesis.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.00033/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 14 February 2020
doi: 10.3389/fmed.2020.00037

Biobank@VITO: Biobanking the General Population in Flanders

Rosette Van Den Heuvel[1]*, *Elly Den Hond*[2], *Ann Colles*[1], *Vera Nelen*[2], *Karen Van Campenhout*[3] and *Greet Schoeters*[1]

[1] Unit Health, Flanders' Research and Technology Organisation on Cleantech and Sustainable Development (VITO), Mol, Belgium, [2] Department of Environment, Provincial Institute for Hygiene (PIH), Antwerp, Belgium, [3] Vlaams Planbureau voor Omgeving (VPO), Flemish Government, Brussels, Belgium

During the last 15 years, VITO has established an infrastructure for biobanking a collection of biological samples from the general population in Flanders (Belgium). This biobank was set up to contribute to future, yet unspecified, research questions in the field of environment and health. Biobank@VITO is a population biobank in which bio-specimen including human peripheral blood, cord blood, and blood derivatives (e.g., serum, plasma, cells, RNA, DNA), urine, hair, nails, exhaled breath condensate, saliva DNA, and human breast milk collected from non-diseased populations are preserved. Currently, the biobank stores about 70,000 samples from 7,700 individuals. These biospecimen were collected since 2002 in different human biomonitoring studies comprising European (e.g., DEMOCOPHES, HBM4EU), national (e.g., WHO human breastmilk studies), Flemish (Flemish Environment and Health Study (FLEHS) campaigns), and local (e.g., hotspots, 3xG project) well-defined and ethically approved research projects. Participants to the surveys included different age groups (newborns, children, adolescents, and adults) and were representatively selected with regard to gender, age class, residence, and/or socioeconomic status (SES). In each campaign, samples were stored in the Biobank@VITO. The registration, preservation, and management of the samples in the biobank were done in a qualitative and uniform manner which guarantees the traceability of all samples. The samples in the biobank have an extended information backbone on the lifestyle, environment, and health status of the donor. The biological samples in the biobank are an invaluable archive that can be used to address specific policy and research questions in the future, to test old samples with new technology and according to the latest methods and insights or to measure newly identified pollutants in old samples looking for long-term trends.

Keywords: population biobank, human biomonitoring, biobank, FLEHS, 3XG

OPEN ACCESS

Edited by:
Michel Goldman, Institute for Interdisciplinary Innovation in Healthcare (I3h), Belgium

Reviewed by:
Sung Won Choi, University of Michigan, United States
Annelies Debucquoy, Belgian Cancer Registry, Belgium

***Correspondence:**
Rosette Van Den Heuvel
rosette.vandenheuvel@vito.be

Specialty section:
This article was submitted to Translational Medicine, a section of the journal Frontiers in Medicine

Received: *28 February 2019*
Accepted: *27 January 2020*
Published: *14 February 2020*

Citation:
Van Den Heuvel R, Den Hond E, Colles A, Nelen V, Van Campenhout K and Schoeters G (2020) Biobank@VITO: Biobanking the General Population in Flanders. Front. Med. 7:37. doi: 10.3389/fmed.2020.00037

INTRODUCTION

Flanders is generally considered to be one of Europe's economic top regions with an extensive transportation network and intensive industrial activity. At the same time, Flanders is one of the most densely populated areas of Europe. Exposure to traffic or industrial emissions remain an important factor for adverse human health effects. Also lifestyle, diet, socio-economic, or physical exercise have been shown to have an impact on the health of the population. All these issues clearly illustrate that the relationship between environmental quality, socio-economic living conditions, individual behavior, and public health is a very complex one. As a response to this and to the societal

challenges, human biomonitoring data and auxiliary personal information are collected and used as science based evidence to underpin measures for safeguarding environmental quality and to minimize the adverse effects of environmental stressors. In 2003, the Flemish government voted the Decree on Preventive Health Care as a legal recognition of environmental health in which the Flemish government imposes itself to perform human biomonitoring (HBM), i.e., the measurement of potentially adverse chemicals in human matrices such as urine or blood. In order to achieve a structured and coordinated approach for human biomonitoring in Flanders, the Flemish Centre of Expertise on Environment and Health was founded by the Flemish government (Department of Economics, Science and Innovation; Flemish Agency for Care and Health; and Department of Environment, Nature and Energy). The Center is the main driving force behind the Flemish Environment and Health Studies, FLEHS. Human biomonitoring is the core of the programme on which several research projects are engrafted. Internal concentrations of a broad range of environmental chemicals and/or associated health effects are monitored. Exposure biomarkers provide information on the internal exposure to chemical substances and effect biomarkers provide information on the biological consequences of the presence of these chemical substances in the human body.

For over 15 years, a large number of human samples (urine, blood, plasma, hair,…) have been collected in the Flemish general population and have been analyzed for the presence of environmental chemicals or their metabolites. This allowed to estimate population reference values (statistically derived numbers that indicate the upper margin of background exposure to a given substance in a defined population at a given time) of exposure to both well-established and new or emerging chemicals (1). Also other projects, typically addressing specific human health issues around areas with specific environmental pressure (hot-spots), or topics of societal concern, use HBM for mapping and benchmarking the levels of potentially adverse chemicals in the otherwise healthy general population (2). In each campaign, additionally to biomarker analyses which were planned to answer the research questions, extra samples were taken and stored in the biobank. These samples may be used in the future for both prospective and retrospective research. The concept of the biobank may be considered as a biological archive of internal human exposure levels in Flanders, and as such allows analysis of the past with future technologies.

This paper describes the mission, the objectives, the management, and the sample collections of the population biobank hosted at VITO.

MISSION

Human biomonitoring in the general population is an intrinsic part of the Flemish approach to science based policy-making in the field of environment and health. The monitoring programs of the Flemish Centre of Expertise for Environment and Health comprise now already a large number of chemicals. However, due to the rapid progress of technology and predicted increases in production volume of chemicals over the next 30 years, we expect increased exposure through environment and use of consumption products and people will come into contact with ever more and new foreign substances. As a result, additional relevant research or policy questions may arise in the future. Therefore, as part of the FLEHS programme and on request of the Flemish authorities, it was decided to build a human biobank in which samples are stored for potential use in the future.

Since 2002, biobank samples were stored in a biorepository. The human samples originate from members of different age groups from the Flemish population that were invited to participate in the studies according to a randomized two stage recruitment strategy and that gave permission to take and store their samples in the biobank. A large number of human biological samples including blood and blood derivatives, urine, hair, nails, exhaled breath condensate, saliva DNA, and human breast milk samples have been collected over the years, resulting from the activities of the Flemish Centre of Expertise for Environment and Health and a number of other biomonitoring activities. The samples are well-documented in terms of individual exposure to environmental chemicals and related health status. Additionally, extensive information on lifestyle, personal characteristics, food consumption, individual risk perception, etc., is available from self-assessed questionnaires at the time of sampling. In this way, the samples are invaluable for new knowledge acquisition in the field of environment and health. In order to preserve this potential optimally for the future and to be able to serve for further research, a well-developed biobank is necessary.

Biobank@VITO aims at setting up a professional, state-of-the-art biobanking structure in which the focus is put on collecting samples of the general population. The goal is 2-fold: (1) provide a facility where the collection of human samples, gathered over the last two decades, are stored under state-of-the-art circumstances; (2) provide a professional storage facility for future (prospective) biomonitoring initiatives in Flanders and elsewhere. Both objectives are implemented with attention to respect the privacy of the participants [GDPR (General Data Protection Regulation) compliant] and ethical aspects.

OBJECTIVES

Additional Measurements to Extend Studies

The biobank allows additional biomarker measurements to expand past and ongoing studies. Supplementary biomarker analyses (both biomarkers of exposure and biomarkers of effect) can be carried out at a later time in an existing cohort of which much information is already available. This saves a lot of costs and work as no new recruitment and sampling should be organized, and maximum use can be made of the questionnaire data and measurements that are already available in the database.

With appropriate storage of samples, retrospective analyses of samples from years or even decades ago can be performed using state-of-the-art analytical technology to assess internal exposure and associated biological effects.

Follow Trends in Time

Following time trends requires different campaigns that are spread over time. The biobank allows screening of new and emerging chemicals in various matrices, once appropriate biomarkers are developed. For these new biomarkers, samples from the past can then be analyzed to check the level of these substances in the body x years ago and to assess how levels evolve over time. These analyzes can be performed on pooled samples as well as on individual samples. Such time trends can best be followed up in reference populations.

By repeating biomonitoring campaigns at regular intervals, human exposure to pollutants over time can be monitored and policy measures can be evaluated (3).

Prospective Studies

Increasingly, results of prospective cohort studies are used in environmental health research. Repetitive sampling in the same individuals is extremely powerful to unravel the often very subtle and complex relationships between environmental (chemical or lifestyle related) stressors and potentially adverse health effects. Follow-up studies allow people to be monitored during successive cycles with a specific pre-defined goal in order to study health effects in the longer term (years). Therefore, questionnaires and additional examinations (e.g., including new blood or urine collections) can be scheduled at regular intervals as well as requesting personal health data from registers. By submitting samples to the biobank at each of the successive cycles, new research hypotheses can be tested afterwards.

Often, these prospective studies are birth cohorts, in which the child is followed from the moment of birth (or already *in utero*) for several years or even decades. Prospective cohorts like the 3xG study, or the various newborn cohorts of the FLEHS cycles (4) are examples of ongoing longitudinal birth cohorts studies in Flanders.

Retrospective Studies

Samples that are stored in the biobank, may be used for retrospective assessment of both exposure and biological effects as new and improved technical innovations become available or to give answer to new research questions. Samples from follow-up studies that were stored under the appropriate conditions could be used for (1) the detection of novel effect biomarkers (e.g., omics) to monitor the early onset of diseases or (2) the identification of historical exposure that is involved in the onset of diseases later in life The availability of biobank samples in combination with extensive information on the participants allows to design nested case-control studies, and hence offers reductions in costs and efforts of data collection and works more efficiently in case of rare outcomes, expensive measurements, or missing covariates.

PRIVACY AND ETHICAL ASPECTS

Since human biomonitoring campaigns involve processing of personal data, the studies were registered at the Belgian Privacy Commission (CBPL = Commissie voor de bescherming van de persoonlijke levenssfeer) until the new GDPR law came into force on 25 May 2018 [(EU) 2016/679]. Following this, the new guidelines on the protection of personal data were applied.

In addition, attention is paid to compliance with the ethical code for dealing with biological material (5). All samples present in the Biobank@VITO have been collected in the context of a predefined research project. Each human biomonitoring study was submitted for approval to the Ethics Committee of the university of Antwerp for all studies, and additionally to the Ethics Committee of local hospitals in some specific cases (e.g., newborn studies). The initial principle of the biobank, being the storage of samples in the long term, was explained to the participant at the start of the study in the information brochure and in the consent form. The study participant gave written and signed permission for the storage of biological samples in the biorepository. In addition, contact details of the principal investigator (PI) and responsible doctor were given on the information letter and consent form (template forms in **Supplementary Material**). Participants gave donor consent which means that the rationale for the use of donors' samples and data is explained in great detail in the consent form. In mother-birth cohorts, the participating mothers filled out an informed consent. In case minor children were involved, parents signed the informed consent on behalf of the children. In the adolescent population (14–16 years), both the minor participant and one of the parents gave written informed consent to participate in the study. In the case children reach the age of majority, they will be asked to re-consent for the storage and use of their samples collected at the minor age.

No names or addresses of participants are registered in the Biobank@VITO. All participants, their samples and additional information are pseudonymized by the use of a unique identification code. The key to this code is only known to the field work team that works under the supervision of the responsible medical doctor. All communication with the participants takes place through the latter. The participant can request at any time information about the state of the research and the samples in the biobank, either through the PI or the medical doctor, but only the responsible study doctor can report back to the participant. Each individual can terminate further participation in the study and/or request at each moment to destroy his/her remaining personal samples. The results that are already in the database remain available for the researchers unless the participant explicitly asks to remove all his/her data. The data manager of the field work team contacts the Biobank manager if the consent status of the participant changes. The Biobank manager takes the necessary actions based on the unique identification code. For specific subgroups, permission from the participant was obtained to request personal health data from registers, e.g., data from child care services in the newborn group, data from school health investigations in the adolescent group, etc. This information is requested by the medical doctor, and can be coupled to the central study database on the basis of the unique identification code.

The participants are aware of the fact that the analyses that are performed on biobank samples are not communicated on an individual level, since this is explicitly mentioned in the

informed consent. However, summary reports of the studies that are performed on biobank samples are communicated via the study websites. Based on these reports, participant can follow the new analyses that are performed. At any moment, a participant has the right to request his/her personal results via the PI or the medical doctor, and this information will then by shared by the medical doctor, either via letter or via telephone, to be able to provide the necessary background information. Also in case of an alarming result for a health parameter that is clearly interpretable, the medical doctor could communicate this result directly to the participant, after discussion and consent by the management board.

METHODS

Sample Management

The human samples are collected, registered, stored, and managed in a qualitative and uniform manner. Procedures for sample collection, sample pre-treatment, aliquoting, primary and secondary sample tubes, temporary storage, transport conditions, and storage method are documented in a detailed manual. Biobank@VITO uses an unambiguous donor identification system which guarantees the traceability of all samples at any time. All samples are uniformly provided with a unique label number. The sample number is linked to a minimal dataset such as the identification code of the participant, collection date, sample type, sample volume, tube code, and sample location. All this information is stored in a database. Since 2015, a computer-based inventory LIMS (Laboratory Information Management System) system is in use for sample registration and management (Labvantage). The inventory enables to identify the location of any sample at all times.

Sample types include whole (cord)blood, (cord)blood derivatives such plasma, serum, red blood cells, white blood cells, RNA and DNA, urine, hair, nails, saliva DNA, exhaled breath condensate, and breastmilk. Samples are stored under optimal conditions in storage facilities at various storage temperatures including −80°C, −20°C and room temperature.

Continuously efforts are made to improve the quality management system of the biobank in order to be compliant with OECD and ISO guidelines for biobanks (6, 7) and the Belgian law on biobanking (8). Biobank@VITO received ethical approval and was notified to FAMHP (Federal Agency for Medicines and Health Products) (notification number BB190064). The organizational structure of Biobank@VITO and the sample flow in the biobank are shown in **Figure 1**. The workflow starts with the notification of a new study at the biobank manager. Proof of ethical review, study design and minimal dataset are needed to proceed the registration of samples in the sample management system (LIMS, Labvantage). Sample release can only take place if the necessary formalities are met, in particular signed MTA's (material transfer agreement).

Quality Assurance Measures

The pre-analytic phase includes the collection, transport and registration of the samples. The different steps are described in detail in a scenario or in a specific procedure (SOPs). The management of material and equipment is described in a SOP and registrations are kept in a database.

Samples are stored under the most appropriate condition depending on the sample type and the biomarker to measure. The quality and the stability of the samples is checked on a regular basis and is dependent on the type of sample and the planned analyses e.g., DNA/RNA integrity check, biomarker stability using control samples kept under the same conditions as the actual samples. Samples are stored in small aliquots and freeze-thaw cycles are kept to a minimum. The storage time can be dependent on the quality of samples, on the number of freeze-thaw cycles, on the participants consent or can be a fixed period set by the PI.

Freezers and cooling systems are equipped with a computer-based temperature monitoring system for temperature control and an automatic alarm system in case of repository failure. Freezers are centralized in separate rooms that are not shared with other activities.

Sample Access Policy

As participants gave donor consent and no broad consent, samples from collections are available for further research within the context of the well-defined research topic that researchers have had the sample donor's consent for. Therefore, novel usage of the samples considering any new research, further uses or new studies, is not possible without first obtaining a new consent of the donor. Supervision on the correct compliance with these terms is the responsibility of the Biobank manager.

In addition, Biobank@VITO hosts different samples collections and access to samples is dependent on the reuse policy of the collection's PI. Sample requests addressed directly to the Biobank@VITO will be referred to the relevant PI. As such, new research projects aiming to use samples from the Flemish Environment and Health Study (FLEHS) collections in the biobank have to apply a request to the Supervisory Board of the Centre of Expertise on Environment and Health. Data/sample transfer can only be carried out after approval by the Supervisory Board. Terms and conditions for data/sample request are defined by the Supervisory Committee. For every new analysis on biobank samples, the approval of an ethics committee is required.

COLLECTIONS

A large amount of samples have been collected from the general population of Belgium (e.g., DEMOCOPHES), Flanders (e.g., FLEHS, HBM4EU) or specific regions (e.g., hotspot studies, 3xG). These human biomonitoring studies have different scopes: they often combine specific research questions and policy-based goals, and are financed by different funders, such as the European Commission, the Flemish government, national, or regional organizations. An overview of the type and amount of samples in the biorepository which were collected in different studies is given in **Table 1**. The main studies are described in detail below.

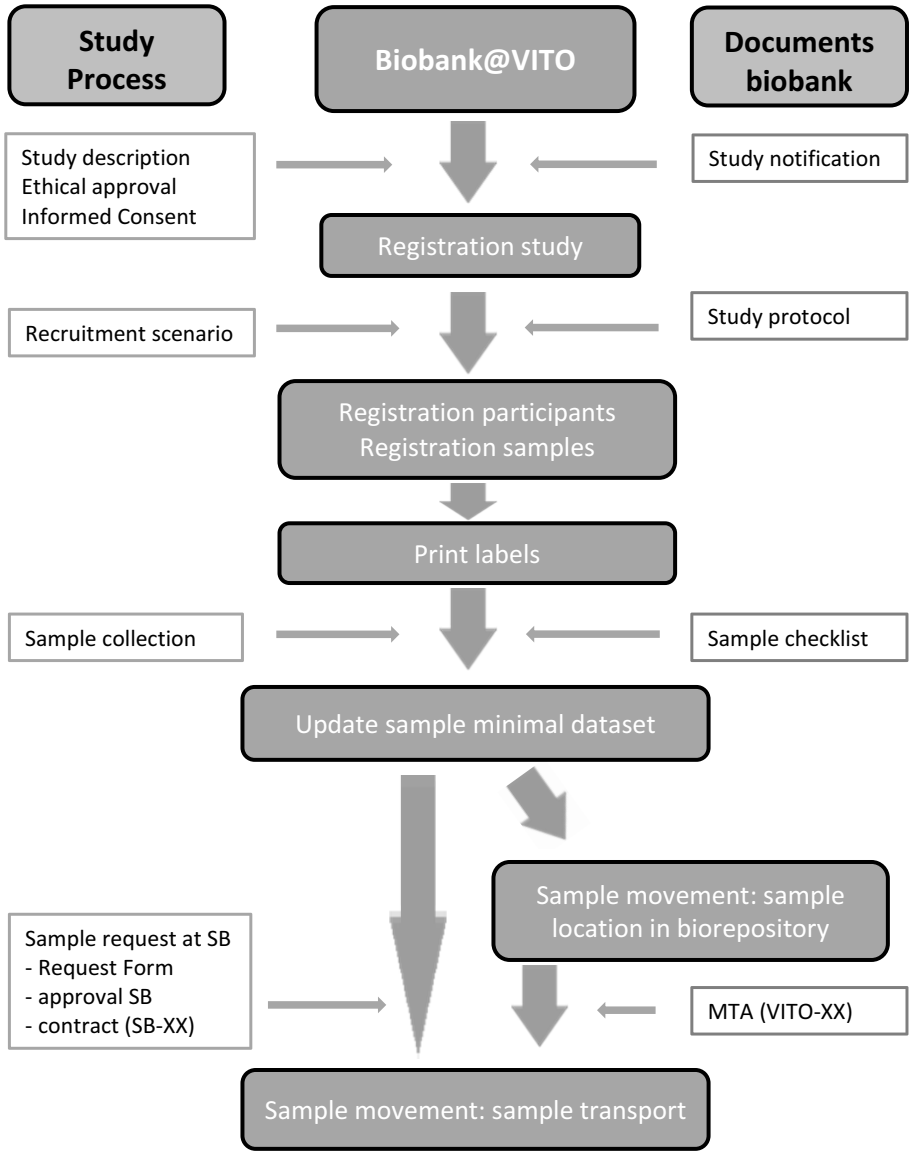

FIGURE 1 | Management structure Biobank@VITO and sample flow chart. (SB, Supervisory Board of the Centre of Expertise on Environment and Health; MTA, Material Transfer Agreement).

Flemish Environment and Health Study (FLEHS)

On behalf of the Flemish government, the Center of Expertise on Environment and Health has studied the human exposure and health effects of environmental pollutants by conducting 5 years human biomonitoring campaigns in Flanders (3, 9, 15). The research consortium, a collaborative effort of VITO, the Provincial Institute for Hygiene (PIH) and teams of the five Flemish universities, has applied human biomonitoring to detect the levels and the effects of environmental pollutants in babies, adolescents and adults. Four cycles of human biomonitoring were conducted up to now.

FLEHS I

The first Flemish Environment and Health Study (FLEHS I 2002–2006) included participants belonging to three different age groups (newborns and their mothers, 14–15 year old adolescents and 50–65 year old adults) recruited in eight regions in Flanders with different environmental characteristics. In total, about 1,600 participants per age group participated in the study. The exposure and health effects of 'classic' historical pollutants [such as heavy metals, dioxin-like compounds, para-dichlorodiphenyldichloro-ethylene (p,p'-DDE), polycyclic aromatic hydrocarbons (PAHs)] were measured. Blood and urine samples were collected. Any leftover specimens after biomarker analyses were stored in a

TABLE 1 | Collections of human biological material in Biobank@VITO.

Study	Collection period	Population	Number participants	Gender (%) (male/female)	Sample types	Biobank samples *N*	Storage
FLEHS I	2002–2004	Newborns	1,196	52/48	Cord blood	1,799	−20°C
					Cord blood plasma	1,974	−20°C
					Cord bloodDNA	117	−80°C
	2003–2004	Adolescents (14–15 y)	1,679	53.1/46.9	Peripheral blood	1,636	−20°C
					Serum	1,445	−20°C
					Urine	1,688	−20°C
	2004–2005	Adults (50–65 y)	1,583	49/51	Peripheral blood	1,526	−20°C
					Serum	1,489	−20°C
					Urine	1,581	−20°C
FLEHS I birth cohort follow-up	2013–2014	Children (10 y)	133	33.1/66.9	Blood white blood cells	99	−80°C
					Red blood cells	300	−80°C
					Plasma	86	−80°C
					Blood DNA	134	−80°C
					Blood RNA	100	−20°C
					Saliva DNA	132	−80°C
FLEHS II	2008–2009	Newborns	255	52/48	Cord blood plasma	219	−80°C
					Cord blood cells	254	−80°C
					Cord blood DNA	726	−80°C
	2008–2009	Adolescentsss (14–15 y)	210	57.6/42.4	Peripheral blood	196	−80°C
					Serum	64	−80°C
					Urine	1,828	−80°C/−20°C
	2008–2009	Adults (20–40 y)	204	47.1/52.9	Peripheral blood	406	−80°C
					Serum	622	−80°C
					Urine	2,033	−80°C/−20°C
FLEHS III	2014	Newborns	281	53.1/48.8	Cord blood	1,710	−80°C/−20°C
					Cord blood plasma	122	−80°C/−20°C
					Cord blood cells	279	−80°C
					Hair mother	196	RT
					Nails mother	159	RT
	2012–2013	Adolescentss (14–15 y)	208	45.7/54.3	Peripheral blood	618	−80°C
					Peripheral blood cells	276	−80°C
					Blood RNA	92	−20°C
					Serum	181	−80°C
					Plasma	202	−80°C
					Urine	496	−80°C
					EBC*	41	−80°C
	2014	Adults (50–65 y)	209	46/54	Peripheral blood	616	−80°C
					Blood DNA	204	−20°C
					Serum	333	−80°C
					Urine	657	−80°C/−20°C
FLEHS IV + FLEHS I birth cohort (14–15 year)	2017–2018	Adolescents (14–15 y)	611	47.2/52.8	Peripheral blood	1,306	−80°C
					Peripheral blood cells	609	−80°C
					Blood RNA	608	−20°C
					Serum	1,879	−80°C
					Urine	3,009	−80°C/−20°C
					Hair	609	RT
3XG	2011–2015	Mother–newborn cohort	301	50.8/19.2	Cord blood	1,039	−80°C/−20°C
					Cord blood cells	1,110	−80°C
					Cord blood plasma	930	−80°C/−20°C
					Peripheral blood	1,211	−80°C
					Serum	721	−80°C/−20°C
					Urine	5,575	−80°C/−20°C
					Breastmilk	196	−80°C/−20°C
DEMOCOPHES	2011–2012	Mother (≤45 y)—child cohort (6–11 y)	129	51.2/48.8	Urine	873	−80°C
					Hair	263	RT
Hotspot study: Genk	2010	Adolescents (14–15 y)	197	45.2/54.8	Serum	169	−80°C/−20°C
					Plasma	552	−20°C
					Urine	1,942	−80°C/−20°C
					Red blood cells	137	−20°C

(Continued)

TABLE 1 | Continued

Study	Collection period	Population	Number participants	Gender (%) (male/female)	Sample types	Biobank samples *N*	Storage
Hotspot study: Menen	2011	Adolescents (14–15 y)	199	57.3/42.7	Serum	177	−80°C/−20°C
					Plasma	585	−20°C
					Urine	2331	−80°C/−20°C
					Red blood cells	195	
Hotspot study: Ghent canal zone	2013	Adolescents (14–15 y)	200	50.5/49.5	Peripheral blood	789	−80°C/−20°C
					Peripheral blood cells	249	−80°C
					Serum	180	−80°C
					Plasma	94	−80°C
					Urine	564	−80°C
					EBC*	752	−80°C

*EBC, exhaled breath condensate.

biorepository. Saliva and blood samples were collected in a subpopulation of the longitudinal birth cohort of FLEHS I at the age of 10 years (4).

FLEHS II

The FLEHS II (2007–2011) biomonitoring campaign aimed to set reference values for a broad range of environmental pollutants in three age groups of the general population (10). Participants were recruited across Flanders. In addition to the historical pollutants, a large number of "new" pollutants (including phthalates, brominated flame retardants, musk's, new pesticides,...) were investigated in three age groups (1) a newborn cohort ($n = 250$), (2) 14–15 year old adolescents ($n = 200$), and (3) adults between 20 and 40 years of age ($n = 200$). Moreover, adolescents of 14–15 years were recruited in two industrial hotspot areas in Flanders (Genk, Menen) ($n = 200$ in each hotspot) (11). Field work, chemical analyses, database management, statistical analysis, interpretation, and communication was performed according to the same standards as in the reference population. In FLEHS II, cord blood, blood, and urine samples were collected and analyzed for the pre-defined measurements. A specific plan to store biobank samples was put into practice: small additional volumes of samples were collected and stored in the biorepository.

FLEHS III

The third human biomonitoring program FLEHS III (2012–2015) continued to build on the broad basis of the first and second cycle. Flemish reference values both for historical and recent pollutants were determined in different age groups: pregnant mothers, adolescents of 14–15 year old, adults (50–65 years). Between 200 and 300 study participants per age group were recruited across Flanders. Specific efforts were made for recruitment of participants from different ethnic origin, low income or low education level. Cord blood, blood, urine, and hair samples were collected. In addition, adolescents were studied in one industrial hotspot area (Ghent canal zone). Similar to FLEHS II, additional samples were taken for long-term storage in the biorepository.

FLEHS IV

The fourth campaign FLEHS IV (2016–2020) aimed to recruit 600 adolescents aged 14 and 15 years across Flanders from which 200 participated earlier in the newborn campaign of FLEHS I, 14 years ago. FLEHS IV will examine the exposure to environmental pollutants among young people from the general Flemish population. The study will address present-day topics: environmental exposure and health in Flanders in relation to use of space and eco-behavior related to consumption of locally grown or organic food and housing conditions (use of healthy building materials and energy efficiency). New and emerging chemicals were prioritized based on their relevance for assessing exposures from green/gray/blue/agricultural space and eco-behavior. Samples of whole blood, serum, blood RNA, hair, and urine were stored in the biorepository.

3xG Study

The 3xG study is a health monitoring pilot study that has been conducted on behalf of the Belgian Agency for Radioactive Waste and Enriched Fossil Materials (NIRAS) and the local partnerships STORA (Dessel) and MONA (Mol) to survey health in relation to life style and environment of children that are born and grow up in 3 Flemish municipalities (Dessel, Mol, and Retie). The 3xG project is programmed as a long term follow up study for children from before birth until the age of 18 years and was initiated in autumn 2009. The project is carried out by the VITO Health team, Provincial Institute of Hygiene of Antwerp and social scientists of the University of Antwerp and collects biomonitoring data on pesticides, heavy metals, substances in consumer goods and on lifestyle. For this study, urine and blood samples of pregnant women and cord blood samples of their babies were collected, analyzed or stored for later analysis. Monitoring newborns from birth and their long term follow up is used as a sentinel for health of the local population. Early warning and sensitive parameters are collected to reflect potential environmental and life style risk factors and to provide advice for improving health. Special focus is on early warnings for obesity, asthma, allergies, growth, and development and heart and vascular diseases. Presently, 300 mothers and their babies are participating in this study. Cord blood, breast milk, blood, plasma, serum, and urine samples were stored in the biorepository. A follow-up of the children at the age of 7 years is planned in 2019–2020.

DEMOCOPHES

The objective of the European Seventh Framework Programme COPHES (Consortium to Perform Human biomonitoring on a European Scale) was to develop a harmonized approach to conduct human biomonitoring in Europe. In 17 European countries, the biomonitoring guidelines and protocols developed by COPHES were tested in a biomonitoring pilot study DEMOCOPHES (DEMOnstration of a study to COordinate and Perform Human biomonitoring on a European Scale) (12, 13). Mercury in hair and cotinine, phthalate metabolites and cadmium in urine of 1,844 children (5–11 years of age) and their mothers were measured. The Belgian participant population consisted in 129 children aged 6–11 years and their mothers ($\leq$45 years) living in urban or rural areas of Belgium (14). Samples were collected over a 5 months period in 2011–2012. Leftover samples of urine and hair were stored in the biorepository.

CONCLUSION

Biobank@VITO has its limitations and strengths. Biobank@VITO is a population biobank and hosts a heterogeneous collection of samples from the general population spread across Flanders and over time and covers different ages. As no continuous monitoring program exist in Flanders the characteristics of the collections are completely dependent on the research question of the biomonitoring study. Over the years, samples have always been collected and stored according to most appropriate conditions at that time with regard to the selected biomarkers. Due to progressive insight into storage conditions and technological developments, some issues must be taken into account when reusing these samples. The quality of the samples collected a long time ago might not be useful to perform all type of newly developed biomarker analyses because of the way they were collected/stored at that time and the uncertainty of biomarker stability over time. However, for specific purposes, these samples are still very valuable. Further, biobank establishment, biobanking of human samples and biobank management and maintenance is costly and involves a significant workload. These expenses have to be taken into account in project application and implementation and have a considerable impact on the study budget.

However, Biobank@VITO is unique in providing a biological archive of human exposure to environmental chemicals in Flanders. The biobank holds great potential for research on the interaction between health, environment and lifestyle to support policy development in the nexus of environment and health. The biobank is a sustainable platform in which the same human samples can be reanalyzed for new technologically advanced exposure and effect biomarkers. The platform will allow to address specific research questions on population health in relation to environmental factors allowing both prospective and retrospective analysis.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

GS, VN, and ED designed and coordinated the Flemish HBM studies FLEHS and 3xG. GS, KV, and ED initiated the biobank initiative at VITO. ED, AC, and RV further elaborated the biobank initiative. RV wrote the paper. All authors evaluated and approved the manuscript.

FUNDING

The initiation, development and maintenance of Biobank@VITO were financed by the Flemish Government (Department of Environment) and VITO. The FLEHS studies of the Flemish Center of Expertise on Environment and Health are commissioned, financed, and steered by the Ministry of the Flemish Community (Department of Environment; Department of Economics, Science and Innovation and Agency for Care and Health). The 3XG study is an initiative of the surface disposal project, commissioned by NIRAS (Belgian Agency for Radioactive Waste and Enriched Fissil Materials) and the partnerships STORA and MONA. DEMOCOPHES (DEMOnstration of a study to COordinate and Perform Human biomonitoring on a European Scale) was co-funded (50:50%) by the European Commission LIFE+ Programme (LIFE09/ENV/BE/000410) and the partners.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.00037/full#supplementary-material

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