The Swedish Cervical Cytology Biobank: Sample Handling and Storage Process

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The Swedish Cervical Cytology Biobank (SCCB) is the first national initiative of a prospective repository for liquid-based gynecological cell samples (LBC) from women participating in organized cervical cancer screening programs. Development and implementation of a nationally standardized method for the handling and long-term storage of cervical cell samples has been a primary goal for the Swedish hub of The Biobanking and Molecular Resource Infrastructure (BBMRI.se, www.bbmri.se). The sample handling protocol was developed through i) review of the literature on biobanking processes, ii) wide consultation within the academic community, and iii) various verification assays in collaboration with the clinical cytology laboratories. A general quality management system, covering all aspects of sample handling and storage, has been established. BBMRI.se financed the development and implementation of SCCB. The protocol established in the pilot project in Stockholm is now being implemented in other counties in Sweden, and during this year, more than 120,000 LBC samples will be processed for biobanking nationwide. SCCB is embedded in a comprehensive cytology diagnostic registry and will be linked with the national cancer registry to constitute a nearly inexhaustible resource for performance of fundamental and applied biologic research.

Introduction

The Swedish National Board of Health and Welfare issues national guidelines for cervical cancer screening. According to these guidelines, it is recommended that all women undergo screening tests every 3 years from the age of 23 to 50, and every 5 years between the ages of 50 to 60. This testing collects 700,000 samples annually. Systematic biobanking of this cohort would greatly improve both clinical diagnostics and biomolecular research on cervical cancer and other diseases that affect women. Liquid-based cytology (LBC) is the recommended method for screening and human papilloma virus (HPV) testing; see www.socialstyrelsen.se/hpv. Healthcare in Sweden is organized regionally by county, each of which must have high-quality and standardized methods for sample collection, transport, and diagnosis at clinical cytology laboratories; see www.vardguiden.se.

The biological samples collected from women who have participated in cervical cancer screening constitute huge cohorts, providing the necessary statistical power to aid researchers in translating basic scientific discoveries into clinical applications. The availability of high-quality cytological specimens for diagnostic and research purposes requires the development of standardized methods for sample handling and long-term storage that will enable the future use of specimens. The Swedish Cervical Cytology Biobank (SCCB) is the first national initiative for a prospective repository for liquid-based gynecological cell samples from women participating in organized cervical cancer screening programs. To develop and implement a unified national biobanking system in this area has been one of driving forces of The Biobanking and Molecular Resource Infrastructure of Sweden, BBMRI.se, www.bbmri.se. The sample handling and storage processes for the SCCB have been developed by and provided to multiple clinical cytology laboratories by BBMRI.se. This biobanking procedure has been advanced as an extension of current cytopathology practices in the laboratories.

The protocols for the processing and archiving of biological samples were developed through a review of current literature on the topic, wide consultation with and peer review by the scientific community, and extensive validation to ensure that the proposed procedures met their intended purposes. The sample processing procedures, defined in a series of standard operating procedures, detail the largest amount of residual cervical cells to be collected for aliquoting and storage in the SCCB, the most advantageous final storage format, the aliquoting volume, storage processing, and storage temperature.
Methods

Approach to sample processing and archiving into the SCCB

Liquid-based cytology has emerged as an alternative to the conventional Pap test and is already used for more than 80% of cervical screening in Sweden.1 In this procedure, cervical cells are collected in the individual clinic, suspended and fixed in Thinprep (TP) containing 20 ml PreservCyt (ThinPrep® Hologic, Boxborough, MA) for subsequent preparation and diagnosis of the cell samples.2 Because only a portion of the collected cells is typically needed for the cytological diagnosis and HPV analysis, the majority of the remaining cells had previously been either archived at uncontrolled ambient temperature or discarded. In this article, biobanking is defined as the process of sample handling from the time the LBC samples are delivered from the cytology laboratories to the biobank centers for archiving of the samples.

One of the crucial goals of establishing a long-term archive of cytological material is the creation of a resource with applicability to a wide range of scientific questions and patient care inquiries. To accomplish this overall goal, the biobank requires a robust laboratory information management system (LIMS) to secure the traceability of its samples to their donors and link the samples with the clinical data of the patient, with proper ethical concern given to the use of these data.

The SCCB processing method aims to i) serve as wide a range of scientific investigations as can be anticipated, ii) develop a national methodology platform with rigorous built-in quality assurance and control procedures, iii) preserve intact sample fractions for re-staining with different diagnostic markers, and genetic studies, and iv) maintain a detailed and secure data audit trail through the use of LIMS and a robust inventory function. Development of the cell sample processing and archiving procedures was constrained by the finite budget determined by BBMRI.se and the key principles of the project.

Process and technology development of the SCCB

The principles of building a biobank of residual samples collected within the health care system to make them available to both health care laboratories and the research communities determined the protocol development, which was then adjusted for feasibility and cost.

The pilot project was developed on a manageable scale in a defined area of clinical laboratory medicine, namely the clinical cytology center at the Karolinska Hospital in Stockholm, which cooperated throughout the development and implementation of the protocol. The method developed in the national pilot project was designed to be transferable to other laboratories throughout the country. Therefore, in this article, the SCCB refers to a unified national biobanking system. The national SCCB was initiated as a pilot project in the Stockholm county council with the goal of processing 100,000 LBC samples annually. We attempted to design the best practices for LBC processing and storage and subsequently implement them into a high-throughput automated operation. These processes have been integrated into a prototype robotic system, which is currently handling patient clinical samples.

Verification analysis of LBC sample handling

In this article, we defined the term “validation” as independent procedures that are used together for checking that our system will meet requirements and specifications that were the intended purpose. Verification analysis was performed using LBC samples that were delivered to the biobank center after clinical diagnosis to develop a standardized national sample handling method.

Assessing of storage volume and format. The cost efficiency of sample handling and storage for different storage formats and volumes was assessed. Collecting the cell population from its suspension in the TP-containers provided a solution to storage of the large sizes of sample vials. LBC samples from 20 patients were anonymized randomly and used in verification studies. The assays were verified and repeated in our laboratory. A range of aspiration volumes (100 µl–4 ml) were tested for yield of the largest total amount of cells, and a volume of 4 ml was chosen. An optimal final storage volume of 300 µl was selected in two steps. In the first step, 4 ml of cell solution was aspirated from the bottom of a patient vial (TP) and transferred into an intermediary conical tube (Cat nr: 401201, Nordic Biolabs AB, Täby, Sweden), which was then allowed to sediment for 30 min. In the second step, 300 µl was aspirated from the conical tube and transferred to the destination vial for storage. Because cells in PreserveCyt medium will not freeze at −25°C, it can easily be aliquoted. Furthermore, cell quantitation determined that the dual sedimentation procedure included the majority (on average about 80%) of cells from the primary vial (see below). Thus, we chose to store only a single 300 µl aliquot of cells from each LBC sample.

An essential requirement of the SCCB for future users will be the efficient availability and traceability of high-quality samples. After a careful investigation of a diverse line of sample archiving tubes, a standard 96-well format of storage vials (0.5 ml Tracker 2D in Loborack-96w low cover, MPW52337BC3, Nordic Biolabs AB, Täby, Sweden) was selected. The individual 0.5 ml piercable tubes are permanently laser-etched by unique two-dimensional (2D) codes. The tubes are 100% quality controlled on all 2D codes. The selection of 96-well plates was based on space efficiency and high traceability that fit the purpose.

Quantitative assessment of real-time cell sedimentation. The same number (20) of anonymized samples was utilized. The cell-sedimentation capability tests were set up at room temperature, and the cell concentration in each of the sediments was measured (using cell counting in a Buerker chamber) at different time points. The largest amount of cells accumulated in the bottom of the tubes after 30 min of incubation time, and the rate of accumulation decreased over time for up to 120 min. The assay was then verified and repeated.

Assessing of storage temperature. To assess the appropriate storage temperature, sedimented cervical cells in the 300 µl final volume were subjected to temperatures of +4°C, −25°C, −35°C, and −80°C, and storage times of 1 week to 4 weeks. After these treatments, the cells were examined microscopically, and cell concentrations were determined. The results demonstrated that cells were intact in fluid at temperatures from +4°C to −35°C but were frozen at −80°C, in all cases after 2 weeks of storage time.
The stability of archived LBC over long periods has been examined in previous epidemiological studies. These studies examined the impact of storage at various temperatures from uncontrolled ambient temperature to controlled ranges between \(+4\)°C and \(-75\)°C. Extensive review of current scientific publications, coupled with wide consultation and peer review by the scientific community and a series of validation and verification assays, ensured that the proposed storage temperature of \(-25\)°C met the purposes of the project; \(-25\)°C was the optimal temperature for long-term storage of cervical cell samples because all of the cells remain intact in a fluid medium, an obvious advantage (e.g., for being able to make new slides for cytological diagnosis, immunohistochemistry, and in situ-hybridizations). At the same time, the low temperature used for storage is advantageous for preserving high quality DNA and RNA for the long term.

Process automation and quality assurances. Our goal was to design a cell handling and storage strategy and integrate it with a highly automated process. Such a process greatly increases the achievable throughput, reduces opportunities for operator error, and increases consistency and reproducibility. Automation also enables the complex structure of the data to be managed. Prototype testing has been used to develop robust, affordable, fully automated methods that prevent unexpected process bottlenecks or incompatibilities between technology and data. An essential requirement of the SCCB for future users will be the creation of a national platform for accessing the high-quality samples in long-term storage. Therefore, each step of the process includes quality assurance procedures, and the data outputs contain the identity of the machine and the operator that processed each sample to enable the rapid identification and rectification of quality problems. The findings from verification analysis were implemented into a robotic system in sequential steps as follows; i) scanning of 96-well storage plates for the sample identification’s numbers, ii) aspiration of 4 ml cell suspension from TP containers into the conical tubes, sedimentation of cells in the bottom of the tubes, iii) aliquoting of 300 μl of sedimented cells as final storage volume into the individual storage vials in the 96-well plate, iv) and finally, manual transportation of the plate to the storage facility and equipment.

The automated processing equipment handles individual sample containers, scans the sample labels, and transmits them to the destination tubes, and senses the liquid levels in both the intermediary conical tubes and final storage vials. This equipment increases the number of samples that can be processed in a given time and reduces the number of defects, providing quality assurance for the biobanking process design. Any defects are controlled either by halting the process or raising an alert to the operator.

The storage equipment (Laboratory Freezer Froster-720, Philipp Kirsch GmbH, Offenburg, Germany) is designed for operation at the \(-25\)°C to \(-30\)°C range and is connected to an automatic monitoring and alarm system readily available to the staff.

LIMS, data structure and data security. The SCCB has implemented a robust, commercially available LIMS system (LabWare-LIMS, www.labware.com) that was initially developed for high-throughput biobank processing. Although this LIMS package is ideal for well-described and repeated operations because of its logical data structure and compatibility with the robotic software database of the cytology biobank, it does require a special configuration to be aligned with the laboratory patient information system. The overall data structure and system has been designed and implemented alongside the biobank sample handling protocol, according to several guiding principles. The use of bar codes (1-dimensional on the patient sample containers and on the 96-well storage plate, 2D on the bottom of the 0.5 ml aliquot storage vials) ensures the accuracy and traceability of all samples from the assessment center to the archive, and links the samples to the patient information data systems at the correlated laboratories. Because the sample handling processes are fully automated, additional information, such as time and date of processing and archiving, storage volume, and position in the biobank, can be attributed to each aliquot and linked to the individual donors. The confidentiality of participants is of the utmost concern: all processed samples and their aliquots are identified only by bar codes and can be linked back to the donors only through a unique 12-digit 2-dimensional bar code. All operator staff should be trained appropriately and approved by the director of the biobank. The data in the LIMS are stored separately from those of the assessment centers (e.g., laboratory registration data systems, and the data that identify each individual biobank sample). All authorized personnel will be identified by LIMS through log-on information. Access to the entire database structure is restricted to the director of the biobank, who has its overall responsibility. The backup storage is hosted on the Karolinska Institute server at the Karolinska Institute in Stockholm.

A summary of work plan developing a national standardized sample handling protocol

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Validation of the SCCB sample handling and storage

We defined the term “validation” as the documented act of demonstrating that a procedure, process, and activity will consistently lead to the expected results. The first step of validation was an extensive series of verification analyses and scientific investigations that led to the sample handling and storage methods. The validation assays were performed on handling processes after the initial clinical diagnosis, from the time when the LBC samples were delivered to the biobank center onward. The second step was the design and implementation of the infrastructure necessary to provide sufficient capacity for processing and archiving cervical cell samples at the required throughput and quality to ensure...
their utility for sample diagnosis and scientific research. Since August 2011, the majority of cervical cell samples have been processed in the SCCB according to this carefully designed, automated infrastructure of sample handling and storage. More than 50,000 samples (August 2012) were stored in the biobank from the Stockholm county council alone.

The third step, conducted during late spring 2012, was the performance of the sample handling validation studies. These studies investigate whether cervical cell samples that were maintained at ambient temperature in the laboratory after initial clinical collection for 1–4 weeks, then transported to the biobank center and processed according to the protocol, were comparable to same samples processed immediately after collection. Several assays were utilized:

- A morphological investigation with the specified diagnostic criteria in a comparative study.
- DNA stability of the processed cervical cells at the storage temperature.

Results of validation. For the morphological investigation, 100 LBC samples archived at −25°C for 10 months in the SCCB were randomly selected and anonymized by the director of the biobank. These samples were defined as biobank samples. A volume (relative to the cell concentration) of 20–40 μl was extracted from the biobank samples and suspended in PreservCyt fixative (ThinPrep®, 20 ml). All the extraction actions were registered in the Cervical Cytology Biobank-LIMS. The test samples were transferred to the clinical cytology laboratory at the Karolinska University Hospital at Huddinge-Stockholm for assessment according to the laboratory procedures determined by a senior cytopathologist. These biobank samples were compared morphologically with the original patient sample slides that had been stained and archived at the laboratory. The morphological criteria for the cells were based on the degree of satisfactory diagnosis, and slides were scored as follows: i) satisfactory: most cells had good nuclear detail with visible chromatin structure; ii) satisfactory but limited: some cells lacked good nuclear detail; iii) marginal: most cells showed nuclear degradation; or iv) unsatisfactory: the specimen could not be evaluated.10 The result of this microscopic analysis demonstrated that 98% of the biobank-processed samples were adequate for diagnosis. The two remaining samples had a limited cell number but were still sufficient for the diagnosis. Figure 1 displays an illustration of a biobank sample (A) and a sample from the same patient prepared immediately after collection (B).

Polymerase chain reaction amplification of β-globin fragments of different size was used as a generic marker of DNA stability, a marker that should be unaffected by HPV infection or low-grade squamous intraepithelial lesions.1 One hundred biobank samples were randomly anonymized, and 10 μl from each sample was extracted for evaluation and diluted in 100 μl of PreservCyt (1:10). All of the extraction actions were registered into the SCCB-LIMS. The test samples were transferred to the molecular biology laboratory at the Karolinska University Hospital, Huddinge, Stockholm, for assessment of the DNA quality.11,12 The results demonstrated that 98 of the biobank-samples had adequate amounts of β-globin, indicating that the DNA of the biobank cell samples was intact.

FIG. 1. Papanicolaou staining on cervical cell samples as a screening test performed for morphological evaluation. Comparative illustration of the biobank-sample (A) and a sample from the same patient prepared immediately after collection (B). Magnification 20X. A color version of this figure is available in the online article at www.liebertpub.com/bio.

The Establishment of Quality Controls for Processed Cervical Cell Samples into the SCCB

The SCCB is an extension of LBC procedures at clinical laboratories; therefore, a quality management system (QMS) of a laboratory accreditation procedure (ISO 15189), including quality assurance (QA) and quality control (QC) programs, covered the full spectrum of biobanking operations. The timeline of the whole biobanking procedure is limited from the time of LBC sample delivery to the biobank center through storage of the samples at −25°C. The previous clinical handling process of LBC was subject to the same QMS; therefore, this process might not affect the biobanking procedures. This system controls the quality of sample handling and storage processes and the traceability of each high-quality sample to the end user communities. One of the primary issues in our biobank has been the establishment of standard operating procedures (SOPs) for the complete sample processing and storage protocols. These SOPs have been documented for the entirety of the sample handling procedures, including the delivery of cervical cell samples from the assessment laboratories to the biobank center, the pre-analytical
variation studies, aliquoting and storage into the biobank freezer at −25°C, and biobank-sample quality. The establishments of standard QMS and SOPs has been crucial due to the nationwide distribution of the biobank. Identical biobanking procedures for LBC samples have already been implemented in multiple Swedish counties. Keeping in mind the national scope of biobanking’s infrastructure, these SOPs are the basic guideline to the other receiver nodes in different county councils as long as the laboratories undertake the same accreditation management system (ISO 15189). These documents will then be embedded into the health care system, and generally will be available for the research groups after ethical approval. However, these SOPs describe handling of practical procedures at laboratories.

**Quality Assurance of the SCCB.** Quality assurance of the biobank was established to verify that all specimens are handled appropriately. The documented SOPs for the biobank describe how the tasks pertaining to repository operations should be handled by the staff assigned to those specific responsibilities. These SOPs allow for uniformity and reproducibility in cervical cell sample handling. The SOPs were written by the biobank director and the quality coordinator of the Department of Clinical Pathology and Cytology Laboratory at the Karolinska University Hospital at Huddinge in Stockholm, Sweden, as the development and implementation of the Cervical Cytology Biobank occurred at this site. The SOPs were reviewed and approved by the department quality coordinators before they were finalized and published on the intranet of the Karolinska University Hospital for accessibility to the staff. The QA documents for the biobank LIMS system were written by personnel with additional expertise in LabWare LIMS and were approved in the same way. Each SOP is marked by a unique title and number that can be used for assay protection and contains the date, the department, the purpose, the protective equipment, and all material and supplies needed to perform the given procedure, step-by-step guidelines, including any safety steps associated with the procedure, and the references.

**Quality Control of the SCCB biobank.** Quality control of the SCCB is the system that measures the quality of the processed samples in the biobank on a regular basis, along with the performance and the quality of the instruments and their calibration, and recommends upgrades and replacement of equipment. All activities were recorded according to the best practice guidelines of the QMS.

The quality control of the processed cell samples was performed recently, and the results have been described in this article.

**Discussion**

The SCCB and its sample handling and storage protocols were developed to establish a standardized national infrastructure for biobanking, as was the main aim of BBMRI.se, which financed the biobank. The sample processing method was developed and implemented to maintain both sample security and cost efficiency. The whole infrastructure of the biobanking procedure as summarized in schematic presentation of work plan has been successfully transferred to multiple laboratory nodes around the country that utilize LBC-methods. Allowing all of them to share the same system is a crucial quality criterion for a truly national biobank. In parallel with the sampling method, fit-for-purpose processes, facilities and technology have been established to ensure the long-term integrity of the samples.

LBC is available in several different commercial systems with different fixatives and volumes, the major ones being ThinPrep and SurePath (BD SurePath™ Liquid based, pap test, BD Diagnostic, Tri Path, Erembodegem, Belgium). The robotic system implemented for aliquoting and storage has the capacity to process both types of LBC samples, as the sample handling robot can use both types of containers. Validation and implementation has so far only encompassed ThinPrep LBC, however.

The SCCB has now been established in seven different counties across the country during 2012 through the support of the BBMRI.se. Many extensive national, regional, and local regulations must be met by this biobank. Due to the quality and the flexibility of the unified biobank infrastructure, Sweden has the potential to construct a truly national platform for cervical cytology biobanking. This national approach allows for the management of multicenter, population-based studies. In designing and commissioning the national cytology biobank, the use of robust established technology and processes has been paramount, as has transfer of the best practices from developed clinical methods to the industry, because failure could lead to loss of this national resource. To date (August 2012), we have processed and stored more than 50,000 cervical cell samples in the Stockholm county council alone. We expect that, by the end of this year, approximately 120,000 cervical cell samples will have been processed and stored in the SCCB at the different nodes across the country.

Use of adequate legal, ethical, and sample governance procedures in large-scale population biobanks such as the SCCB are critical for the success of biobank-based biomedical research. Therefore, these will be the subject of a second report. In brief, the key principles used are including patient information in the screening invitation letters, use of broad consent for biobanking, and use of an Open Access principle that does not discriminate against or favor any requesting organization and makes samples available for health-related research that can be performed without jeopardizing patient integrity and has Institutional Review Board (IRB) approval.

The SCCB is a population-based biobank that aims to collect gynecological cell samples from all Swedish women. These samples will be banked to build up a resource that will be used by the health care system and research groups to work towards the selection of optimum targeted diagnosis and treatment for individuals.

In summary, the SCCB has adopted a unified sample handling and storage process that is fit-for-purpose for the large sample sizes, the goals of the project, and can provide standardized, nationwide biobanking in spite of the differences between and within different counties in Sweden.

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Author Disclosure Statement

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