VITILIGO BIOBANK GUIDELINES
for collection and storage of blood and serum samples

1 OVERVIEW

This document describes sample collection within Vitiligo Biobank initiative hosted by the VR Foundation, which aims to collect biological samples of vitiligo patients which are accompanied by detailed description of disease course and treatments outcomes.

The procedures described in this document are applicable to collection and storage of blood and serum samples. Blood sample stored in aliquots is designated for genomic DNA isolation for genetic research and is collected only once. Serum samples stored in aliquots are designated for analysis of various serum-derived moieties (such as cytokines, growth factors, microRNAs, etc), and are collected either once from a patient not being recently treated for vitiligo (see Criteria for biosample collection section) or (preferably) additional consecutive serum samples are collected from the patient during and/or after vitiligo treatment course.

Data associated with patient's biosamples are collected at the time of the primary visit of the patient and (preferably) additional data are collected during the course of vitiligo treatment(s) and/or after its (their) completion to document results of particular treatment(s). For data collection, specific Patient Biobank Profile forms are used. Collected data should to be transferred to a central database hosted by the VR Foundation to enable data search and sample retrieval based on specific criteria.

This document describes procedures for data and biosample collection.

2 DEFINITIONS

Terms described below are used in this document.

Biobank code – a unique digital identifier to be assigned to each combination of patient's data and samples.

Dataset – collection of data describing patient, its vitiligo course, treatments received and their outcomes.

Local biobank – remote facility operating according to a standard Vitiligo Biobank protocols where datasets and biosamples are collected.

Central database – centralized database of patient and biosample associated data derived from all local biobanks.

Primary visit – a first visit of patient not enrolled previously in Vitiligo Biobank project.

Follow-up visit – any consecutive visit of patient after the primary visit.

3 GENERAL DESCRIPTION OF THE PROCEDURE

Basic procedure of biobank sample collections includes 5 basic steps at primary or follow-up visit(s), and is strictly associated with the visit(s) of patient to a professional dermatologist.

3.1 Primary visit

Step 1. Professional dermatologist assesses that a patient meets criteria for biosample collection (see relevant section below).

Step 2. If patient meets criteria for biosample collection, professional dermatologist (or his assistant) familiarizes a patient with basic aims of Vitiligo Biobank, biosample collection procedure and answer his questions. If the patients voluntary agrees to participate in the Vitiligo Biobank (to donate samples for research use and agree with using his de-identified medical information in research purposes), and only under this condition, get the Informed consent form signed by the patient followed by the signature of a person who is taking the Informed consent form.

Step 3. Professional dermatologist, based on information provided by the patient and results of patient's examination fills out the Patient Biobank Record form and assigns unique Biobank code to the record. The same code should be entered into dedicated field on the last page of the Informed consent form signed by the patient.

Step 4. Blood samples (venous blood) are collected from the patient. *To avoid unnecessary patient's compliance, it is desirable to combine sample collection for biobanking with the routine blood test required for patient's examination.*

Step 5. Samples for biobanking should be immediately processed according to protocols outlined below in relevant sections.

3.2 Secondary visit

Step 1. Professional dermatologist assesses that a patient meets criteria for biosample collection (see relevant section below).

Step 2. If patient meets criteria for biosample collection, professional dermatologist (or his assistant) confirms with the patient that he (she) remains voluntary agreed to participate in the Vitiligo Biobank and to donate samples for research use, and agrees with using his de-identified medical information in research purposes.

Step 3. Professional dermatologist, based on information provided by the patient and results of patient's examination fills out the Patient Biobank Record Follow-up form which should be labeled with assigned to patient earlier unique biobank code.

Step 4. Blood sample (venous blood) is collected from the patient. *To avoid unnecessary patient's discomfort, it is desirable to combine sample collection for biobanking with the routine blood test required for patient's examination.*

Step 5. Samples for biobanking should be immediately processed according to protocols outlined below in relevant sections.

4 ETHICAL ASPECTS

VRF follows existing ethical aspects in Vitiligo Biobank projects. Therefore datasets and biosamples could be collected only if patient voluntary agrees to contribute to the Vitiligo Biobank project which has to be confirmed be signing him the Informed consent form. Adopted be VRF Informed consent form could be used, or it might be adjusted to meet specific local requirements.

5 CRITERIA FOR BIOSAMPLE AND DATA COLLECTION

5.1 Primary visit

At primary visit, the Patient Biobank Record form should be filled out in full (see Data collection section), including data on previous vitiligo treatments and their outcomes. If patient is prescribed new vitiligo treatment, description of prescribed treatment should also be documented, leaving respective Treatment outcome section of the Patient Biobank Record form blank.

Biosamples which can be collected at primary visit are blood sample designated for DNA extraction and serum sample.

Blood sample designated for DNA extraction could be collected under any conditions of the patient (refer to Blood for DNA collection procedure).

Serum sample (refer to Serum collection procedure) might be collected only if
(i) patient is in a good general health condition (i.e. no symptoms of acute disease such as flu, etc., are present) and
(ii) patient currently (within last 3 month or more) was not under any vitiligo treatment. If any of the above is not true, serum sample should not be collected.

5.2 Secondary visits

At secondary visits, the Patient Biobank Record Follow-up form should be filled out in full (see Data collection section), which is designed to record information on the current treatment efficiency and outcome.

Biosamples which can be collected at secondary follow-up visit(s) are serum samples only unless blood sample for DNA isolation has not been collected during the primary visit. In this case, blood sample has to be collected at the follow-up visit.

Serum sample (refer to Serum collection procedure) might be collected only if patient is in a good general health condition (i.e. no symptoms of acute disease such as flu, etc., are present).

We recommend that follow-up data and serum samples were collected not earlier than three month after previous visit to avoid overloading database with intermediate data.

6 BIOBANK CODE

6.1 Biobank code description

Samples collected from a single patient and his Patient Biobank Record should have unique identifier. This identifier should be of the same type used in other local biobanks to enable data incorporation into a single database.

Vitiligo Biobank uses 8-digit code to uniquely identify biosamples and datasets.

The biobank code has a format XX-YYYY-ZZ. Barcoding of biobank code might also be implemented if desired.

The first two-digit block of the code, XX, is assigned by VRF and identifies particular local biobank.

The middle four-digit block YYYY is used to consequently enumerate unique patients whose biosamples and data are collected, starting from 0001.

NOTE: *it is responsibility of personnel at each local biobank to develop and implement suitable procedure of record-keeping for already used biobank codes to assure their consecutive and non-redundant use.*

The last two-digit block of the code, ZZ, is used to indentify type of labeled item derived from one patient.

When blood and serum are collected, following values of this block should be used:

20 - for labeling dataset (i.e. filled out patient profile) and blood sample(s). Also is used to identify items derived from a particular patient (for example, during blood processing for serum production, etc).

22 - for labeling serum samples collected at the primary visit.

23, 24, ..., 29 - for labeling each consecutively collected serum sample, if any (for example, collected after completing treatment, etc), collected at follow-up visits.

21 - is reserved for labeling DNA samples isolated from blood, if any.

6.2 Items labeled by biobank code

1. Patient Biobank Record and Patient Biobank Record Follow-up forms
2. Stored blood samples
3. Stored serum samples
4. Patient's medical record
5. Temporal items (tubes) used during preparing collected biosamples for storage

6.3 Patient's personal information and biobank code

VRF will not store patient's personal information allowing his identification such as name and postal address. However we would advise physicians at local collection points to confidentially keep a record where biobank codes assigned to patient's personal information such as name, etc., and listed in an ordered manner are juxtaposed with patient's personal details allowing contacting them in case such a need would arise (for example, when recruiting for clinical trails).

7 ENTERING DATA INTO PATIENT'S MEDICAL RECORD

We strongly recommend labeling patient's routinely used medical record with assigned to his biosamples and datasets biobank code at a time of completing Patient Biobank Record. This would simplify updating Patient Biobank Record during patient's follow-up visits to record treatment results.

It also is highly recommended to enter information about blood for DNA isolation and serum sample collection in patient's routinely used medical record in a dedicated space.

For blood collected for DNA isolation, the fact of collection has to be reflected.

When serum samples are collected, date of serum collection and assigned consecutive number of serum samples should be entered (encoded in the last block of biobank code, i.e. -21, -22, -23, etc, see section Biobank code description). Entering these data is critical to ensure correct numbering of consecutively collected serum samples from the same patient in a course of treatment(s).

8 LABELING BIOSAMPLES

Collected biosamples are stored in 0.5/1.5/2.0 ml screw cap tubes (see below description of collection procedures under sections Serum collection procedure and Blood for DNA collection procedure) which should be labeled with assigned biobank code. The procedure of tube labeling should be worked out locally depending on existing capacities.

The procedure of tube labeling should assure tube labeling with biobank codes complying with the following requirements:

- numerical code general readability;
- preserving numerical code readability during prolonged storage under particular storage conditions;
- numerical code integrity with the labeled item during prolonged storage under particular storage conditions.

9 DATA COLLECTION

Data collection comprises from filling out Patient Biobank Record which is contained in PBR_xx-xxxx-xx.docx file. This should be done by a professional dermatologist during patient visit to assure the most accurate data are entered.

Filling this form by a patient alone is not allowed.

Based on the existing experience, we found that physicians generally prefer to enter data into hard (paper) copy of the form, with data converted into digital format later using hard copy filled out during the visit by a physician. At the moment, the most appropriate way is entering data into MS Word file PBR_xx-xxxx-xx.docx file by typing in text fields and selecting appropriate options by changing color of the appropriate selection(s) from black to red. File than is saved under the name PBR_xx-xxxx-xx.docx, where xx-xxxx-xx is a unique biobank code.

Filling Patient Biobank Record Follow-up form which is contained in PBR_FU_xx-xxxx-xx_dd-mm-yy.docx file, is done in a similar way, and the file is saved under the name PBR_FU_xx-xxxx-xx_dd-mm-yy.docx, where xx-xxxx-xx is a unique biobank code assigned previously to a patient, and dd-mm-yy is a current date which would allowing unique identification of each follow-up record.

If standard protocols are used for treatment, in order to save time on treatment description, they could be entered under specific codes in a designated field on Treatment description section in Patient Biobank Profile, with only patient-specific deviations from a standard protocol entered in detailed protocol description section. To use this option, standard protocols should be send to central database (please use e-mail address i.korobko@vrfoundation.org) for assignment of a special code. Based on this code, protocols details in full will be entered into central database by VRF staff.

10 DATA MANAGEMENT

Collected data should be transferred to VRF to be included in the central database. Currently this should be done by sending MS Word files with patient's data to e-mail address i.korobko@vrfoundation.org. Sending files should be done periodically (beweekly or monthly).

11 BLOOD FOR DNA COLLECTION PROCEDURE

To avoid unnecessary patient's discomfort, it is desirable to combine sample collection for biobanking with the routine blood test required for patient's examination.

If serum sample is to be collected along with blood sample for DNA collection procedure (see Criteria for biosample and data collection section), these two collection procedure should be done consecutively, within a single blood collection procedure. Please follow manufacturer's recommendation on the order of blood collection into different tube types.

We strongly recommend to use for blood collection single-use venous blood collection systems compatible with specific blood collection tubes.

For blood collection designated for DNA isolation, a one 4-ml blood collection tube with EDTA is used sufficient amount of blood.

For blood for DNA isolation collection, follow the protocol below.

BLOOD FOR DNA ISOLATION COLLECTION PROTOCOL

1. Label appropriate blood collection tube (4 ml volume, with EDTA) with a biobank code assigned to a patient.
2. Collect blood and close tube if necessary.
3. Gently invert tube 5-10 times to dissolve anti-coagulant in the tube.
   Note: *Tubes might be temporally stored at -20^{o}C at this point prior to further processing.*
4. Prepare five sterile 2.0 or 1.5 ml screw-cap tubes. Label them with assigned to a sample biobank code, with last two-digit block of the code being -20 (see section Labeling biosamples for labeling requirements).
5. *(Skip this if blood sample was not frozen after collection)* Thaw completely blood sample if it was frozen. Gently invert tube several times to mix its content.
6. Transfer 800 µl of blood to each of four prepared screw-cap tubes. Transfer remaining blood into fifth tube and record the amount of the blood transferred.
7. Place tubes for a long-term storage to -70^{o}C freezer (-20^{o}C freezer without non-frost regime is allowed but is less preferable).
8. Send information about amount of the archived blood samples to a central database (e-mail address i.korobko@vrfoundation.org) to trace an amount of available biomaterial (unless this information was entered in "Type of sample(s) collected" field on the page 1 of Patient Biobank Record form). Include in a message (this might be done periodically in bulk using Excel file or table in MS Word file) following information:
   - biobank code,
   - number of 800 µl aliquots archived,
   - volume of blood archived in a fifth aliquot.

12 SERUM COLLECTION PROCEDURE

To avoid unnecessary patient's discomfort, it is desirable to combine sample collection for biobanking with the routine blood test required for patient's examination.

If blood sample for serum preparation is to be collected along with blood sample for DNA isolation (see Criteria for biosample and data collection section), these two collection procedure should be done consecutively, within a single blood collection procedure. Please follow manufacturer's recommendation on the order of blood collection into different tube types.

We strongly recommend to use for blood collection single-use venous blood collection systems compatible with specific blood collection tubes.

For serum collection, TWO 4-ml (or 5 ml) blood collection tube with clot activator are used for blood collection. It is desirable that tubes contain an inert barrier gel separating serum from clot after centrifugation during serum preparation available from various suppliers.

For serum collection, follow the protocol below.

SERUM COLLECTION PROTOCOL

1. Label appropriate blood collection tube (4 or 5 ml volume, with clot activator and - if available - with polymer gel) with a biobank code assigned to a patient.
2. Collect blood and close tube if necessary.
3. Gently invert tube 5-10 times.
4. Leave tubes at room temperature for clot to form. *Do not exceed 30 min of incubation time!*
5. Prepare appropriate number (12-15) of 0.5, 1.5 or 2.0 sterile screw-cap tubes. Label them with assigned to a sample biobank code, with last two-digit block of the code being either:
   - -21 for the first serum collection from the patient (see section Labeling biosamples for labeling requirements), or
   - -22, -23, etc., for consecutively collected serum samples during/after treatment course, depending on the code value used to label last collected serum samples (records in patient's medical record could be used to trace correct numbering of consecutively collected serum samples; see section Entering data into patient's medical record for recommendations).
6. When incubation for clot formation is complete, centrifuge tubes for 10-15 min at 1800-2200g to separate serum.
7. Transfer serum from two blood collection into a single sterile 15 ml (or equivalent) tube labeled with biobank code to trace sample identity.
8. Close the tube and invert several times.
9. Dispense 220 µl of serum nto prepared screw-cap tubes. Collecting blood into two 4 ml tubes usually provides enough serum to fill 12 to 15 screw-cap tubes with 220 µl of serum.
10. Record quantity of tubes with 220 µl of serum.
11. ***Immediately*** place tubes for a long-term storage to -70^{o}C freezer. *No freeze-thawing of serum samples is allowed as it voids serum samples for use specific assays*.
12. Send information about amount of the archived serum aliquots to a central database (e-mail address i.korobko@vrfoundation.org) to trace an amount of available biomaterial

(unless this information was entered in "Type of sample(s) collected" field on the page 1 of Patient Biobank Record form). Include in a message (this might be done periodically in bulk using Excel file or table in MS Word file) following information:

- biobank code of serum samples (with relevant value of the last two-digit block of the code reflecting consequence of serum sample collection),
- number of 220 μl serum aliquots archived.

13 BIOBANK SAMPLE USE IN RESEARCH

Biobank samples are generally and *a priori* intended for use in specific research projects aiming to address vitiligo problem. Central database where data attributed to each archived biosample are stored, enables virtual recruitment of patients with specific characteristics for their biomaterial use in research projects. Such projects might be either initiated by external researchers or by researchers associated with local biobank.

If biosamples are required for an externally initiated research projects, their availability from a local biobank should be separately discussed (in terms of logistics, reimbursement of financial burden related to biosample collection and storage, etc).

Researcher(s), associated with local biobank, can use biosamples for their research projects. However, information on biosample use should be delivered to a central database hosted by VRF. This is required to maintain up-to-date information about biosamples availability and to trace research projects in terms of ***(i)*** adding biosample data obtained in a course of a research project to database and ***(ii)*** monitoring demands on Vitiligo Biobank resources and their use.

Therefore, if biosamples are used in locally initiated research project, it is necessary to provide an information on:

- biosamples used in a study (i.e. their biobank codes) and quantity/amount of biosamples used;
- brief description of the study in which biosamples were used (study aim, methods applied).

This information should be forwarded to an e-mail address i.korobko@vrfoundation.org for central database update.