IMI2 821520 - ConcePTION

ConcePTION

WP4 – Establishment of a non-commercial, Europe-wide breast milk biobank and analytical centre

D4.3 Sampling, storage and handling standards, consent procedures and templates

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Document History

Version	Date	Description
V1.0	10.10.2019	First draft procedure for blood and breast milk collection
V1.1	14.10.2019	Work instructions – Shipping
V1.2	20 02.2020	Bioanalytical process to support IMI demonstrational studies
V1.3	04.04.2020	Information to potential participants and consent form
V1.4	04.05.2020	Information to potential participants and consent form, revised version
V1.5	05.05.2020	Procedure for blood and breast milk sampling, aligning the first draft with the bioanalytical process
V1.6	07.06.2020	merges revised versions of the procedure document and the information to potential participants

Abstract

4.3 has developed information and consent procedures for recruitment of potential participants in collection of breast milk samples for storage in a European Biobank and subsequently being used for pharmacokinetic analyses of if, and to what extent, medical drugs transfer from breast milk to the breast feeding child. In addition, procedures for collecting breast milk and blood have been developed to facilitate research collecting blood and breast milk. The procedures and templates are designed to fit five demonstration studies within the following therapeutic areas and for the following drugs:

- Diabetes – metformin
- Bacterial Infection – amoxicillin
- Allergy – cetirizine
- Depression – venlafaxine
- Rheumatologic and Inflammatory Bowel Diseases – infliximab.

This report provides a description of the procedures, including materiel and equipment needed to prepare samples for analysis. To reduce patient burden and to promote feasibility, we provide procedures for sampling at the clinical sites as well as for sampling at the participants' homes. These procedures should be used to harmonize sample collection when it is performed at multiple sites. The procedures provide a general description of how to collect samples of blood and breast milk and require adaption to apply to the specific study, local conditions and national legislations and regulations. The procedures will be validated with regard to bioanalytic properties and conditions and changed accordingly if needed before inclusion in study protocols.

Methods

The deliverable report is based on reviews of the literature and collection of best practice related to breast milk sampling for lactation studies and best practices for shipping and storage of samples as established by BBMRI-ERIC. Information and consent procedures is based on requirements in national legislations for those clinical sites where the demonstration studies will take place (Norway, France, Switzerland and Sweden) in addition to publications in the bioethics literature.

Results

1. Information to potential research participants and consent forms

All eligible research participants are informed in person by their caregiver. The "patients" are given information orally as well as in written before giving their consent. Please note that the information and consent form have to be adapted to apply the specific conditions of the study and the local legislations and regulations. Details concerning the collection of breast milk and blood may have to be revised depending on the results from the upcoming bioanalytical validation process.

1.1 Template of information given to potential research participants collecting breast milk samples at home

Information to potential research participants concerning the study [Name of the study]

You are hereby asked if you would like to participate in the research study [Name of the study] and if you allow us to store any samples you provide for future research. This document gives you information about the study and what it means to participate.

What is the study about?

There is not enough knowledge about how much of the medications that a mother takes gets into her breast milk. ConcePTION is a public-private research project, funded by the Innovative Medicines Initiative (IMI2 JU) (https://imi-conception.eu), with the purpose to increase this knowledge about medications taken during pregnancy and breastfeeding. Within the project, breast milk and blood from mothers taking medication are collected, analyzed and stored in a biobank to enable medical research.

Within the research study [Name of study], we are now measuring how much of the medication [Name of medication] also called [tradename/s]) that gets into the breast milk. This will be compared with the intake [Applicable to studies also collecting blood:] and how much medication is in the mother's bloodstream. The results of the study will be reported at group level in scientific papers, without disclosing the identity of study participants. You are asked to participate in the study on [Name of medication] because you

have visited [the participating clinical sites], are breastfeeding and at the same time using the medication, or plan to do so soon. The main person/organisation responsible for the study is [Name of organization].

What will happen if I decide to participate?
Participation means that you will express breast milk for us at [X] occasions and [Applicable to studies also collecting blood:] once let us draw blood. The time-points for sampling depends on when you take your medication. We will contact you to plan the sampling.

Breast milk sampling
When you collect breast milk, you express all the milk from one of your breasts, until it feels empty. You express breast milk at home with the help of a breast pump you get to borrow from us. After gently inverting the expressed breast milk, you freeze a breast milk sample of 20 ml. You choose what to do with the remaining breast milk. When you express, you fill in and submit an [online-questionnaire and then are interviewed by phone] with the sampling time-point, the medication(s) you are using and when you have taken it/them, the condition or disease that you are treated for, details concerning how you went about sampling breast milk, age, ethnicity, your height, weight, and the age of your child[1]. You store the samples of breast milk in your freezer at home until all samples have been collected. You will receive detailed instructions on how to collect the breast milk.

[Applicable to studies collecting blood:]
Blood sampling
You will have blood samples taken only at one time. Two tubes will be taken, together 15ml of your blood. If you already have a venous catheter and want us to use it to draw blood, additional 7,5 ml of blood will be drawn (this requires that the catheter has not previously been used to administer the medicine). When the blood sample is taken, you fill in and submit an [online-questionnaire/questionnaire/interviewed by phone].

You may not participate until two weeks after childbirth. A participation is estimated to take about [X] minutes.

Possible consequences and risks of participating in the study
[Applicable to studies also collecting blood:] When blood is collected, you usually feel a slight sting. You may get a bruise from it and there is always a small risk of nerve damage, which may be expressed as pain or numbness. You are not expected to benefit for the participants directly from participating.

What happens to my information?
The study will collect and save information about you. We will collect your contact information, information concerning the sampling time-points, the medication(s) you are using, when you have taken your medications, the condition/disease that you are treated for, details concerning breast-feeding and how you went about sampling breast milk, age, ethnicity, your height, weight, and the age of your child[2].

Your contact information will be stored at [X] to be able to contact you to plan the sampling. This contact information will be discarded when all information and samples have been collected.

The health-related information you provide will be labeled with a code and stored in a database at Uppsala University, Sweden. [Applicable if paper questionnaires are used: The paper questionnaires will be stored at [the local site] for ten years before it is discarded]. Your coded information will be transferred to a database at the University Medical Center in Utrecht, the Netherlands. Your health-related information will be saved for 10 years after the study has finished. Then it is discarded.

The blood and breast milk samples, information on the time-points of sampling and the handling of the samples will be sent to Uppsala Biobank in Sweden. This material will be labeled with a code. The code will be used to connect the samples you have provided with the information that is stored at the database at the University Medical Center in Utrecht but it will not be possible to link the code to you after the samples have been collected.

The purpose of the processing of your personal data is to investigate how much of [Name of the medication] that goes into breast milk and to be able to review the research afterwards. Your data will be processed in confidence and there is no access for unauthorized persons.

[1] If any additional data will be collected in the specific study, what data and how the data will be collected has to be described.

Responsible for your personal data is [X]. According to the EU General Data Protection Regulation, you have the right to know what information is stored about you in the study free of charge, and if necessary, to correct any errors. You can also request your data to be deleted or to restrict the processing of your data. If you would like to access the information, please contact [X]. The Data Protection Ombudsman at [X] may be reached at [X]. If you are dissatisfied with how your personal data is handled, you have the right to submit a complaint to the [X], which is the supervisory authority.

What will happen to my samples?

The collected blood and breast milk samples will be sent to [X] to prepare the samples for storage and later shipped and stored at Uppsala Biobank in Uppsala, Sweden (registration number 827 at the Swedish Health and Social Care Inspectorate). Responsible for the biobank are Region Uppsala and Uppsala University in Sweden. The levels of [Name of medication] in blood and breast milk will be analyzed at [UDOPP (Department of Pharmacology, Uppsala University)/ Centre Hospitalier Universitaire de Toulouse] but may be sent abroad to another EU country for analysis. Your samples are used and saved for the purpose of this study for 10 years. Then they will be discarded unless you have consented to saving them for future research.

If you consent to saving your samples for future research, they will be made available for medical research purposes that are considered to be of benefit to society. At present, it is not possible to know what these specific purposes will be. It may refer to specific medical conditions or treatments, unknown diseases or genetic disorders. Genetic analyzes may be performed in which your entire genome (your DNA) may be determined. The samples will be made available for future medical research within the EU for an undetermined period of time. You decide if you want to make them available for public as well as commercial research.

You have the right to decline to saving the samples. If you consent to it, you have the right to later withdraw (undo) that consent. Then your samples will be discarded or de-identified. If you wish to withdraw your consent, please contact the Principal Investigator (PI) for the demonstration study [contact person at clinical site and contact information].

The samples may only be used in the manner you have consented to. Should there be any additional research that is not yet planned, an Ethical Review Board will decide if they can be used and if additional information needs to be communicated to the donor.

How do I get information about the results of the study?

You will not receive any individual results from the analyses of your samples. The results will be published in scientific journals. When this happens, it will be communicated on our website (https://imi-conception.eu).

Insurance and compensation

As a study participant, you are covered by a patient insurance through [X]. You will not receive any financial compensation for your participation.

Participation is voluntary

Your participation is voluntary and you can choose to cancel the participation at any time. If you choose to cancel your participation, you do not need to explain, nor will it affect your future care or treatment. If you want to cancel your participation, please contact the person responsible for the study (see below).

Contact information

If you have any questions about the study, please contact the research team:

[Principal Investigator for the demonstration study]

Contact person for sample collection]

1.2 Template of information given to potential research participants collecting breast milk samples at the clinical site

Information to potential research participants concerning the study [Name of the study]

You are hereby asked if you would like to participate in the research study [Name of the study] and if you allow us to store any samples you provide for future research. This document gives you information about the study and what it means to participate.

What is the study about?

There is not enough knowledge about how much of the medication/s that a mother takes gets into her breast milk. ConcePTION is a public-private research project, funded by the Innovative Medicines Initiative (https://imi-conception.eu), with the purpose to increase this knowledge about medication/s taken during pregnancy and breastfeeding. Within the project, breast milk and blood samples from mothers are collected, analyzed and stored in a biobank to enable medical research.

Within the research study [Name of study], we are now measuring how much of the medication [Name of medication] (also called [tradename]) gets into the breast milk. This will be compared with the intake [Applicable to studies also collecting blood:] and how much medication is in the mother's bloodstream. The results of the study will be reported at group level in scientific papers, without disclosing the identity of study participants. You are asked to participate in the study on [Name of medication] because you have visited [the participating clinical sites], are breastfeeding and at the same are using medication, or plan to do so soon. The main responsible for the study is [Name of organization].

What will happen if I decide to participate?

Participation means that you will visit us at [Name of the clinic] to express breast milk at [X] occasions and [Applicable to studies also collecting blood:] once let us draw blood. The time-points for sampling depends on when you take your medication. We will contact you to plan the sampling.

Breast milk sampling

When you collect breast milk, you express all the milk from one of your breasts, until it feels empty. You express breast milk with the help of a breast pump you get to borrow from us. After gently inverting the expressed breast milk, we freeze a breast milk sample of 20 ml. You choose what to do with the remaining breast milk. When you express, you fill in and submit an [online-questionnaire/questionnaire/interviewed by phone] with the sampling time-point, the medication(s) you are using and when you have taken it, the condition or disease that you are treated for, details concerning how you went about sampling breast milk, age, ethnicity, your height, weight, and the age of your child[2]. You will receive detailed instructions on how to collect the breast milk.

[Applicable to studies collecting blood:]

Blood sampling

You will have blood samples taken once. Two tubes will be taken, together 15ml of blood. If you already have a venous catheter and want us to use it to draw blood, additional 7,5 ml of blood will be drawn (this requires that the catheter has not previously been used to administer the medicine). When the blood sample is taken, you fill in and submit an [online-questionnaire/questionnaire/interviewed by phone]

You may not participate until two weeks after childbirth. A participation is estimated to take about [X] minutes.

Possible consequences and risks of participating in the study

[Applicable to studies also collecting blood:] When blood is collected, you usually feel a slight sting. You may get a bruise from it and there is always a small risk of nerve damage, which may be expressed as pain or numbness. You are not expected to benefit directly from participating.

What happens to my information?

The study will collect and save information about you. We will collect your contact information, information concerning the sampling time-points, the medication(s) you are using, when you have taken your medications, the condition/disease that you are treated for, details concerning breast-feeding and how you went about sampling breast milk, your age, ethnicity, height, weight, and the age of your child[2].

Your contact information will be stored at [X] in order to be able to contact you to plan the sampling. This contact information will be discarded when all information and samples have been collected.

The health-related information you provide will be labeled with a code and stored in a database at Uppsala University, Sweden. [Applicable if paper questionnaires are used: The paper questionnaires will be stored at [the local site] for ten years before it is discarded]. Your coded information will be transferred to a database at the University Medical Center in Utrecht, the Netherlands. Your health-related information will be saved for 10 years after the study has finished. Then it is discarded.

[2] If any additional data will be collected in the specific study, what data and how the data will be collected has to be described.

The blood and breast milk samples, information on the time-points of sampling and the handling of the samples will be sent to Uppsala Biobank in Sweden. This material will be labeled with a code. The code will be used to connect the samples you have provided with the information that is stored at the database at the University Medical Center in Utrecht but it will not be possible to link the code to you after the samples have been collected.

The purpose of the processing of your personal data is to investigate how much of [Name of the medication] goes into breast milk and to be able to review the research afterwards. Your data will be processed without access for unauthorized persons.

Responsible for your personal data is [X]. According to the EU General Data Protection Regulation, you have the right to know what information is stored about you in the study free of charge, and if necessary, to correct any errors. You can also request your data to be deleted or to restrict the processing of your data. If you would like to access the information, please contact [X]. The Data Protection Ombudsman at [X] may be reached at [X]. If you are dissatisfied with how your personal data is handled, you have the right to submit a complaint to the [X], which is the supervisory authority.

What will happen to my samples?

The collected blood and breast milk samples will be sent to [X] to prepare the samples for storage and later shipped and stored at Uppsala Biobank in Uppsala, Sweden (registration number 827 at the Swedish Health and Social Care Inspectorate). Responsible for the biobank are Region Uppsala and Uppsala University in Sweden. The levels of [Name of medication] in blood and breast milk will be analyzed at [UDOPP (Department of Pharmacology, Uppsala University)/ Centre Hospitalier Universitaire de Toulouse] but may be sent abroad to another EU country for analysis. Your samples are used and saved for the purpose of this study for 10 years. Then they will be discarded unless you have consented to saving them for future research.

If you consent to saving your samples for future research, they will be made available for medical research purposes that are considered to be of benefit to society. At present, it is not possible to know what these specific purposes will be. It may refer to specific medical conditions or treatments, unknown diseases or genetic disorders. Genetic analyzes may be performed in which your entire genome (your DNA) may be determined. The samples will be made available for future medical research within the EU for an undetermined period of time. You decide if you want to make them available for public as well as commercial research.

You have the right to decline to saving the samples. If you consent to it, you have the right to later withdraw (undo) that consent. Then your samples will be discarded or de-identified. If you wish to withdraw your consent, please contact the PI for the demonstration study [contact person at clinical site and contact information].

The samples may only be used in the manner you have consented to. Should there be any additional research that is not yet planned, an Ethical Review Board will decide if they can be used and inform you.

How do I get information about the results of the study?

You will not receive the results from the analyses of your samples. The results will be published in scientific journals. When this happens, it will be communicated on our website (https://imi-conception.eu).

Insurance and compensation

As a study participant, you are covered by a patient insurance through [X]. You will not receive any financial compensation for your participation.

Participation is voluntary

Your participation is voluntary and you can choose to cancel the participation at any time. If you choose to cancel your participation, you do not need to explain, nor will it affect your future care or treatment. If you want to cancel your participation, please contact the person responsible for the study (see below).

Contact information

If you have any questions about the study, please contact us.

[Principal Investigator for the demonstration study]

[Name of R.N./Contact person for sample collection]

1.3 Template of consent form

Consent to participate in the research study [Name of study]

I have received oral and written information about the study and have had the opportunity to ask questions and get them answered. I get to keep the written information.

☐ I consent to participate in the research study ”[Name of study]”.

☐ I consent to having my information handled in the way described in the information.

☐ I consent to having my samples stored in a biobank as described in the information.

Place and date	Signature

Contact information (This information is used to contact you to plan the collection of [breast milk/blood and breast milk] samples. The information is discarded when the sampling is finished).

Name: _____

Address:_____

_____

Phone:_____

E-mail:_____

Consent to saving samples for an undetermined time and use them for future broad medical research purposes

☐ I consent to having my blood [applicable only for studies collecting blood] and breast milk samples stored and saved for future medical research, of which the specific purposes have not been described here, as described in the patient information. If applicable, the research will first be reviewed and approved by an Ethical Review Board.

☐ I consent to having genetic analyses performed on the blood and breast milk samples.

☐ I consent to making the samples available for commercial medical research.

Place and date	Signature

Signature from the Care provider

☐ I confirm that I have given oral and written information about the study and that I have given the patient a copy of the patient information.

Place and date	Signature

2. Procedures for collecting and processing breast milk samples

The following procedures describe the process of collecting breast milk samples from women that have consented to participate in the demonstration studies, and the process to prepare the samples for analysis. The procedures include the materiel and equipment needed. Procedures for collecting breast milk at the clinical site and at the participants' homes are presented separately. Uppsala Biobank provides the local labs with pre-labeled aliquot tubes.

Please note that the procedures have to be adapted to apply the specific conditions of the study and the local legislations and regulations. Details concerning the collection of breast milk are finally determined after the upcoming bioanalytical validation process.

2.1 Procedure for collecting and processing samples of breast milk at the clinical site

General Considerations

The breast milk sample collection is carried out by the mother at the clinical site. Sampling should take place after the development of mature milk (two weeks postpartum). The timing of the sampling in relation to medicine intake depends on the substance that is investigated. Sampling details and health-related details are collected using an online/paper questionnaire/by telephone.

Milk samples are put in the freezer at the clinical site and transferred to the local lab. If the lab does not have the capacity to do the aliquoting, the samples are stored locally and then sent to Uppsala Biobank, where the aliquoting is performed.

Material and Equipment

The material and equipment required for the participating woman to collect one sample of breast milk are:

- Electric breast pump (washed and sanitized according to the instructions). Preferably, all participants use pumps of the same brand and model.
- Instructions on how to assemble and sanitize the breast milk pump
- Milk study sampling kit:
  - One 100ml milk container labeled with the sample ID. The material should support -20C°, be compatible with the medicine, and able to be shipped without being damaged.
  - 1 small plastic bag
  - Sterile compresses
  - Sterile NaCl solution
  - Paper towels
  - 1 hand sanitizer
  - Soap
- [Applicable if information is collected using an online questionnaire:] A smartphone/tablet/computer with Android or iOS and Internet connection used for reporting sampling details and health information in an online-questionnaire.
- [Applicable if information is collected using an online questionnaire:] The web address to the online-questionnaire.
- [Applicable if information is collected using paper:] Paper questionnaire and pen.
- [Applicable if information is collected by telephone interviews]: Telephone
- Pleasant room temperature
- Convenient chair or any other furniture that enables a convenient sitting position while breast feeding
- Icepacks

Preparing for Breast Milk Samples

1. Decide together with the mother when she will visit the clinic for sampling.

2. [Applicable if information is collected by telephone interviews:] Decide when to perform the telephone interview to collect information (Annex 1). This has to be done the same day as the sample is taken.
3. The study contact person checks that the patient's identity corresponds to the informed consent and the assigned donor ID and the sample ID (printed on the label of the milk container).
4. The study contact person/personnel hands over the material to the participating woman
5. The participating woman is recommended to contact the study contacts person/personnel if she meets any problems when sampling. She should be able to quickly get assistance without having to leave the room while expressing milk.
6. Assemble the electric breast pump according to the instructions.

Breast Milk Sampling at the Clinical Site

1. Wash hands with water and soap and let them dry.
2. Disinfect hands with the hand sanitizer.
3. Wet the provided sterile compresses with the sterile NaCl solution and use it to wipe and clean one of your breasts, the breast you will be pumping from. Start with the nipple and then wipe in circles in a direction moving away from the nipple.
4. Use the breast pump according to the instruction manual provided by the manufacturer. Pump milk from the breast until it starts to feel empty. This usually takes about 10 to 20 minutes.
5. [Applicable if information is collected by questionnaires]: Continuously fill in the [online-questionnaire/questionnaire] while breast-feeding (Annex 1). If this is not possible, it is filled in afterwards, as soon as possible.
6. Disconnect the container provided with the breast pump.

The following steps can be conducted by the mother or personnel at the clinical site:

7. Invert the container 10 times to homogenize the milk.
8. Pour 20 ml of the breast milk in the provided 100ml study milk container labeled with the study donor ID and sample ID. If the full breast emptying yielded less than 20ml, pour this breast milk into the container. It is important that the container is not filled with more than 60ml as the milk expands when it is frozen. You decide what to do with the remaining milk.
9. Place the container in the plastic bag.
10. Hand the breast milk sample [and any paper questionnaire] to the study contacts person/personnel.
11. [Applicable if information is collected by questionnaires:] The participating woman makes sure that she has responded to all required information in the questionnaire [if online-questionnaire:] and submits it.

The following steps are performed by personnel at the clinical site:

12. The plastic bag with the container is put in the freezer and stored at -20 C°.
13. Sanitize the breast milk pump according to the instructions.
14. [Applicable if information is collected by telephone interviews:] The mother is called during the day to respond to the questions (Annex 1).
15. The milk is stored in a freezer until all samples have been collected and are then transported to the local lab (preferably) or Uppsala Biobank on dry ice.
16. The personnel submit the time-point of putting the sample in the freezer (Annex 2). [If paper questionnaires have been used:] The personnel submits the participant's responses to the paper questionnaire using the online-questionnaire (Annex 1).

Preparing the Samples for Analysis and Storage at the Local Lab or Uppsala Biobank

1. The sample is thawed and stirred gently.
2. The sample is aliquoted into the pre-labeled aliquot tubes leaving sufficient room in the tubes for liquid expansion during freezing. A maximum 20 ml of breast milk is aliquoted according to an algorithm developed during the validation process of the bioanalytics.
3. [Applicable if the aliquoting is performed at the local lab:] The samples are freezed at a temperature of -20C°.
4. The study donor ID, sampling time, sample ID, freezing time, and SPREC code for all aliquots are registered using an online-questionnaire.
5. The samples are shipped on dry ice to Uppsala Biobank. When samples are ready for shipment, please notify Uppsala Biobank by sending an e-mail to info@uppsalabiobank.uu.se (subject: ConcePTION: Sample shipment).
6. [Aliquots arriving/aliquoted] at Uppsala Biobank are frozen at a temperature of -80C°.

2.2 Procedure for collecting and processing samples of breast milk at the participant's home

General Considerations

The breast milk sample collection is carried out by the mother in her home. Sampling should take place after the development of mature milk (two weeks postpartum). The timing of the sampling in relation to medicine intake depends on the substance that is investigated. The milk samples are put in the freezer at the mother's home and are brought to the [local lab/clinical site] after the last sample has been collected. Sampling details and health-related information are reported by the mother using an online/paper questionnaire. The aliquoting is performed at the local lab. If the local lab does not have the capacity to do the aliquoting, the samples are stored locally and sent to Uppsala Biobank for aliquoting.

Material and Equipment

The material and equipment required for the participating woman to collect one sample of breast milk are:

- Electric breast pump (washed and sanitized according to the instructions). Preferably, all participants use pumps of the same brand and model.
- Instructions on how to assemble and sanitize the breast milk pump
- Milk study sampling kit:
  - One 100ml milk container labeled with the sample ID. The material should support -20C°, be compatible with the medicine, and able to be shipped without being damaged.
  - 1 small plastic bag
  - Sterile compresses
  - Sterile NaCl solution
  - Paper towels
  - 1 hand sanitizer
  - Soap
- [Applicable if information is collected using an online questionnaire:] A smartphone/tablet/computer with Android or iOS and Internet connection used for reporting sampling details and health information in an online-questionnaire.
- [Applicable if information is collected using an online questionnaire:] The web address to the online-questionnaire.
- [Applicable if information is collected using paper:] Paper questionnaire and pen.
- [Applicable if information is collected by telephone interviews]: Telephone
- Pleasant room temperature
- Convenient chair or any other furniture that enables a convenient sitting position while breast feeding
- A freezer
- Icepacks
- Shipping documents and material needed for transportation (according to local routines and any shipping company involved)

Preparing for Breast Milk Sampling

1. Decide together with the mother the time-points for sampling and when the samples will be transported to the local lab.
2. [Applicable if information is collected by telephone interviews:] Decide when to perform the telephone interview to collect information (Annex 1). This has to be done the same day as the sample is taken.
3. Order transportation if needed.
4. The study contacts person checks that the patient's identity corresponds to the informed consent and the assigned study donor ID and the sample ID (printed on the label of the milk container).
5. The study contacts person [hands over/sends] the material to the participating woman
6. The participating woman is recommended to contact the study contacts person if she meets any problems when sampling. She is provided a telephone number to quickly get assistance.
7. The woman assembles the electric breast pump according to the instructions.

Breast Milk Sampling at the Participant's Home

1. Wash hands with water and soap and let them dry.
2. Disinfect hands with the hand sanitizer.
3. Wet the provided sterile compresses with the sterile NaCl solution and use it to wipe and clean one of your breasts, the breast you will be pumping from. Start with the nipple and then wipe in circles in a direction moving away from the nipple.
4. Use the breast pump according to the instruction manual provided by the manufacturer. Pump milk from the breast until it starts to feel empty. This usually takes about 10 to 20 minutes.

5. Continuously fill in the [online-questionnaire/questionnaire] while breast-feeding (Annex 1). If this is not possible, it is filled in afterwards, as soon as possible.
6. Disconnect the container provided with the breast pump.
7. Invert the container 10 times to homogenize the milk.
8. Pour 20 ml of the breast milk in the provided 100ml study milk container labeled with the study donor ID and the sample ID. If the full breast emptying yielded less than 20ml, pour this breast milk into the container. It is important that the container is not filled with more than 60ml as the milk expands when it is frozen. You decide what to do with the remaining milk.
9. Place the container in the plastic bag
10. Put the plastic bag with the container in the freezer.
11. [Applicable if information is collected using questionnaires:] Make sure that you have responded to all required information in the questionnaire (Annex 1), including the time-point for putting the sample in the freezer in the questionnaire.
12. [Applicable if information is collected using online-questionnaire:] Submit the questionnaire.
13. [Applicable if information is collected by telephone interviews:] The mother is called during the day to respond to the questions (Annex 1).
14. Sanitize the breast milk pump according to the instructions.
15. The milk is stored in the freezer until all samples have been collected.
16. The samples are transported on ice [If paper questionnaires are used: together with the questionnaires and breast milk pump] to the [local lab for aliquoting/clinical site for storage] using the ice packs, shipping material and shipping documents needed according to local routines.

Preparing the Samples for Analysis and Storage at the Local Lab or Uppsala Biobank

1. The sample is thawed and stirred gently.
2. The sample is aliquoted into the pre-labeled aliquot tubes leaving sufficient room in the tubes for liquid expansion during freezing. A maximum 20 ml of breast milk is aliquoted according to an algorithm developed during the validation process of the bioanalytics.
3. [Applicable if the aliquoting is performed at the local lab:] The samples are freezed at a temperature of -20C°.
4. The study donor ID, sampling time, sample ID, freezing time, and SPREC code for all aliquots are registered using an online-questionnaire.
5. The samples are shipped on ice to Uppsala Biobank. When samples are ready for shipment, please notify Uppsala Biobank by sending an e-mail to info@uppsalabiobank.uu.se (subject: ConcePTION: Sample shipment).
6. The aliquots are freezed at Uppsala Biobank at a temperature of -80C°.

3. Procedures for Collecting and Processing Blood Samples

The following procedures describe processes of collecting blood samples from women that have consented to participate in the demonstration studies, and the processes of preparing the samples for analysis. The procedures include the materiel and equipment needed. Procedures for collecting blood at the clinical site and at the participants' homes are presented separately. To ease the burden on the mother, the blood samples are preferably taken in her home or during an appointment to the recruiting clinic.

Please note that the procedures have to be adapted to apply the specific conditions of the study and the local legislations and regulations. Details concerning the collection of blood are finally determined after the upcoming bioanalytical validation process.

3.1 Procedure for collecting and processing blood samples at the clinical site

General considerations

The blood drawings are carried out by trained personnel used to draw blood. The timing of the blood draw in relation to medicine intake and breast milk sampling depends on the specific study and substance being investigated. The tubes required depends on the specific study and if plasma or serum is being collected. A maximum time between drawing the blood to putting the aliquots in the freezer is decided based on the stability of the specific drug. Uppsala Biobank provides pre-labeled aliquot tubes.

Material and Equipment

The material and equipment required to collect blood are delivered with the Blood Sampling Kit provided by the study contacts person.

- Blood Sampling Kit:
  - 1 alcohol hand sanitizer
  - 1 pair of non-sterile gloves, in a well-fitting size
  - 1 tourniquet
  - Alcohol swabs for skin disinfection
  - 1 set of venipuncture equipment used at the local site. E.g., needle and a tube holder or a butterfly needle set.
  - [Applicable when plasma is collected:] Use 2 Li Hep tubes (à 7 ml) labeled with the study donor ID.
  - [Applicable when serum is collected:] Use two SST tubes (à 7 ml) labeled with study donor ID.
  - Cotton wool balls, compresses or similar materials used at the clinical site to stop bleeding.
  - Skin tape
  - One disposal unit
  - Extra material (gloves, swabs, venipuncture equipment, tubes, labels, compresses, and skin tape) in case the blood drawing fails.
  - [Applicable when an existing venous catheter is used to draw blood from:] One 7,5ml slush tube
  - [Applicable when an existing venous catheter is used to draw blood from:] The syringes, saline solution, heparin and catheter plugs necessary to flush and lock the catheter according to the local routines.
- [Applicable if information is collected using an online questionnaire:] A smartphone/tablet/computer with Android or iOS and Internet connection used for reporting sampling details and health information in an online-questionnaire.
- [Applicable if information is collected using an online questionnaire:] The web address to the online-questionnaire.
- [Applicable if information is collected using paper questionnaires:] Paper questionnaire and pen.
- [Applicable if information is collected by telephone interviews:] Telephone.
- Pleasant room temperature
- Convenient chair with armrests or any other furniture that enables a convenient sitting position with the arm relaxed.

Preparing for Blood Sampling

1. Decide together with the mother when she will visit the clinic for sampling.
2. [Applicable if information is collected by telephone interviews:] Decide when to perform the interview to collect information (Annex 3)
3. The study contacts person checks that the patient's identity corresponds to the informed consent and the assigned study donor ID (printed on the label of the blood tubes).

Blood Sampling at the Clinical Site

1. Prepare yourself and the required material and equipment according to the local routines. Make sure to have the material you need within an arm's reach.
2. Draw the blood in accordance with local routines and manufacturer instructions. Please note that existing venous catheters cannot be used if they have previously been used to administer [Name of medication]
3. [Applicable if existing venous catheters are used:] Make sure that a slush tube is filled completely before sampling, to avoid getting diluted samples.
4. [Applicable if information is collected using questionnaires:] Ask the woman to report sampling details and medicine intake (Annex 3) using the [paper questionnaire/online-questionnaire].
5. Respond to the online-questionnaire (Annex 4).
6. [Applicable if the participant responds to a paper questionnaire:] Submit her responses using the online-questionnaire.
7. The blood is transported to the local lab in a temperature that is consistent with the stability conditions.
8. [Applicable if information is collected by telephone interviews:] Information is collected by telephone interview afterwards, the same day as sampling (Annex 3)

Preparing the Samples for Analysis and Storage at the Local Lab

1. The LiHep tube is centrifuged at 2400g for 7 min.
2. Pipette the supernatant into aliquot tubes (à 225µl, pre-labeled with the sample ID). As many aliquot tubes as possible should be filled completely. However, no more than 8 aliquot tubes should be filled.
3. The study donor ID, sampling time, sample ID, freezing time, and SPREC code for all tubes are registered using an online-questionnaire.
4. The eight aliquots and the whole blood tube are frozen at -20C°.
5. The samples are shipped on dry ice to Uppsala Biobank. When samples are ready for shipment, please notify Uppsala Biobank by sending an e-mail to info@uppsalabiobank.uu.se (subject: ConcePTION: Sample shipment).

6. The aliquots and whole blood tube arriving at Uppsala Biobank are freezed at a temperature of -80C°.

3.2 Procedure for collecting and processing blood samples at the participant's home

General considerations

The blood drawings are carried out by trained personnel used to draw blood. The timing of the blood draw in relation to medicine intake and breast milk sampling depends on the specific study and substance being investigated. The tubes required depends on the specific study and if plasma or serum is being collected. The maximum time from drawing the blood to putting the aliquots in the freezer is decided based on the stability of the specific drug. Uppsala Biobank provides pre-labeled aliquot tubes.

Material and Equipment

The material and equipment required to collect blood are delivered with the Blood Sampling Kit provided by the study contacts person.

- Blood Sampling Kit:
  - 1 alcohol hand sanitizer
  - 1 pair of non-sterile gloves, in a well-fitting size
  - 1 tourniquet
  - Alcohol swabs for skin disinfection
  - 1 set of venepuncture equipment used at the local site. E.g., needle and a tube holder or a butterfly needle set.
  - [Applicable when plasma is collected:] Use 2 Li Hep tubes (à 7 ml) labeled with the study donor ID.
  - [Applicable when serum is collected:] Use two SST tubes (à 7 ml) labeled with study donor ID.
  - Cotton wool balls, compresses or similar materials used at the clinical site to stop bleeding.
  - Skin tape
  - One portable disposal unit
  - Extra material (gloves, swabs, venepuncture equipment, tubes, labels, compresses, and skin tape) in case the blood drawing fails.
  - [Applicable when an existing venous catheter is used to draw blood from:] One 7,5ml slush tube
  - [Applicable when an existing venous catheter is used to draw blood from:] The syringes, saline solution, heparin and catheter plugs necessary to flush and lock the catheter according to the local routines.
- [Applicable if information is collected using an online questionnaire:] A smartphone/tablet/computer with Android or iOS and Internet connection used for reporting sampling details and health information in an online-questionnaire.
- [Applicable if information is collected using an online questionnaire:] The web address to the online-questionnaire.
- [Applicable if information is collected using paper questionnaires:] Paper questionnaire and pen.
- [Applicable if information is collected by telephone interviews:] Telephone
- Transportation equipment according to local routines.
- Pleasant room temperature
- Convenient chair with armrests or any other furniture that enables a convenient sitting position with the arm relaxed.

Preparing for Blood Sampling

1. Decide together with the mother when you will visit her to draw blood.
2. [Applicable if information is collected by telephone interviews:] Decide when to perform the interview to collect information (Annex 3)
3. Check that the patient's identity corresponds to the informed consent, the assigned study donor ID and the sample ID (printed on the label of the blood tubes).

Blood Sampling at the Participant's Home

1. Prepare yourself and the required material and equipment according to the local routines. Make sure to have the material you need within an arm's reach.

2. Draw the blood in accordance with local routines and manufacturer instructions. Please note that existing venous catheters cannot be used if they have previously been used to administer [Name of medication].
3. [Applicable if existing venous catheters are used:] Make sure that a slush tube is filled completely before sampling, to avoid getting diluted samples.
4. [Applicable if the participant responds to a paper questionnaire:] Ask the woman to report sampling details and medicine intake (Annex 3) using the [paper questionnaire/online-questionnaire].
5. Respond to the online questionnaire (Annex 4).
6. [Applicable if the participant responds to a paper questionnaire:] Submit her responses using the online-questionnaire
7. The blood is transported to the local lab in a temperature that is consistent with the stability conditions.
8. [Applicable if information is collected by telephone interviews:] Information is collected by telephone interview afterwards, the same day as sampling (Annex 3)

Preparing the Samples for Analysis and Storage at the Local Lab

1. The LiHep tube is centrifuged at 2400g for 7 min.
2. Pipette the supernatant into aliquot tubes (à 225µl, pre-labeled with the sample ID). As many aliquot tubes as possible should be filled completely. However, no more than 8 aliquot tubes should be filled.
3. The study donor ID, sampling time, sample ID, freezing time, and SPREC code for all aliquot tubes are registered using an online-questionnaire.
4. The eight aliquots and the whole blood second tube are frozen at -20C°.
5. The samples are shipped on dry ice to Uppsala Biobank. When samples are ready for shipment, please notify Uppsala Biobank by sending an e-mail to info@uppsalabiobank.uu.se (subject: ConcePTION: Sample shipment).
6. The aliquots and whole blood tube arriving at Uppsala Biobank are freezed at a temperature of -80C°.

Discussion

Procedures and templates have been designed in order to be fit for purpose in establishing a Europe-wide biobank for breastmilk and blood, as well as bioanalytic centres fulfilling regulatory standards in accordance with FDA's criteria for Bioanalytic Method validation (BMV). This is a necessary requirement if order to bring future lactation studies up to date with those requirements that drug manufacturers will have to attend to. During the first months of the project, experts from the Mommy's Milk Biorepository for human milk research in San Diego, CA (*https://mommysmilkresearch.org/*) were contacted and a collaboration was initiated in order to attain consensus regarding procedures as far as is possible considering different international regulatory frameworks. There has been no deviations from what is described in the DoA.

Conclusion

The consent procedures and templates for the patient information and the procedures that specifies the minimum quality requirements for sampling of human breast milk and blood are instructive for not only the demonstration studies performed by WP 4 but for all future lactation studies collecting reast milk samples with the aim of providing data that is fit for label.

4. Annexes

4.1. Annex 1

[Questionnaire/Online-questionnaire] for mother to respond to when breast milk sampling

The first seven questions are only posed at the first breast milk sampling.

1. What is the age of your baby?

[Free text:] _____

2. At what gestational age was your baby born?

At ____ weeks and _____ days

3. What is your age?

____ years

4. What is your ethnicity?

[Free text:] _____

5. For what medical condition are you taking [Name of medication] (also called [tradenames])?

[Free text:]_____

6. What is your height?

____ cm

7. What is your current weight?

____ kg

8. What medications are you taking?

[Free text:] _____

9. Are you using any traditional medicinal products?

□ No □ Yes, I use the following: [Free text:]_____

10. What dose of [Name of medication] (also called [tradenames]) do you take?

[Free text:] _____ □ I do not know

11. How often do you take [Name of medication] (also called [tradenames])?

[Free text:] _____

12. Please indicate when you took your last dose of your medication.
Date: ____/_____/___ex. 20/01/2012 time: _____h_____ ex. 12h52

13. Please indicate if you recall missing a dose during the last [7 days/other appropriate time interval]

□ I have not missed a dose during that time □ I am not sure [Should be adjusted to the specific medication studied:] □ yes,

____ days ago at ____h___ ex. 12h52 PM or AM

14. Please indicate when your last breastfeeding occurred

Date: ____/_____/___ex. 20/01/2012 time: _____h_____ ex. 12h52

15. At what time did you begin pumping breast milk?

Date: ____/_____/___ex. 20/01/2012 time: _____h_____ ex. 12h52

16. Where did you take the breast milk sample from?

□ left breast □ right breast

17. Time of removal?

□ before breastfeeding

□ immediately after breastfeeding
□ breastfeed □ left side □ right side □ both sides

□ Combination of both (sample was partially taken before and after breastfeeding)
□ breastfeed □ left side □ right side □ both sides

18. Have you [frozen the breast milk sample/given the breast milk sample to the personnel]?

□ Yes, at Date: ____/_____/___ex. 20/01/2012 time: _____h_____ ex. 12h52

4.2. Annex 2

Online-questionnaire for personnel to respond to when breast milk sampling has been performed at the clinical site

19. Has the breast milk sample been frozen?

□ Yes, at Date: ____/_____/___ex. 20/01/2012 time: _____h_____ ex. 12h52

4.3. Annex 3

[Questionnaire/Online-questionnaire] for mother to respond at blood sampling

1. What medications are you taking?

[Free text:] _____

2. Are you using any traditional medicinal products?

□ No □ Yes, I use the following: [Free text:]_____

3. What dose of [Name of medication] (also called [tradenames]) do you take?

[Free text:] _____ □ I do not know

4. How often do you take [Name of medication] (also called [tradenames])?

[Free text:] _____

5. Please indicate when you took your last dose of your medication.
Date: ____/_____/___ex. 20/01/2012 time: _____h_____ ex. 12h52

6. Please indicate if you recall missing a dose during the last [7 days/other appropriate time interval]

□ I have not missed a dose during that time
□ I am not sure
[Should be adjusted to the specific medication studied:]
□ yes, ____ days ago at ____h___ ex. 12h52 PM or AM

7. Please indicate when your last breastfeeding occurred.

Date: ____/_____/___ex. 20/01/2012 time: _____h_____ ex. 12h52

4.4. Annex 4

Online-questionnaire for personnel to respond to after blood sampling

1. When was the blood sample drawn?

Date: ____/_____/___ex. 20/01/2012 time: _____h_____ ex. 12h52

2. Where did you take the blood sample from?

□ left arm □ right arm □ both sides □ Other location:_____

3. How was the blood drawn?

□ By puncturing the skin
□ From a peripheral venous catheter
□ From a port-à-cath
□ From a PICC-line
□ From another catheter: _____

4. What type of tube was used? _____

5. What type of venipuncture equipment was used? _____

Repository for primary data[3]

Samples will be stored at Uppsala Biobank according the following:

- Name of site
- Study Donor Id
- Sample Id
- Sampling date and time of sample collection
- SPREC code (type of sample and sample handling)
- All biobank data will be in LIMS and traceability of samples and shipments will be managed in the LIMS

Bioanalytical data will be stored at UDOPP (Uppsala university)

- Drug concentration value in breast milk
- Drug concentration value in plasma from mother
- Drug concentration value in plasma/blood from infant
- Analytical methods validation results
- Quality data

PK data will be stored in ConcePTION database in Utrecht

- AUCτ: Area under the curve over a dosing interval
- Cav: Average concentration over a dosing interval, equal to Cav=AUCτ/τ
- Cmax: Maximum observed drug concentration
- tmax:Time of the maximum observed concentration
- λz: First-order terminal elimination rate constant, calculated from a semi-log plot of the milk (plasma) concentration vs time curve
- t1/2: First-order terminal elimination half-life, calculated as 0.693/λz

Clinical data and documentation by women will be stored in the ConcePTION database in Utrecht.

Population PK modeling data will be stored in ConcePTION database

Calculated infant dose and relative infant dose will be stored in ConcePTION database

WP 7 is responsible for setting up the ConcePTION data base.