PREDICT-TBI

(Prediction and Diagnosis using Imaging and Clinical biomarkers Trial in Traumatic Brain Injury)

Study Standard Operating Procedures:

Biospecimen Management

Version 9.3
12 July 2023

Table of Contents

TABLE OF CONTENTS

List of abbreviations

AI	Associate Investigator
AIBN	Australian Institute for Bioengineering and Nanotechnology
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme Linked Immunosorbent Assay
GE	Genome Equivalent
ICU	Intensive Care Unit
MRI	Magnetic Resonance Imaging
PAH	Princess Alexandra Hospital
PI	Principle Investigator
QBI	Queensland Brain Institute
RBWH	Royal Brisbane and Women's Hospital
SST	Serum Separating Tube
TBI	Traumatic Brain Injury
TUH	Townsville University Hospital
UQ	The University of Queensland
USI	Unique Study Identification

1 Purpose

The collection of biofluids is central to the goals of the PREDICT-TBI study. The purpose of this manual is to provide PREDICT-TBI staff (PIs, AIs, study coordinators, phlebotomists) at the various study sites with instructions for collection and submission of biological samples for the PREDICT-TBI study. It includes instructions for biospecimen submission to the PREDICT-TBI sample repositories at QBI.

The following samples will be collected:

- Serum
- Plasma
- Red Blood Cells

This manual includes instructions for collection of blood, fractionation of blood from vacutainer tubes, aliquoting, labelling, storage prior to shipping to the PREDICT-TBI biorepositories.

2 Biorepositories Laboratory Information

2.1 Contacts

Attn: Fatima Nasrallah – email f.nasrallah@uq.edu.au
Queensland Brain Institute
University of Queensland
St Lucia
Queensland 4072

Attn: Project Manager – email predict-tbi@uq.edu.au
Queensland Brain Institute
University of Queensland
St Lucia
Queensland 4072

2.2 Shipping Days

QBI:

Frozen Samples must be shipped on Mondays, Tuesdays, or Wednesdays only to allow them to be received during the week.

2.3 Holiday Schedules

Please be sure to verify with the courier's schedule prior to shipping close to a public holiday. This chart represents national public holidays, please be aware of any state public holidays which may impact shipping or receiving of samples.

New Year's Day	1 January
Australia Day	26 January
Good Friday	Varies
Easter Monday	Varies
ANZAC Day	25 April
Christmas Day	25 December
Boxing Day	26 December

Additionally, UQ is closed from the 25th of December till the 2nd of January each year. Do not ship any samples during these periods.

2.4 Shipping Frequency and Conditions

Frequency of shipments will depend on the enrolment rate and on the storage capacity of the hospital laboratory at each study site. Frozen samples should be shipped at least quarterly. Please ensure adequate storage at -80°C prior to shipment. Check the weather report to make sure that impending weather events (hurricanes etc) will not impact shipping of delivery of the samples.

3 Biorepository Research Sample Collection

IMPORTANT NOTE: In order to ensure the highest quality samples are collected, processed and stored, it is essential to follow the specific collection, processing and shipment procedures detailed in the following pages. Please read the following instructions first before collecting any specimens. Have all your supplies and equipment out and prepared prior to drawing blood. Please follow the order of draw instructions.

3.1 Study Sampling Procedure,

Study bloods to be taken by either registered nurses with venepuncture training or phlebotomists. If the participant is in ICU and has a central/arterial line, blood will be drawn using that line. When possible, study blood samples will be taken at the same time as any routine clinical bloods to minimise the need for separate venepuncture.

3.2 Materials and Equipment Required at the Study Site for Local Processing Prior to Shipping

The following materials and equipment are necessary for the processing of specimens at the collection site and are to be **supplied by the local site**:

- Personal Protective Equipment
- Tourniquet
- Cleansing Prep Pad
- Gauze Pad
- Bandage
- Needles
- Microcentrifuge tube rack
- Gloves
- Sharps bin and lid

In order to process samples consistently across all projects and ensure the highest quality samples possible, project sites must have access to the following equipment:

- -80°C Freezer
- Centrifuge capable of 1900g spin
- Ideally, also a centrifuge capable of 14,000g spin

3.3 Order of Draw

Please follow this order of draw:

1. SST tube
2. EDTA tubes

3.4 Biospecimens to be sent to the PREDICT-TBI Biorepositories

Biospecimens are whole blood collected into two different types of vacutainer tubes. Please use:

- **2 x 10ml EDTA Tubes** (supplied by QBI to each study site)
- **1 x 5ml SST Tube** (supplied by QBI to each study site)

Guidelines for the processing and storage of all samples are specified in the table below:

Samples		Tubes required	Instructions	
		2 x 10ml EDTA	Deliver to Site Pathology lab on wet ice	
		1 x 5ml SST	Deliver to Site Pathology lab at Room Temperature	
Tube type	**Size**	**Instructions for hospital pathology laboratory**		**Shipping**
K2 EDTA 1	**10ml**	• Take to pathology on wet-ice if feasible. • Spin within 4 hours of blood draw if stored at room temperature, 8 hours if at 4°C. • First spin at 1,900*g* for 10 minutes at 4°C with brake off. • Transfer plasma to small graduated centrifuge tubes. Carefully retrieve to avoid contamination by the buffy coat. • Second spin at fastest rotor speed (14,000–16,000g – small centrifuge) for 10 minutes with brake on. • Aliquot plasma into 4 x 1.5 ml Nunc tubes. • Aliquot red cells into 1x1.5 ml Nunc tube. • Store in -80°C freezer.		***Send all aliquots to QBI
K2 EDTA 2	**10ml**	• Take to pathology on wet-ice if feasible. • Spin within 4 hours of blood draw if stored at room temperature, 8 hours if at 4°C. • First spin at 1,900*g* for 10 minutes at 4°C with brake off. • Transfer plasma to yellow graduated 10ml centrifuge tube. • Second spin at 1,900g for 10 minutes at 4°C with brake on.		***Send all aliquots to QBI

		• Aliquot plasma into 4 x 1.5 ml Nunc tubes. • Aliquot red cells into 1x1.5 ml Nunc tube. • Store in -80°C freezer.	
SST	**5ml**	• Take to pathology at Room Temperature • Spin and freeze within 8 hours • Single spin at 1,900g for 10 minutes at 4°C with brake on. • Aliquot serum into 1 x 6ml P6 Serology tube.	Send to QBI

***Site Research Coordinator to ensure samples are organised for shipping to destination.

For PAH Site Only

Due to variations in hospital laboratory processing facilities, **samples collected at PAH must be transferred to Qld Pathology at RBWH** for processing and storage. Once drawn, PAH samples will be sent to Qld Pathology Laboratory at PAH who will then arrange to transfer them to RBWH Qld Pathology Laboratory.

Samples collected at the PAH will be sent to RBWH via the routine courier at 4°C. Below is the courier schedule for departure and arrival times; there would need to be enough time for the samples to be received in the lab at PAH and prepared for shipment to RBWH which would include sample receipt, registration, distribution to sendaways department, prepared for shipment to RBWH, including accommodating busy workload. A last blood collection of 2 pm would be recommended.

PAH Depart	RBWH Arrive
8:30	9:00
8:45	9:35
9:45	10:05
11:55	12:15
13:40	13:55
15:50	16:15

3.5 Blood Sample Timepoints

Blood samples will be collected at the time points as per this schedule.

Important note: **please do not collect samples from Friday at 1600hr through to Monday at 0400hrs**, even if this means that the first sample (within 24 hours of injury) is missed. Samples cannot be processed over the weekends and are not able to be stored for more than 8 hours prior to processing.

Missed sample timepoints should be recorded as missed in REDCap. All other sample timepoints should be timed for a weekday collection.

TIMEPOINT	TEST IF INPATIENT	TEST IF OUTPATIENT
Timepoint 1: Within 36 hours of injury	✓	✗
Then at the following days post injury		
Timepoint 2 (between 48 and 72 hours post injury (dpi)	✓	✗
Timepoint 3 (Day 4 post injury + 24hours)	✓	✗
Timepoint 4 (Day 7 post injury +/- 24 hours)	✓	✗
Timepoint 5 (Day 14 post injury +/- 24hours)	✓	✗
STOP then:		
3 months post injury (+/- 14 days)	✓	✓
6 months post injury (+/- 21 days)	✗	✗

IMPORTANT NOTES:

- If the participant is undergoing MRI, study bloods should be taken on the same day, preferably prior to the MRI scan.

3.6 Blood Sample Study Collection Kits

Each site will be provided with the below kits from QBI:

- 2 x 10ml EDTA tubes
- 1 x 5ml SST tube
- 1 x 5ml serology tube
- 10 x 1.5 ml tubes
- 1 x specimen bag
- Study labels

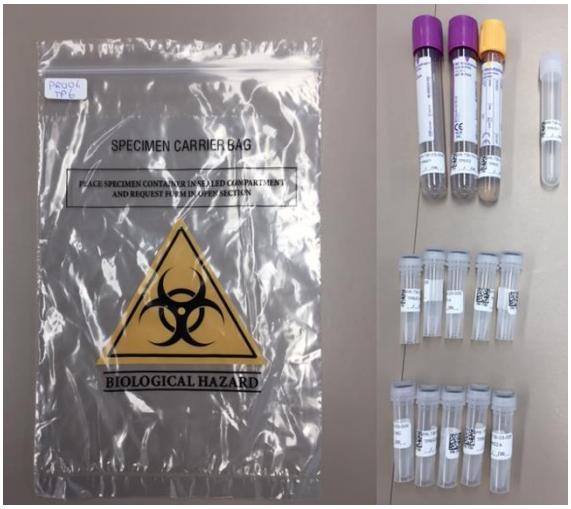
Please be sure to order additional kits before you run out, so you are prepared for both scheduled sampling and new patients. Please allow at least 15 days for additional EDTA tubes to be delivered.

4 Blood Sample Labelling, Storage and Shipping

4.1 Labelling and Storage of Blood Samples

In order to ensure the label adheres properly and remains on the tube, please follow these instructions.

Each kit is supplied with labels for the specimens shipped to the PREDICT-TBI Biorepository. The tubes and pre-printed labels will be supplied by QBI to each participating site. Labels will be prepopulated with PREDICT-TBI Site Number, plasma sample number (i.e., EDTA1, EDTA2, or SST), Nunc tubes for aliquoting, and the study identifier (see below for the identifier).



Each vial will be identified with an alphanumeric string derived from this system:

Study	Site	Subject ID	Blood Draw	Specimen Vial
PR-TBI	01	001-299	*TP1-TPn* TP1=First sample at timepoint 1 TP2=Second sample at timepoint 2 … TPn=nth sample at timepoint n	E1 (ETDA 1) E2 (EDTA 2) S (SST)

For example: PR-TBI-01-001-TP1-E1 ← SPECIMEN VIAL

PREDICT-TBI SITE PATIENT ID BLOOD DRAW

Therefore, based on the above, each patient will have six kits in total, one for each timepoint of blood collection. Each kit will contain 1 x E1, 1 x E2, 1 x SST, 10 x 1.5 ml Nunc tubes and 1 x 5 ml serology tube. A list of all tubes is below:

1. E1: 1st EDTA blood collection of 10 ml
2. E2: 2nd EDTA blood collection of 10 ml
3. S: 5.0 ml blood collection
4. E1-a: 1.5 ml plasma after processing from EDTA1
5. E1-b: 1.5 ml plasma after processing from EDTA1
6. E1-c: 1.5 ml plasma after processing from EDTA1
7. E1-d: 1.5 ml plasma after processing from EDTA1
8. E1-RC: 1.5 ml tube for red blood cells of EDTA1
9. E2-a: 1.5 ml plasma after processing from EDTA2
10. E2-b: 1.5 ml plasma after processing from EDTA2
11. E2-c: 1.5 ml plasma after processing from EDTA2
12. E2-d: 1.5 ml plasma after processing from EDTA2
13. E2-RC: 1.5 ml tube for red blood cells of EDTA2
14. S-1: 5.0 ml tube for Plasma deriving from SST tube after processing

Where Sites are coded based on the following:

- 01 = The Royal Brisbane and Women's Hospital (RBWH)
- 02 = The Princess Alexandra Hospital (PAH)
- 03 = Royal Darwin Hospital (RDH)
- 04 = Liverpool Hospital (LH)
- 05 = Gold Coast University Hospital (GCUH)
- 06 = Townsville University Hospital (TUH)
- 07 = The Alfred Hospital (AH)

All samples will be stored at the hospital pathology storage facility with study labels provided by QBI. The label will also include the Date and Time that the sample was drawn.

Write on the labels with a ballpoint pen and place labels on ALL serum and aliquot tubes BEFORE any sample collection, processing or freezing - this should help ensure that the label adheres to the tube before exposure to moisture or different temperatures.

Labels need to be fixed horizontally (wrapped around sideways if the tube is upright) so that they can be read while standing the tube on its base and placed just below the ridges of the aliquot tube. Please take a moment to ensure that the label is completely adherent to each tube. Sample boxes will be labelled with the study and site name and the box number (e.g. PREDICT-TBI-TUH-01).

Finalised samples are to be placed immediately into a biohazard bag. Put wet ice into another biohazard bag and place this inside the bag containing the finalised samples. Do not put the ice directly in the bag with the samples, as the ice could contaminate the samples as it melts. Transfer the samples to the hospital pathology laboratories where they will be stored at -80°C.

A Biospecimen Record Summary and Shipment Notification (Appendix A) is to be completed for all specimens to be transported.

4.2 Transport of Plasma Samples

Samples will be collected by a specialist courier company which will be arranged by QBI and shipped on dry ice to QBI at the expense of UQ.

To arrange for the packaging and transport of samples, please contact the Trial Research Manager, at predict-tbi@uq.edu.au

Transport of plasma samples to QBI will occur as follows:

- Samples will remain housed in their boxes during sample transport
- Electronic copies of the completed Biospecimen Record Summary and Shipment Notifications shall be sent to QBI
- The courier will collect all samples at the request of the site study coordinator
- The courier will provide all packing materials.
- The courier will pack samples to be compliant with IATA requirements.

5 Management of blood samples for withdrawal of consent or absence of SDM consent.

Should there be no SDM available for the foreseeable future, and the participant has not regained the capacity to provide their own Consent to Continue, study blood samples will be collected while consent is being sought within a 5-day working period from the time the patient has been identified as eligible for the study. If an SDM is not available or does not consent, or if the patient does not consent, to participating in the study, all biological samples will be destroyed commencing on working Day 6 from enrolment.

Samples from patients where consent to analyse the samples has been revoked, should be destroyed at participating sites. This will be documented in REDCap.

6 Analysis of Biospecimens

Samples will be analysed for the following:

CSIRO	Quantification of ccfDNA levels (GE/ml) and its temporal pattern.
QBI - Biobanking for Simoa Quanterix	Quantification of Glial Fibrillary Acidic Protein (GFAP) levels (pg/ml) and temporal pattern. Quantification of Ubiquitin Carboxyl-terminal Hydrolase Isozyme L1 (UCH-L1) protein levels (pg/ml) and temporal pattern Quantification of total Tau protein levels (pg/ml) and temporal pattern Quantification of Phospho-Tau protein levels (pg/ml) and temporal pattern Quantification of NF-Light (NF-L) (pg/ml) and temporal pattern Quantification of amyloid β 1-40 (Aβ40) protein (pg/ml) and temporal pattern Quantification of amyloid β 1-42 (Aβ42) protein (pg/ml) and temporal pattern
AIBN to analyse – but send samples to QBI	Quantification of neural-derived exosomes (particles/mL) and neural exosome biomarker profile.

7 Deviations from Prescribed Sample Collection and Storage

The following may each be considered a deviation from the PREDICT TBI study Protocol:

- samples are collected outside of the prescribed collection times
- samples have not been processed and/or stored as outlined in this Operations Manual

In each of the above circumstances, the samples may still be valid, however, the following will need to be completed:

- document the reason for this deviation in REDCap
- notify the Trial Research Manager

If samples are received at the Biorepositories that do not conform to study guidelines the Biorepository personnel will complete a Non-Conformance Report (Appendix B).

Please do not destroy samples – send to QBI as per normal process.
Note: the above deviations DO NOT require to be reported as protocol deviations for the main PREDICT-TBI study.

8 Safety Reporting

In this sub-study, adverse events are likely to be extremely rare due to a lack of intervention. However, it is possible for adverse events to arise from the interactions required for participation in the study (i.e. directly resulting from the procedure of blood collection) and these will be reportable to the coordinating centre and local ethics committee where applicable.
Clinically significant procedural related adverse events will be reported on the relevant form and submitted to the Central Coordinating Centre within 72 hours. These will be reviewed by the coordinating centre staff and recorded in a safety database.
Only those reactions that are thought to have a direct causal relationship with the PREDICT-TBI study procedures should be reported as an Adverse Event (AE).

Reporting requirements
AEs including serious adverse events (SAEs) and suspected unexpected serious adverse reactions (SUSARs) will be reported from the time of enrolment into the PREDICT-TBI study until completion of the trial.

9 Monitoring

Study monitoring will be conducted with a focus on study sampling and sample integrity as follows:

Study Sampling:

- Confirm consent for sample collection
- On site – verify study procedures are performed in accordance to the protocol and recorded in REDCap according to the Biospecimen SOP.
- Remotely – verify study procedures are performed in accordance to the protocol and recorded in REDCap according to the Biospecimen SOP.

Sample Integrity

- Confirm that each site has the capacity to store samples in a monitored -70 °C to -80 °C freezer
- Verify freezer log if available
- Confirm all samples have been processed and stored according to the protocol
- If there has been a storage failure, a note to file must be recorded in the site folder outlining subject number and explaining the situation and a copy forwarded to the project manager.

10 Appendices

10.1 Appendix A - Biospecimen Record Summary and Shipment Notification

Site Name and Number_____ PQ Services_____

Phone number_____ Email_____

Please list only ONE Subject per Sample Record Summary and Shipment Notification Form and use ONE form for each separate sample destination (cross out not applicable samples/destinations).

PREDICT TBI USI_____

Date samples shipped_____

Courier Tracking Number_____

Instructions: Ship frozen shipments on Mondays, Tuesdays, or Wednesdays ONLY

This form must be completed for shipment of all research samples.
Notify PREDICT TBI by email preferred in advance of shipment (predict-tbi@uq.edu.au)

1. Place a copy of this form in the shipment box for EACH DESTINATION,
2. Email a copy to the respective Biorepository
3. File a copy of the completed form in the study binder.

Ensure all frozen shipments are filled with DRY ICE.

To be completed by SITE/SENDER				**To be completed by PREDICT TBI Biorepositories**
Dates of Draw	Draw Numbers	Specimen Type	Destination	Notification of Problems
		P6 Serology	QBI, UQ, St Lucia, QLD 4072	
		1ml aliquots to QBI	QBI, UQ, St Lucia, QLD 4072	
		1ml 200µL aliquots	QBI, UQ, St Lucia, QLD 4072	
		4x0.5ml Nunc tubes	QBI, UQ, St Lucia, QLD 4072	

10.2 Appendix B - Biorepository Biomarker Sample Submission Non-Conformance Report

This form is to be completed by the PREDICT-TBI Biorepository personnel when a sample has been received and issues are noted. Completed forms are to be emailed or faxed to the submission site coordinators and the central study coordinator.

Site Name:
Site Number:
USI:
Sample number:
Received by:
Date:

Your shipment was received with the observed problem(s) checked below. Please take note so that your future shipments are received without incident.

Tick relevant section/s	
	Samples shipped on the incorrect days
	Samples arrived on a weekend
	Advanced notice of the shipment was not provided
	Shipment notification does not match shipment notification form received with samples
	No shipment form included in the package
	Shipment notification form incomplete
	Package contents do not match shipment notification form
	Package received has little or no dry ice
	Signs of samples thawing present
	Samples submitted in incorrect tubes
	Sample tubes damaged/cracked
	Samples not labelled appropriately or labels peeling off
	Samples received with low volume
	Unexpected sample received (specified in comments below)
	Other (specified in comments below)

Comments:_____
_____
_____
_____
_____