



ICONIC: improving outcomes
through collaboration in osteosarcoma

LABORATORY MANUAL

For the collection, processing, storage and shipping of samples
FOR ALL ICONIC PATIENTS

Version v5, 20/05/2022



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VERSION	DATE	SUMMARY OF MAIN CHANGES	AUTHOR
1	25/10/2019	Not Applicable	Krystyna Reczko
2	19/11/2019	Clarification on collection of blood samples on a Friday/Bank Holiday	Sharon Forsyth
3	13/11/2020	Addition of blood collection for CTCs at Newcastle; Clarification of timepoint for Germline DNA sample	Krystyna Reczko
4	15/04/2021	<ul style="list-style-type: none"> Integration of surgery and chemotherapy lab manuals into single lab manual for all ICONIC sites. Clarification on collection of ctDNA samples for all patients Addition of blood collection for CTCs at Sheffield New clarification on collection of blood samples on a Friday 	Krystyna Reczko
5	20/05/2022	<ul style="list-style-type: none"> ctDNA follow-up timepoint frequency clarified in section 2.5 Sheffield lab address and email contact for shipping CTC samples updated RNOH lab address updated Cryolabel details & examples updated for consistency with actual labels 	Kate Sparksman

1 GLOSSARY

Acronym	Meaning
AP	Doxorubicin, cisplatin chemotherapy
ccfDNA	Circulating cell free DNA
ctDNA	Circulating tumour DNA
CTCs	Circulating Tumour Cells
FFPE	Formalin-fixed paraffin-embedded
ISF	Investigator Site File
MAP	Methotrexate, doxorubicin, cisplatin chemotherapy
Mtx	Methotrexate
RNOH	Royal National Orthopaedic Hospital
TC	Trial Coordinator
UCL CTC	CR UK & UCL Cancer Trials Centre

2 INTRODUCTION

This laboratory manual covers the collection, processing, storage and transportation of blood and tissue samples for the ICONIC study.

2.1 Site staff delegation of duties and training

In order to preserve the integrity of patient samples, they must be collected, handled, processed and stored in accordance with the instructions in this manual and the current approved version of the study protocol. Designated staff must receive adequate training and be delegated authority to carry out these tasks (duty O) in the ICONIC Site Staff Delegation Log.

2.2 Sample audit trail and documentation

A complete audit trail must be maintained for all samples and it should be possible to locate any sample at any point in time.

For each sample, complete the relevant processing or shipping form which can be found in the laboratory section of the Investigator Site File (ISF). Electronic versions of these forms are also provided.

The sample processing or shipping forms and sample logs must be available for review during onsite monitoring visits, and at other times as requested by UCL CTC.

2.3 Temperature tolerances

The following are the acceptable temperature ranges for the conditions specified in this laboratory manual, unless specified otherwise.

Table 1: Temperature tolerances

Storage condition specified	Temperature range (°C)
Ambient or room temperature	15 - 30
Fridge	2 - 8
Freezer or -80	-70 or colder

2.4 Contact Details

All ICONIC queries should be directed to the ICONIC Trial Coordinator.

Contact: **ICONIC Trial Coordinator**
Address: Cancer Research UK & UCL Cancer Trial Centre
90 Tottenham Court Road
London
W1T 4TJ
Email: ctc.iconic@ucl.ac.uk
Telephone: 020 7679 9878

2.5 Summary of Sample Collection

SAMPLE	REQUIRED FROM:	SAMPLE TIMEPOINTS	PROCESSING	SHIPPING TIMELINE	SHIP TO
Archival FFPE tumour tissue	All patients	<ul style="list-style-type: none"> Biopsy/Surgery Preregistration 	None	On request from UCL CTC	RNOH, Stanmore
FFPE tumour tissue	All patients	<ul style="list-style-type: none"> Routine surgery post registration 			
Stored frozen tissue	All patients (collected as per SOC)	<ul style="list-style-type: none"> Baseline, surgical resection, 	Freezing (per local practice)	On request from UCL CTC (courier provided)	RNOH, Stanmore
New relapse/mets tumour biopsy	Patients with SoC local recurrence or mets resection or consenting Patients with resection of local recurrence or mets if not SoC.	Relapse/resection of local recurrence or metastases	FFPE	On request from UCL CTC	RNOH, Stanmore
			Freezing (per local practice)	On request (courier provided)	
Blood for CTCs	Neoadjuvant chemotherapy patients only. <ul style="list-style-type: none"> All samples must be collected 	<ul style="list-style-type: none"> Baseline (NB if patient has started chemotherapy prior to study entry NO samples can be collected) C2D21 MAP or C3 AP* Relapse* 	None	On day of collection	Newcastle University
					Sheffield University
Blood for ctDNA, methylation profiles, future research	All patients (optional).	<ul style="list-style-type: none"> Baseline (NB if patient has started chemotherapy prior to study entry this baseline sample may still be collected) C2D21 MAP or C3 AP End of treatment** Follow up - no more than 6 monthly Relapse/mets 	None	On day of collection	RNOH, Stanmore
Blood for germline DNA	All patients	<ul style="list-style-type: none"> Baseline (NB if sample is missed at baseline the sample may still be collected at a later visit) 	None	On day of collection	RNOH, Stanmore

* (NB only if baseline collected)

** End of treatment: after surgery for neoadjuvant and surgery only patients, after end of adjuvant chemotherapy for patients receiving adjuvant chemotherapy.

2.6 Consumables and shipping provided by UCL CTC

The following consumables will be provided to Sites by UCL CTC:

- ccfDNA PAXgene tubes (10 mL)
- Streck tubes (10 mL)
- Royal Mail Safeboxes
- Sample labels
- Sample shipping forms
- Freezer boxes (on request)
- 2 mL Cryovials (on request)

Shipping

For samples to be shipped on dry ice, UCL CTC will arrange and pay for the courier. The courier will provide dry ice and packaging required for shipment.

3 ARCHIVAL FFPE TUMOUR TISSUE SAMPLES

For the timing of sample collection please refer to [section 2.5](#) and protocol section 10.1.

Archival FFPE tissue blocks from previous surgery/biopsy and other samples from routine surgery taking place after registration will be collected.

NB: it is expected the site storing these samples would be responsible for submitting to the central laboratory (regardless of which site registered the patient). The collection of samples will be coordinated centrally by UCL CTC.

3.1 Equipment required

None.

3.2 Processing procedure

- Sites should obtain at least one and ideally two formalin fixed paraffin embedded (FFPE) tumour tissue block(s).
- Review the pathology report and select blocks as follows:
 - Biopsy cores: Choose cores that have the highest tumour tissue content.
 - Surgical resection tissue: Choose blocks containing the most tumour tissue.
- At least one block must be sent – two is preferable

Labels will be provided with the following pre-printed:

- Sample: **FFPE AB**
- Timepoint: **PreT**

Add the following information (in bold) using non-water-soluble ink:

- Pt ID (patient study ID): **ICO-XXX**
- Pt Initials (Patient Initials): **XXX**
- Date (date of sample collection): **DD/MM/YYYY**
- Block Number: **1** or **2**
- Example label below:

Pt ID: ICO-001	ICONIC	Sample: FFPE AB Timepoint: PreT
Pt Initials: HP		
Date: 01/01/2000		
Block No: 1		

Labels will be provided with the following pre-printed:

- Sample: **FFPE AS**

Add the following information (in bold) using non-water-soluble ink:

- Pt ID (patient study ID): **ICO-XXX**
- Pt Initials (Patient Initials): **XXX**
- Date (date of sample collection): **DD/MM/YYYY**
- Block Number: **1** or **2**
- Timepoint:
 - PreT – Pre Treatment
 - OT – On Treatment

- Example label below:

Pt ID: ICO-001 Pt Initials: HP Date: 01/01/2000 Block No: 1	ICONIC	Sample: FFPE AS Timepoint: OT
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- Complete sections 1 & 2 of the *Archival FFPE Sample Shipping form*
- Store until requested by UCL CTC.

3.3 Shipping

- Post tumour blocks in a padded envelope.
- Take a copy of the *Archival FFPE Sample Shipping Form* to keep.
- Send the original form with the tissue blocks.
- At the time of posting, email a scanned copy of the form to UCL CTC at ctc.iconic@ucl.ac.uk, and copy in the RNOH lab at moh.biobank@nhs.net

Post to:

RNOH Biobank Team
 Histopathology Department
 UCL Institute of Orthopaedics
 Royal National Orthopaedic Hospital
 Brockley Hill
 Stanmore
 Middlesex
 HA7 4LP

On receipt RNOH will complete section 3 of the *Archival FFPE Sample Shipping Form* and return to UCL CTC. Any discrepancies or inconsistencies will be raised with the Site by UCL CTC.

3.4 Return of FFPE samples

All FFPE samples will be stored at RNOH. Sites may request return of routine samples if required for clinical purposes but must return the samples to RNOH after this.

4 STORED FROZEN TUMOUR

For the timing of sample collection please refer to [section 2.5](#) and protocol section 10.2.

During the Study, information will be captured about routine collection and storage of frozen tumour specimens.

NB: it is expected the site storing these samples would be responsible for submitting to the central laboratory (regardless of which site registered the patient). The collection of samples will be coordinated centrally by UCL CTC.

4.1 Shipping

Shipping of these stored samples is not required until the end of the study.

UCL CTC will arrange for the samples to be collected by courier. The courier will provide the dry ice and packaging required for shipment.

On the day of shipping:

- Complete sections 1 and 2 of the *Dry Ice Shipping form* for the samples to be shipped
- Take a copy of the form to keep
- Send the original form with the samples (but NOT in with the dry ice)
- At the time of posting, email a scanned copy of the *Dry Ice Shipping form* to the UCL CTC at ctc.iconic@ucl.ac.uk, and copy in the RNOH lab at rnoh.biobank@nhs.net
- Record details of shipping on the sample inventory log
- Check the shipping container is addressed correctly (this will be provided by UCL CTC to courier at time of booking):

RNOH Biobank Team
Histopathology Department
UCL Institute of Orthopaedics
Royal National Orthopaedic Hospital
Brockley Hill
Stanmore
Middlesex
HA7 4LP

On receipt RNOH will complete section 3 of the *Dry Ice Shipping form* and return to UCL CTC. Any discrepancies or inconsistencies will be raised by UCL CTC with the Site.

5 NEW RELAPSE/METS BIOPSIES FOR RESEARCH

For the timing of sample collection please refer to [section 2.5](#) and protocol section 10.3.

At relapse/resection of local recurrence/metastases a new biopsy should be collected for the ICONIC Study, **if not collected routinely as part of standard of care**.

Collection of this biopsy for research purposes is **optional** for all patients.

If possible two cores should be obtained:

- One core should be frozen according to local practice
- One core should be formalin fixed paraffin embedded

If it is not possible to obtain two cores, then one is acceptable. The preference for this would be frozen, but if not possible FFPE is permitted.

5.1 Fresh frozen samples

5.1.1 Equipment required

ITEM	SUPPLIED BY UCL CTC?
Cryovials	On request
Freezer storage box	On request
Freezer storage space (-70°C or below)	No
Labels for samples	Yes
Non-water-soluble ink pen	No
Fresh Frozen Biopsy Sample Form	Yes

5.1.2 Processing procedure

The sample should be frozen according to local practice. Cryovials can be provided by UCL CTC if required.

Labels will be provided with the following pre-printed:

- Sample: **FFBx**
- Timepoint: **Relapse**

Add the following information (in bold) using non-water-soluble ink:

- Pt ID (patient study ID): **ICO-XXX**
- Pt Initials (Patient Initials): **XXX**
- Date (date of sample collection): **DD/MM/YYYY**
- Example label below:

Pt ID: ICO-001	ICONIC	Sample: FFBx Timepoint: Relapse
Pt Initials: HP		
Date:		
01/01/2001		

Complete the *New Biopsy Frozen Tissue Sample Form*.

Store at -70°C or colder. Freezer boxes are available from UCL CTC on request.

5.1.3 Shipping

Refer to [section 4.1](#) for details on shipping of these samples.

5.2 FFPE sample

5.2.1 Equipment required

None.

5.2.2 Processing procedure

Process sample to FFPE.

Labels will be provided with the following pre-printed:

- Sample: **NBx**
- Timepoint: **Relapse**

Add the following information (in bold) using non-water-soluble ink:

- Pt ID (patient study ID): **ICO-XXX**
- Pt Initials (Patient Initials): **XXX**
- Date (date of sample collection): **DD/MM/YYYY**
- Example label below:

Pt ID: ICO-001 Pt Initials: HP Date: 01/01/2001	ICONIC	Sample: NBx Timepoint: Relapse
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- Complete sections 1 & 2 of the *New Biopsy FFPE Sample Shipping form*
- Store until requested by UCL CTC.

5.2.3 Shipping

- Post tumour blocks in a padded envelope.
- Take a copy of the *New Biopsy FFPE Sample Shipping Form* to keep.
- Send the original form with the tissue blocks.
- At the time of posting, email a scanned copy of the form to UCL CTC at ctc.iconic@ucl.ac.uk, and copy in the RNOH lab at rnoh.biobank@nhs.net

Post to:

RNOH Biobank Team
Histopathology Department
UCL Institute of Orthopaedics
Royal National Orthopaedic Hospital
Brockley Hill
Stanmore
Middlesex
HA7 4LP

On receipt RNOH will complete section 3 of the *New Biopsy FFPE Sample Shipping Form* and return to UCL CTC. Any discrepancies or inconsistencies will be raised by UCL CTC with the Site.

6 WHOLE BLOOD FOR CIRCULATING TUMOUR CELLS (CTCs)

Blood samples for CTCs will be obtained from patients receiving neoadjuvant chemotherapy. For the timing of sample collection please refer to [section 2.5](#) and protocol section 10.4.

Where possible these should be collected at the same time as routine bloods.

NB: All 3 timepoints are required for CTC blood collection. If the baseline pretreatment sample is missed do not take further samples.

6.1 Equipment required

ITEM	SUPPLIED BY UCL CTC?
10 mL Streck tubes	Yes
Royal Mail Safeboxes	Yes
Labels for samples	Yes
Non-water-soluble ink pen	No

6.2 Processing procedure

- Label 2 x 10 mL Streck tubes using labels supplied. The following will be pre-printed:
 - Sample: **CTC**
 - Timepoint:
 - PreT** - Pre Treatment
 - OT** - On Treatment
 - Relapse**
- Add the following information (in bold) using non-water-soluble ink:
 - Pt ID (patient trial ID): **ICO-XXX**
 - Pt Initials (patient initials): **XXX**
 - Date (date of sample collection): **DD/MM/YYYY**
 - Time (time of collection): **HH:MM** (in 24-hour format)
 - Example label below:

Pt ID: ICO-001 Pt Initials: HP Date: 01/01/2000 Time: 14:30	ICONIC	Sample: CTC Timepoint: PreT
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- Collect blood into 2 x 10 mL Streck tubes (fill tubes) – avoid backflow by ensuring the patient's arm is in the downward position and that the tube contents do not touch the stopper or end of the needle during collection.
- If collecting at the same time as routine bloods Streck tubes should be drawn after EDTA tubes and before fluoride oxalate (glycolytic inhibitor) tubes
- Record time and date of collection
- Immediately mix the sample by gently inverting 10 times

- No further processing of this sample is required
- Complete sections 1, 2 & 3 of the CTC Sample Shipping Form.
- Send sample **on day of collection** in a Royal Mail Safebox. See [Appendix 1](#) for Safebox packing instructions.
- Safeboxes must be taken directly to your Hospital's post room or posted in a Post Box (do not use internal mail). Ensure the sample is sent before the last post on the day of collection.
- If this sample has been collected but cannot be shipped according to the timeline specified, **contact the ICONIC Trial Coordinator immediately**.

6.3 Shipping

- One sample is to be sent to Newcastle University and one sample to Sheffield University.
- On the day of sample collection/posting:
 - Take copies of *CTC Sample Shipping Form* to keep.
 - Send the original forms with the blood samples.
 - At the time of posting:
 - email a scanned copy of the Newcastle form to UCL CTC at ctc.iconic@ucl.ac.uk, and copy in the Newcastle labs at Kenneth.Rankin@newcastle.ac.uk
 - email a scanned copy of the Sheffield form to UCL CTC at ctc.iconic@ucl.ac.uk, and copy in the Sheffield labs at tatenda.gochera@nhs.net; sheila.harris2@nhs.net; a.cooney@sheffield.ac.uk
- Check the Safeboxes are addressed correctly:

Mr Kenneth Rankin – ICONIC Study Northern Institute for Cancer Research Paul O'Gorman Building Framlington Place Newcastle University Newcastle upon Tyne NE2 4HH	Lab Manager – ICONIC Study Cancer Clinical Trials Centre Weston Park Hospital Whitham Road Sheffield S10 2SJ
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On receipt the labs will complete section 4 of their *CTC Sample Shipping form* and scan copy to UCL CTC. Any discrepancies or inconsistencies will be raised by UCL CTC with the Site.

7 WHOLE BLOOD FOR ctDNA, METHYLATION PROFILES AND FUTURE RESEARCH

Collection of these blood samples is **optional** for all patients.

NB if patient has started chemotherapy prior to study entry this baseline sample may still be collected

For the timing of sample collection please refer to [section 2.5](#) and protocol section 10.5. Where possible these should be collected at the same time as routine bloods.

7.1 Equipment required

REQUIRED ITEM	SUPPLIED BY UCL CTC?
10 mL BD Vacutainer® PAXgene ccfDNA blood collection tube	Yes
Royal Mail Safeboxes	Yes
Labels for samples	Yes
Non-water-soluble ink pen	No

7.2 Processing procedure

- Ensure tubes are stored at room temperature
- Label 2 x 10 mL PAXgene tubes using labels supplied. The following will be pre-printed:
 - Sample: **ctDNA**
 - Timepoint:
 - **PreT** – Pre Treatment
 - **OT** – On Treatment
 - **EoT** – End of Treatment
 - **FUP__m** - Follow-up __m (number of months from end of treatment, e.g. 6m, 12m etc.)
 - **Relapse**
- Add the following information (in bold) using non-water-soluble ink:
 - Pt ID (patient trial ID): **ICO-XXX**
 - Pt Initials (patient initials): **XXX**
 - Date (date of sample collection): **DD/MM/YYYY**
 - Time (time of collection): **HH:MM** (in 24-hour format)
 - Tube (Tube number): **1 or 2** (label tubes sequentially)
 - Timepoint (for Follow-up only)
 - **FUP XXm** (number of months from end of treatment)
 - Example label below:

Pt ID: ICO-001 Pt Initials: HP Date: 01/01/2000 Time: 14:30 Tube No: 1	ICONIC	Sample: ctDNA Timepoint: FUP-06m
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- Collect blood into 2 x 10 mL PAXgene tubes (fill each tube, if this is not possible ensure at least 5ml are supplied in each tube)
- Record time and date of collection
- Immediately mix the samples by gently inverting 10 times and store upright
- No further processing of these samples is required
- Complete sections 1 & 2 of the *ctDNA Sample Shipping Form*
- Send samples **on day of collection** in Royal Mail Safeboxes. See [Appendix 1](#) for Safebox packing instructions.
- Safeboxes must be taken directly to your Hospital's post room or posted in a Post Box (do not use internal mail). Ensure the samples are sent before the last post on the day of collection.
- **Samples must not be refrigerated**
- If this sample has been collected but cannot be shipped according to the timeline specified, **contact the ICONIC Trial Coordinator immediately**

7.3 Shipping

- On the day of sample collection/posting:
 - Take a copy of the *ctDNA Sample Shipping Form* to keep.
 - Send the original form with the blood samples.
 - Email a scanned copy of the form to UCL CTC at ctc.iconic@ucl.ac.uk, and copy in the RNOH lab at rnoh.biobank@nhs.net at the time of posting
- Check the Safebox is addressed correctly:

RNOH Biobank Team
 Histopathology Department
 UCL Institute of Orthopaedics
 Royal National Orthopaedic Hospital
 Brockley Hill
 Stanmore
 Middlesex
 HA7 4LP

On receipt RNOH will complete section 3 of the *ctDNA Sample Shipping Form* and return to UCL CTC. Any discrepancies or inconsistencies will be raised by UCL CTC with the Site.

8 BLOOD SAMPLE FOR GERMLINE DNA

For the timing of sample collection please refer to [section 2.5](#) and protocol section 10.6. Where possible this should be collected at the same time as routine bloods.

This sample is to be collected for all patients.

NB: This sample should be collected at baseline, however, if this is not possible it may be collected at any timepoint post registration.

8.1 Equipment required

REQUIRED ITEM	SUPPLIED BY UCL CTC?
10 mL BD Vacutainer® PAXgene ccfDNA blood collection tube	Yes
Royal Mail Safeboxes	Yes
Labels for samples	Yes
Non-water-soluble ink pen	No

8.2 Processing procedure

- Ensure tubes are stored at room temperature
- Label 1 x 10 mL PAXgene tubes using labels supplied. The following will be pre-printed:
 - Sample: **Germline**
- Add the following information (in bold) using non-water-soluble ink:
 - Pt ID (patient trial ID): **ICO-XXX**
 - Pt Initials (patient initials): **XXX**
 - Date (date of sample collection): **DD/MM/YYYY**
 - Time (time of collection): **HH:MM** (in 24-hour format)
 - Timepoint:
 - PreT – Pre Treatment
 - OT – On Treatment
 - EoT- End of Treatment
 - FUP__m - Follow-up __m (number of months from end of treatment, e.g. 6m, 12m etc.)
 - Relapse
- Example label below:

Pt ID: ICO-001 Pt Initials: HP Date: 01/01/2000 Time: 14:30	ICONIC	Sample: Germline Timepoint: PreT
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- Collect blood into one 10 mL PAXgene tube (fill the tube, if this is not possible ensure at least 5ml are supplied in the tube)
- Record time and date of collection
- Immediately mix the sample by gently inverting 10 times and store upright
- No further processing of this sample is required
- Complete sections 1 & 2 of the *Germline DNA Sample Shipping Form*
- Send sample **on day of collection** in a Royal Mail Safebox. See [Appendix 1](#) for Safebox packing instructions.
- Safeboxes must be taken directly to your Hospital's post room or posted in a Post Box (do not use internal mail). Ensure the samples are sent before the last post on the day of collection.
- **Samples must not be refrigerated**
- If this sample has been collected but cannot be shipped according to the timeline specified, **contact the ICONIC Trial Coordinator immediately**

8.3 Shipping

- On the day of sample collection/posting:
 - Take a copy of the *Germline DNA Sample Shipping Form* to keep.
 - Send the original form with the blood samples.
 - Email a scanned copy of the form to UCL CTC at ctc.iconic@ucl.ac.uk, and copy in the RNOH lab at roh.biobank@nhs.net
- Check the Safebox is addressed correctly:

RNOH Biobank Team
 Histopathology Department
 UCL Institute of Orthopaedics
 Royal National Orthopaedic Hospital
 Brockley Hill
 Stanmore
 Middlesex
 HA7 4LP

On receipt RNOH will complete section 3 of the *ctDNA Sample Shipping Form* and return to UCL CTC. Any discrepancies or inconsistencies will be raised by UCL CTC with the Site.

APPENDIX 1: ROYAL MAIL SAFEBOX PACKING INSTRUCTIONS

>1



Step one

Place sample in a leak-proof container. Send one 50ml container per pack.

>2



Step two

Wrap container in absorbent material and place in plastic self-seal bag and seal.

>3



Step three

Put the whole package in the clear plastic compartment. Place any documentation in the other compartment.

>4



Step four

Important: Check that all the contents you want to send are inside the package before closing as once it has been closed, it can't be reopened without destroying it.

>5



Step five

Remove the cardboard separator from the lid and dispose of it.

>6



Step six

Place the lid over the top of the container and firmly press shut.

>7



Step seven

Peel the outer backing from the self-adhesive label. Then wrap around the Safebox™.

>8



Step eight

Make sure the package is addressed correctly and the return address has been completed. **It's ready to go.**

>9



Step nine

You can put First Class Safebox™ in any post-box. Safebox™ posted using Special Delivery™ needs to be taken to any Post Office.