



Laboratory Manual

Trial Title: A randomised, multi-centre, biomarker-stratified, open-label trial in patients newly diagnosed with Crohn's disease.

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BLOOD TUBES

2.5 mL PAXgene RNA tube



9 mL white-top serum tube



2.6 mL red-top EDTA tube



SCREENING

Screening – Summary of Samples – Blood, urine and stool

- Blood sample for Biomarker assessment
 - One 2.5 mL PAXgene RNA tube.
- Blood samples for research
 - One 2.5 mL PAXgene RNA tube.
 - One 9 mL white-top serum tube.
 - One 2.6 mL red-top EDTA tube.
- Blood samples for clinical assessment – through your local laboratory
 - Full blood count.
 - Urea & electrolytes.
 - Liver function tests.
 - C-reactive protein.
 - TPMT (if not done in last 3 months).
 - Hepatitis B and Hepatitis C (if not done in last 3 months).
 - Varicella zoster serology (if not done in last 3 months).
 - Tuberculosis blood test (if this is part of local protocols and if not done in last 3 months).
- A urine sample for pregnancy test for women with child bearing potential
- Stool samples

- Participants provided with stool collection pots for unbuffered (faecal calprotectin) and buffered (research) samples which they produce and post from home (see below).

Biomarker assessment

One 2.5 mL PAXgene RNA tube for the Biomarker assessment.

Procedure

The sample bottles will be provided by the PROFILE team and will work with the Vacutainer System. Alternatively blood can be taken into a syringe and then the needle attached to fill blood tubes (please do NOT remove the lid from the PAXgene RNA tube). The sample should be collected with other blood samples at the screening visit. Fill tubes completely.

*Thoroughly mix the blood by **inverting** the tube eight to ten times.*

Labelling

PAXgene RNA tubes will be labelled, please complete participant's trial number date of birth and first and last initial. (see appendix 1)

Screening biomarker request card

Each Screening biomarker blood sample will be accompanied with the PROFILE "Screening Biomarker Sample" request card containing participant's trial number, date of birth and first and last initial and the contact details of delegated PI/research nurse/site staff to be contacted if necessary. (see appendix 2)

Storage

Samples can be stored at room temperature for no longer than 24 hours following collection but it is recommended to *post them on the day they are collected*.

Shipment

Samples should be posted at room temperature on the day they are collected, in the provided plastic postal boxes 'Safeboxes' (see Appendix 5) with pre-paid postage according to UN3373. Please ensure the shipment log is completed and filed in the investigator site file.

Samples should be shipped to:

PROFILE trial
East Anglian Medical Genetics Service
Molecular Genetics Laboratories
Box 143, Level 6 Addenbrooke's Treatment Centre
Addenbrooke's Hospital
Cambridge
CB2 0QQ

Blood samples for Research

The following blood samples for research should be obtained at screening and sent to Addenbrooke's Hospital, Cambridge

- One 2.5 mL PAXgene RNA tube (in addition to the above biomarker sample).
- One 9 mL white-top serum tube.
- One 2.6 mL red-top EDTA tube.

Procedure

The sample bottles will be provided by the PROFILE team and will work with the Vacutainer System. Alternatively blood can be taken into a syringe and then the needle attached to fill blood tubes (please do NOT remove the lid from the PAXgene RNA tube). Samples should be collected with other routine blood samples. Fill tubes completely.

*Thoroughly mix PAXgene & EDTA tubes by **inverting** each eight to ten times.*

Labelling

Serum, EDTA and PAXgene RNA tubes will be pre-labelled in an anonymised manner and identified using the participant's trial number. The relevant blank details will need to be completed, including date of sample. (see appendix 1)

Research samples request card

The Research blood samples will be accompanied with the PROFILE Blood Sample Card containing participant's trial number, date of birth and first and last initial and the contact details of site staff/research nurse to be contacted if necessary. (see appendix 2)

Storage

Samples can be stored at room temperature for no longer than 24 hours following collection but it is recommended to *post them on the day they are collected*.

Shipment

Samples should be posted at room temperature on the day they are collected, in the provided plastic postal boxes 'Safeboxes' (see Appendix 5) with pre-paid postage according to UN3373. Please ensure the shipment log is completed and filed in the investigator site file.

Samples should be shipped to:

PROFILE trial
East Anglian Medical Genetics Service
Molecular Genetics Laboratories
Box 143, Level 6 Addenbrooke's Treatment Centre
Addenbrooke's Hospital
Cambridge
CB2 0QQ

Blood samples for clinical assessment

The blood tests required for screening and are to be processed through local laboratories:

- Full blood count
- Urea & electrolytes
- Liver function tests
- C-reactive protein
- Thiopurine methyltransferase (TPMT)
- Hepatitis B, Hepatitis C
- Varicella zoster serology
- Tuberculosis blood test (only where this is part of local protocols for patients starting on anti-TNF therapy).

These should be sent to your local laboratory and processed according to local policies and procedures. Where TPMT, Hep B / C, varicella and TB tests have been done within 3 months these do not need to be repeated.

Screening tests will be performed in accordance with local policies and procedures. Blood samples should ideally be taken at a single time point to minimise burden on the participant.

The local trials team are responsible for tracking and confirming receipt of all local results. The laboratory order sets and normal reference ranges should be filed in the participants patient notes/trial notes section as appropriate.

URINE

A urine pregnancy test will need to be performed at Screening in women of child bearing potential (use local kits, as per usual practice).

STOOL

Two stool samples are required.

- Faecal calprotectin (no buffer – blue top) and
- Research stool sample using the DNA Genotek kit (Purple top - contains buffer liquid).

These samples should be produced at home after the screening visit **and sent to the trial team in “Safeboxes” provided with prepaid postage.**

Pots

One blue-top plastic sample pot with collection spoon – no buffer – for Faecal Calprotectin.



One DNA Genotek Purple-top plastic sample pot with buffer solution and collection spoon – for research.



Faecal calprotectin stool sample – no buffer

Procedure

The stool sample will be produced at home by study participants and sent to the trial team in "Safeboxes" with prepaid postage. This should take place as soon as possible after the visit.

The study participant needs to place approximately five grams (roughly 5 mL = a teaspoon) of stool into the blue-top plastic sample pot with no additive.

The participant will need clear verbal instructions at the trial visit (written instructions will also be contained in the 'Stool sample packs' provided).

Participants should contact their local trial team/research nurse to advise of date the sample was posted. If no call is received within 7 days of the respective trial visit, the local trial team will get in touch with the participant and encourage the sample be produced and sent.

Labelling

Stool sample specimen pots (and request cards) will need to be labelled with the participant's trial number prior to them being given to the participant to take home. *Participants should write on the tube the date the sample is produced.* (see appendix 1)

Faecal Calprotectin Card

Each stool sample will be accompanied with a PROFILE faecal calprotectin request card containing participant trial ID, date and time of the faecal calprotectin stool sample collection. (see appendix 4)

Shipment

Stool samples should be posted at room temperature on the day they are produced, in the provided plastic postal boxes 'Safeboxes' (see Appendix 5) with pre-paid postage according to UN3373. Where two samples are required buffered (DNA Genotek) and unbuffered (blue top bottle) samples should be placed in the same Safebox. Subjects should notify their research team once they have posted the stool samples and this info should be filed in the investigator site file

Samples should be shipped to:

PROFILE trial,
Laboratory 05.830, Department of Medicine, Box 157,
Level 5 Addenbrooke's Hospital,
Cambridge Biomedical Campus,
Cambridge,
CB2 0QQ.

Research stool sample – DNA Genotek kit, with buffer

Procedure

The stool sample will be produced at home by study participants and sent (together with the unbuffered stool sample) to the trial team in "Safeboxes" with prepaid postage. This should take place as soon as possible after the screening visit.

The study participant needs to place approximately five grams of stool (roughly 5 mL = a teaspoon) into the Purple-top plastic sample pot with buffered solution.

The participant will need clear verbal instructions at the trial visit (written instructions will also be contained in the 'Stool sample packs' provided).

Participants should contact their local trial team/research nurse to advise of date the sample was posted. If no call is received within 7 days of the respective trial visit, the local trial team will get in touch with the participant and encourage the sample be sent.

Labelling

Stool sample specimen pots (and request cards) will need to be labelled with the participant's trial number prior to them being given to the participant to take home. *Participants should write on the tube the date the sample is produced.* (see appendix 1)

Shipment

Stool samples should be posted at room temperature on the day they are produced, in the provided plastic postal boxes 'Safeboxes' (see Appendix 5) with pre-paid postage according to UN3373. Where two samples are required buffered (DNA Genotek) and unbuffered (blue top bottle) samples should be placed in the same Safebox. Subjects should notify their research team once they have posted the stool samples and this info should be filed in the investigator site file.

Samples should be shipped to:

PROFILE trial,
Laboratory 05.830, Department of Medicine, Box 157,
Level 5 Addenbrooke's Hospital,
Cambridge Biomedical Campus,
Cambridge,
CB2 0QQ.

SAMPLES REQUIRED AT SUBSEQUENT VISITS

See below for details of monitoring bloods, and blood samples for research.

Where stool samples are specified participants should be given the appropriate stool collection pots at the visit. *The stool sample will be produced at home by study participants* as soon as possible after the visit and sent to the trial team in "Safeboxes" with prepaid postage. Methodological details for Faecal calprotectin and for Research stool samples are as described above under 'screening visit'. The week 48 stool samples should be produced and posted prior to taking bowel prep for the end of trial colonoscopy.

Baseline

- Monitoring bloods.

Week 4

- Monitoring bloods.

Week 16

- Monitoring bloods.
- Blood samples for research.
- Stool sample for Faecal calprotectin only (blue top / no buffer).

Week 32

- Monitoring bloods.
- Blood samples for research.
- Stool sample for Faecal calprotectin only (blue top / no buffer).

Week 48

- Monitoring bloods.
- Blood samples for research.
- Stool samples for Faecal calprotectin (blue top / no buffer) and Research DNA Genotek (Purple top, with buffer).

Monitoring blood tests

The following monitoring blood tests are required at Baseline, Week 4, Week 16, Week 32, Week 48, and ad-hoc visits. They should be sent to your local laboratory and processed according to local policies and procedures:

- Full blood count,
- Urea & electrolytes,
- Liver function tests,
- C-reactive protein,

- Thiopurine metabolites (6 thioguanine nucleotide and 6 methyl mercaptopurine) - for subjects on azathioprine or low-dose mercaptopurine with allopurinol.

Monitoring blood tests will be performed in accordance with local policies and procedures. Blood samples should ideally be taken at a single time point to minimise burden on the participant.

The local trials team are responsible for tracking and confirming receipt of all local blood results. The laboratory order sets and normal reference ranges should be filed in the participants patient notes/trial notes section as appropriate.

Blood samples for Research

The following blood samples for research should be obtained at weeks 16, 32 and 48.

- One 2.5 mL PAXgene RNA tube.
- One 9 mL white-top serum tube.
- One 2.6 mL red-top EDTA tube.

Where possible please avoid doing these visits / posting samples on a Friday.

Procedure

The sample bottles will be provided by the PROFILE team and will work with the Vacutainer System. Samples should be collected with other routine blood samples. Fill tubes completely.

*Thoroughly mix PAXgene & EDTA tubes by **inverting** each eight to ten times.*

Labelling

Serum, EDTA and PAXgene RNA tubes will be pre-labelled in an anonymised manner and identified using the participant's trial number. The relevant blank details will need to be completed, including date of sample. (see appendix 1)

Research samples request card

The Research blood samples will be accompanied with the PROFILE Blood Sample Card containing participant's trial number, date of birth and first and last initial and the contact details of site staff/research nurse to be contacted if necessary. (see appendix 2)

Storage

Samples can be stored at room temperature for no longer than 24 hours following collection but it is recommended to *post them on the day they are collected.*

Shipment

Samples should be posted at room temperature on the day they are collected, in the provided plastic postal boxes 'Safeboxes' (see Appendix 5) with pre-paid

postage according to UN3373. Please ensure the shipment log is completed and filed in the investigator site file.

Samples should be shipped to:

PROFILE trial
Laboratory 05.830, Department of Medicine, Box 157,
Level 5 Addenbrooke's Hospital,
Cambridge,
CB2 0QQ.

Appendices

Appendix 1 - Labels

Screening Biomarker Assessment Label

Trial Number: XXXX – XXX	
DOB: __ / __ / __	Initials: _ _
PROFILE – Biomarker	First & last only

Paxgene Research Sample Label

Trial Number: XXXX – XXX	
Collection date: __ / __ / __	
PROFILE – Paxgene	

EDTA Research Sample Label

Trial Number: XXXX – XXX	
Collection date: __ / __ / __	
PROFILE – EDTA sample	

Serum Research Sample Label

Trial Number: XXXX – XXX	
Collection date: __ / __ / __	
PROFILE – serum sample	

Stool Sample In Pot With No Additive Solution




Trial Number: XXXX – XXX	
Collection date: __ / __ / __	
PROFILE – Unbuffered stool sample	

Stool Sample In Pot With Buffered Solution




Trial Number: XXXX – XXX	
Collection date: __ / __ / __	
PROFILE – Buffered stool sample	

XXXX-XXX denotes the trial number.




Appendix 2 – Screening biomarker assessment request card

 UNIVERSITY OF CAMBRIDGE  PROFILE 		
Screening Biomarker Sample		
PROFILE participant ID		INVESTIGATION REQUIRED
		IBDhi / IBDlo Status
Participants initials (first and last only)		LABORATORY TO REPORT RESULTS TO:
Blood sample time (hh:mm)		add-tr.profile@nhs.net

Appendix 3 - Research samples request card

 UNIVERSITY OF CAMBRIDGE  PROFILE 		
Research Samples		
PROFILE participant ID		PROFILE TRIAL
Blood sample collection date (dd/mmm/yyyy)		{Participating site investigator email contact information}
Blood sample time (hh:mm)		

Appendix 4 - Faecal calprotectin request card

				Cambridge University Hospitals  NHS Foundation Trust	
Research Analysis Request - Faecal Calprotectin					2059
PROFILE Trial Number	PROJ2059 N_ _ _ - _ _ _	Consultant: Miles Parkes			
Date of Birth		Project 2059			
Gender					
Sample collection date (dd/mm/yyyy)		Contact Name: Francis Dowling Daytime Number: 01223-254666			
Sample time (hh:mm)					
Faecal Calprotectin	LAB2785	Participating site: {email contact information for result}			
Cambridge University Hospitals NHS Foundation Trust Pathology Projects 01223 216925 / 0333-103-2220 (Helpdesk) V1 – 22/11/2017					

Appendix 5 - Safebox

safe and sound...


Safebox™: the only pre-paid and secure packaging solution for sending specimens.



When you send specimens, you want them to arrive safe and sound. Safebox™ fits the bill.

Safebox™ from Royal Mail has been specifically designed to provide an easy-to-use, all-in-one secure package for anyone wishing to send specimens. Doctors, vets, universities, researchers, police forces and pharmaceutical companies all over the country have found Safebox™ offers everything they need in one handy pre-paid box.

- > **Everything included:**
Postage, packaging, absorbent wadding, self-seal bag that can be written on, "O" ring to ensure inner container is water tight, address and return label.
- > **Easy to use:**
Quick to put together, simple to take apart, full instructions in every pack.
- > **Provides an element of safety for employees:**
Tamper evident seal eliminates danger of contamination for employees.
- > **Choice of posting options:**
Every Safebox™ comes with pre-paid postage and the address labels attached. Choose from First Class or Special Delivery™.



- Tamper evident seal >
- Absorbent wadding inside >
- Address label >

Safebox™ was developed by working closely with healthcare professionals, so you can be sure it's designed with your health and safety in mind.

- > **Compliant with legislation:**
Fully compliant with packing instruction 650
Fully compliant with the requirements of UN3373
Compliant with the performance tests of packing instructions 602.
- > **Virtually indestructible:**
Passes drop test, puncture test and pressure test.
- > **Small, safe and reduces risk:**
Requires less manual handling than with other packaging reducing the risk of accidental spills. As it is single use, it prevents contamination of contents.

Safebox™ step by step

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

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

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

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.

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

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

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

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

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.

Safebox™ saves you time, money and storage space. Simply order the quantity you need, as and when you need it.

Safebox™ is the only UN3373 compliant medical solution to include postage. With a minimum order of just **12 Items**, and a delivery turnaround target of just 24 hours, it will:

- > **Save time:** Easy to use and only takes a few seconds to get a sample ready for posting.
- > **Save money:** No hidden costs or additional items to include.
- > **Save on storage space:** Delivered in cartons of 12 or 48 and can be stored flat, saving shelf space.
- > **Choice of posting options:** Every Safebox™ comes with pre-paid postage and the address labels attached. You simply choose the delivery option that's easiest for you.
 - **First Class:** A delivery target of next day. Safebox™ can be posted in any post-box, so even after your mailroom has closed for the night, you can catch the last post.
 - **Special Delivery™:** Guaranteed next working day delivery or your money back. Incorporates the Royal Mail Track and Trace facility. Good for time-sensitive samples.

Both First Class and Special Delivery™ Safeboxes can also be included in your usual business collection.

Stored flat

Quick and easy assembly

No hidden costs

First Class

Special Delivery™ next day

Fully recyclable

Safebox™: the technical details

- > **Capacity**
 - 2 x 30ml universal containers
 - 4 x 9ml vacuettes
 - 4 x 7.5ml monovettes
 - 6 x 2.7ml monovettes
 - 5 x 2.5ml glass vials/bottles
 - 4 x 9ml serum containers
 - 3 x 15ml vacuameters
 - 1 x 50ml square tissue box (must be placed upside down)
 - 2 x slide boxes
- > **Materials**
 - Lid: Polypropylene
 - Inner chamber: Polycarbonate
 - Base: Polypropylene
 - Self seal bag
 - Plastic O-ring
 - Absorbent material
 - Cardboard separator (To prevent accidental closure)
 - Pre-paid label
- > **Dimensions**
 - Height: 175mm
 - Width: 138mm
 - Depth: 30mm

(All dimensions are approximate)
- > **Packing instructions**
 - Fully compliant with Packing Instructions 650
 - Fully compliant with the requirements of UN3373
 - Compliant with the performance tests of Packing Instructions 602
 - Meets all the 602 performance criteria except 100mm in the smallest dimension*

Safebox™ is available in boxes of 12 and 48.
Order yours from Royal Mail by calling **0845 778 2677**.

* The UN mark cannot be given to Safebox™ simply because it is too small to meet the minimum size requirements, but makes it perfect for you as it takes less storage space.