FORECEE SAMPLE COLLECTION SOP

1. Purpose of SOP

This SOP describes the standard operating procedures detailed in the FORECEE Study protocol (REC 14/LO/1633; IRAS 53431). Samples will be stored under the custodianship of the UCL Biobank (10/H1306/42), as part of on-going collection (*NC09.13 - Prospective collection and analysis of tissue specific biological samples for translational research of women specific cancer*).

We aim to recruit from four different groups of women:

a. Women who have just been diagnosed with either breast, ovarian or endometrial cancer.
b. Women with a *BRCA1* or *BRCA2* mutation or Lynch syndrome with no disease at present.
c. Women with benign breast or gynaecological conditions.
d. Healthy volunteers from the general population.

A key recruitment target for 4C is the inclusion of 'poor prognostic' cancer cases. Table 1 below provides details poor prognostic cancer types in more detail. Wherever possible please focus recruitment on poor prognostic cancer types.

CANCER	POOR PROGNOSTIC CRITERIA
Endometrial Cancer	• Grade 3 endometrioid • Grade 3 clear cell • Grade 3 serous
Ovarian Cancer	• High Grade Serous • Endometrioid • Mucinous • Clear Cell
Breast Cancer	• Grade 3 of any type • Any node positive • Any ER/PR negative • Any type where the tumour size is ≥ 2 cm

Table 1 Table of poor prognostic conditions that should be prioritised during collection

2. Patient Recruitment

Patients and research volunteers will be identified by the local research and/or clinical team and invited to dedicated research clinics or approached in the clinical setting. The purpose of the study is explained to the patient. Patients will be given adequate time to understand and absorb the information contained in the Patient Information Sheet and will be given the opportunity to ask questions about the research. Consent will only be obtained if freely given. Should the patient wish to participate in the study they receive a copy of the study information sheet (Participant Information Sheet, V4) and a consent form to sign (Consent Form, V4). Once the consent form is signed the patient receives a copy of this form and a second copy is added to the patient's notes (if appropriate/applicable at the local collection centre). **Note the importance of appending a 4C Participant barcode to the retained consent form** (refer to section 4 below). The patient is also asked to answer an epidemiological questionnaire which is filled out via an app called 'Qualtrics' on an iPad (NOTE: Paper questionnaires will not be accepted).

3. Collection kit components

Unless cytology is being performed locally at your centre each kit contains the following items[1]:

- 2 Participant labels: One to be added to the patient paper consent form and 1 spare
- A pre-labelled LBC Thinprep pot (note this may be omitted from the kit if you are performing cytology locally)
- One pre-labelled 2.5ml PAXgene Blood DNA tube
- 2 x pre-labelled 'Copan' flocked buccal swabs

4. Labelling explanations

All collection components are labelled with a 2D barcode label that includes the 'participant' ID number circled in red in Figure 2a below. This number has an 'ST' prefix followed by an 8 digit code and is also visible on all sample labels as well as the 'Participant' label. All labels also have a collection centre code. In the example below this is 4C-UCLH for standing for: 4C – University College London Hospital.

Append the 'Participant' label to the consent form when recruiting patients (note spare labels are provided in the event of using paper questionnaires however **we strongly encourage** the use of the provided iPad to fill out the questionnaire directly using the Qualtrics application). Key documents and online surveys are available at: www.forecee.info. The current version of the questionnaire is sign (Non-standardised Questionnaire, V4).

The Participant ID 'ST code' is also entered into the Qualtrics app when performing the online questionnaire (Figure 2b). Note that it is imperative that each collection centre constructs and maintains a 'link' file that will link the Participant ID with a patient identifier e.g. hospital number so that pseudo-anonymised clinical data can be retrieved from partners as required. The link file **is therefore very important** and should be stored securely and regularly backed up in line with the specific institution's policy for data protection.

[1] Note if cytology is being performed locally at GP clinics a Liquid Based Cytology (LBC) pot will not be provided however a sample label will be provided that should be appended to the LBC pot used for cervical screening.

Figure 2a – Explanation of FORECEE participant & sample labelling

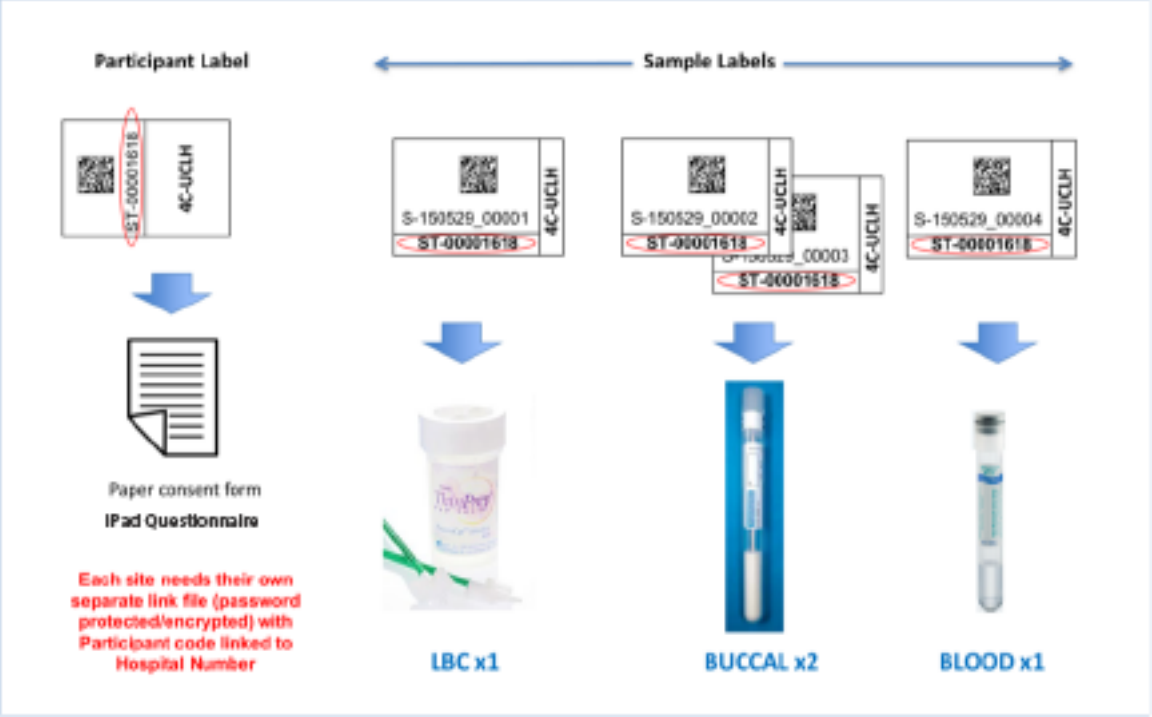
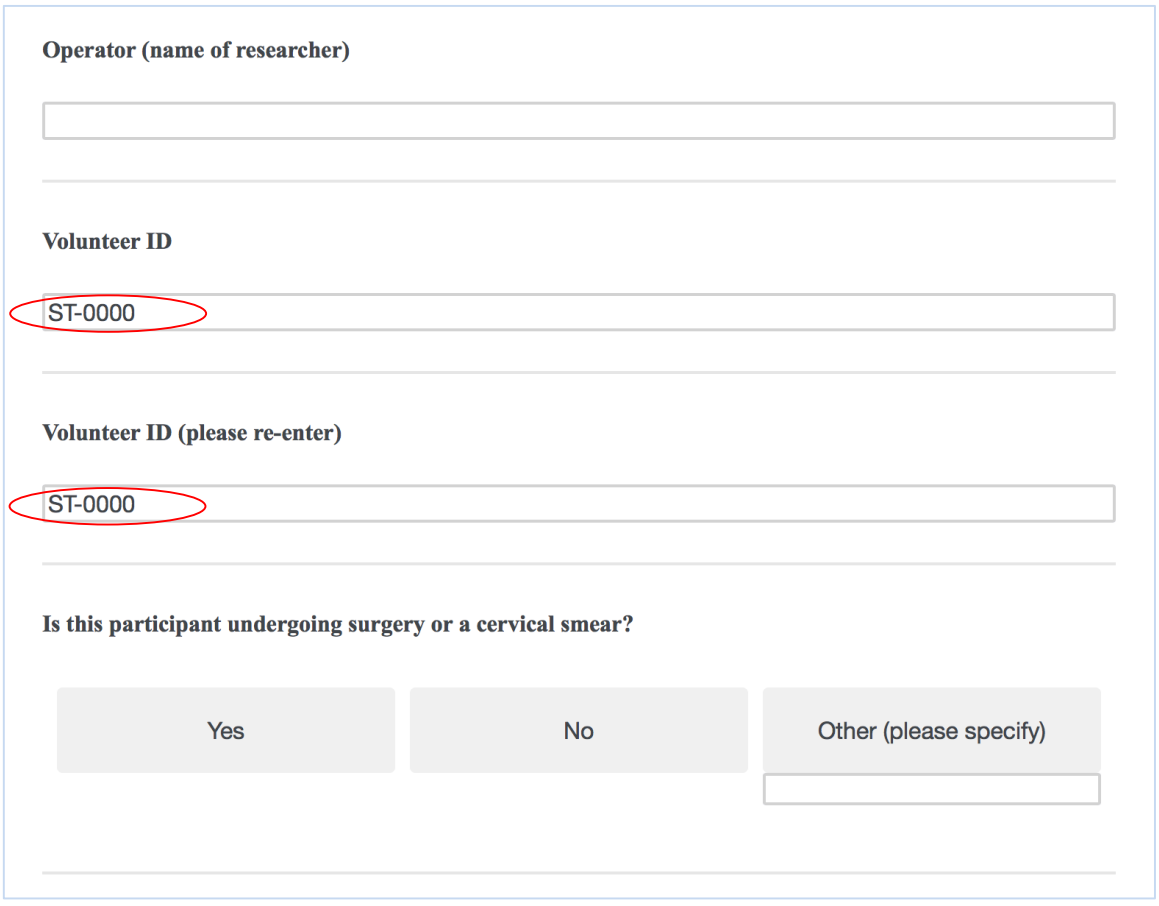


Figure 2b – The Qualtrics online questionnaire with data entry fields for the Participant ID.



5. Collection Inclusion/Exclusion Criteria

Please adhere to the following conditions when recruiting participants.

1. The participant should consent and agree to give all 3 required tissue types i.e. LBC cervical smear, blood and buccal. **If the participant does not wish to give all 3 tissue types they should not be recruited for the study**.
2. Aim to schedule the appointment to **avoid menstruation** at LBC sampling.
3. It is advised that if the patient **should not have had a cervical smear within the 3 months** prior to recruitment and sampling.
4. The participant should have **no history of hysterectomy.**
5. The participant should be **at least 18 years of age – there is no upper age limit**.
6. The participant should have **no prior history of ANY cancer** except non-melanoma skin cancer.
7. The participant should not be recruited if they have started a **current regime of chemotherapy or have received chemotherapy for cancer in the past**. Please exclude patients with Primary Chronic Polyarthritis (PCP) as they tend to be treated with methotrexate which affects DNA methylation.

6. Cervical smear collection

Obtain the specimen in accordance with ThinPrep collection instructions (please refer to the 4C Instructional Video available at www.forecee.info).

a. Water-soluble gel lubricant sparingly applied to the posterior blade of the speculum can be used if necessary[2]. Note: avoid adding gel to the tip of the speculum. Insert the central bristles of the Rovers cervix brush (Cat number:70671-001) into the endocervical canal deep enough to allow the shorter bristles to fully contact the ectocervix. Push gently, and rotate the broom in a clockwise direction five times.

b. Rinse the brush as quickly as possible into the PreservCyt solution vial by pushing it into the bottom of the vial 10 times, forcing the bristles apart. As a final step, swirl the brush vigorously to further release material. Discard the collection device.

c. Tighten the cap so that the torque line on the cap passes the torque line on the vial.

d. Ensure that the Participant number on the LBC pot is recorded against a local patient identifier in your site specific link file.

e. Ensure you have recorded the date the sample was taken in your link file.

f. Store the LBC pot at room temperature or at 5oC if fridge space allows (we recommend the latter simply in order to keep all participant samples in the original kit bag provided). in a secure local collection facility until shipment to UCL. Each shipment must contain a sample inventory list of sample codes (e.g. S-130930-00001) and corresponding sampling date.

[2]Cervicovaginal Cytology Based on the Papanicolaou Technique; Approved Guideline – Third Edition (Clinical and Laboratory Standards Institute GP15-A3).

7. Buccal swab collection

NOTE! The person providing the buccal cell samples should not eat or drink immediately prior to giving the sample. Recommendations are that no food is consumed 30 minutes prior to sampling. If food has been eaten within 30 minutes before sampling then it is recommended that the volunteer swills their mouth with water before a sample is attempted.

Note **2 swabs** are provided per subject: one for each cheek. There is **no need** to label these 'Left' or 'Right' as they will be processed and pooled prior to testing at the lab.

a. Open the swab by twisting the cap.

b. Carefully remove the swab from the tube rub the cotton bud firmly against the inside of the cheek in an up and down motion at least 5-6 times. **Ensure that the strokes used are long, moving high up and down the inside of the cheek. This can be facilitated by placing your free hand against the side of the participants face against the cheek being sampled.**

c. After taking the sample carefully replace the swab back into the pre-labelled collection tube and **push the cap firmly downwards to close the tube securely nb. When the tube is securely closed an audible 'click' will be heard.**

d. Repeat the sampling procedure with the second swab using the other cheek.

e. Ensure you have recorded the date the sample was taken in your link file.

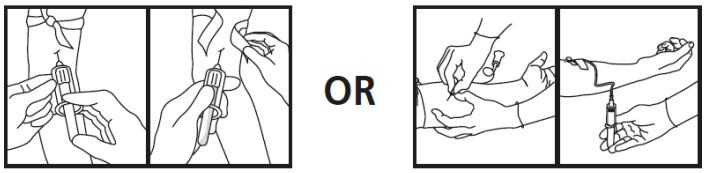
f. Store the buccal swab at room temperature or at 5oC if fridge space allows (we recommend the latter simply in order to keep all participant samples in the original kit bag provided) in a secure local collection facility until shipment to UCL. Each shipment must contain a sample inventory list of sample codes (e.g. S-130930-00001) and corresponding sampling date.

8. PAXgene Blood Tube Collection

Blood is drawn directly into the PAXgene Blood DNA tubes via standard phlebotomy technique. Note needles and vacutainer holders are not provided in the kit.

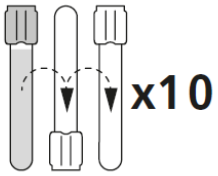
a. Ensure that the PAXgene blood tube is at room temperature (18-25^{o}C) prior to use

b. Collect blood into the PAXgene Blood DNA Tube using your institution's recommended procedure for standard venipuncture technique:



c. Ensure the blood has stopped flowing into the tube before removing the tube from the holder

d. After blood collection gently invert the PAXgene blood tube 8-10 times



e. Record the date and time of blood sampling (for ease this can be added to the link file but can be recorded in a separate dedicated file if required, e.g. excel format)

f. Blood MUST be stored at 2–8°C and for a **maximum of 20 days** before shipment to UCL in a secure local collection facility until shipment to UCL. Each shipment must contain a sample inventory list of sample codes (e.g. S-130930-00001) and corresponding sampling date.

9. Sample Storage

After taking all the participant samples ensure that all are returned to the bag from which they were removed (the bag is labelled with the ST-participant code). Sample bags containing the specimens **should be stored locally at** 5oC for up to a maximum of 20 days before return to the IfWH lab at 72 Huntley Street for processing.

10. Shipment

For details of packaging and shipment please refer to the appropriate protocol:
FORECEE Shipping SOP_International_V4_100217 (For import of shipments **into the UK**)
FORECEE Shipping SOP_Domestic_V6_100217 (For domestic shipments **within the UK**)

11. Follow Up

Clinicopathological update within 6 weeks

All cases (referrals to colposcopy clinic, breast, ovarian and endometrial cancer cases) require an update on minimum clinical data (where applicable) **within 6 weeks after recruitment maximum**:

- Histology
- Stage - if applicable
- Grade - if applicable
- ER status - if applicable
- PR status - if applicable
- HER2 status - if applicable

Further questionnaires

Participants may be sent further questionnaires at regular intervals. All volunteers will be sent a repeat questionnaire 1, 5 and 10 years after joining the study. The questionnaire will ask for details of any hospital admission or serious illness since registration with the study. Where necessary further efforts to contact volunteers will be made by telephone or via the general practitioner.

Local cancer registry

If available, all research participants should be registered with a national Central Registry. This will enable a notification to the study directors in the event of new cases of cancer and deaths from cancer in any individual.

12. Contact for queries

For general collection based queries including kit provision:

Allison Jones (Senior Research Associate)
Institute for Women's Health
UCL
Paul O'Gorman Building
72 Huntley Street
London WC1E 6DD
Tel: + 44 203 108 2001
Email: allison.jones@ucl.ac.uk

Taraneh Ghazali (Clinical Research Practitioner)
Address as above
Tel: +44 203 108 2002
Email: shohreh.ghazali.12@ucl.ac.uk

For R&D/Ethics issues & Qualtrics-related queries:

Dan Reisel (NIHR Clinical Research Fellow)
Address as above
Tel: +44 203 108 2003
Mob: 07870590036
Email: d.reisel@ucl.ac.uk