ESCAPE Laboratory Manual V3 29-05-26

Trial Title	ESCAPE: Exacerbation and Symptom Control After Pseudomonas Eradication Treatment in Adult Bronchiectasis: a multicentre randomized controlled trial
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Associated Documents	ESCAPE Research Sputum Sample Log ESCAPE Excess Clinical Sputum Sample Log ESCAPE Sputum Postal Form ESCAPE Sputum Sample Postage Instructions

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1. Procedures

- Ensure all equipment used is within expiry date
- Obtain sputum samples as per local Standard Operating Procedure.
- Dispose of all clinical equipment as per local policy.

2. Equipment

Sputum

- Sputum sample pots (provided)
- Sample labels (provided)
- Sample logs (provided)
- Storage boxes for excess clinical sputum (provided)
- Postage boxes for research sputum samples (provided)
- Pre-paid postage boxes with labels for fresh sputum sample shipment (provided)
- -80°C freezer (for optional excess sputum sample storage)
- 2 ml tubes for aliquoting excess sputum sample storage and additional excess sputum sample labels (if requested)

3. Sample ID

- Sample ID is made up of:

  ES – site number – participant number – visit number

  e.g. sample ID ES-01-01-02 would be site 01 - participant 01- visit 02

4. Sample collection

Two sputum samples will be collected at visits 2, 4 5 & 6:

- One clinical research sample for *P. aeruginosa* testing at local NHS labs (excess sample to be stored at site, if possible).
- One research sputum sample which will be shipped fresh to the research team laboratory for analysis (either posted from site on day of visit or posted by participant post-visit).

Samples highlighted in **blue** should be carried out using local NHS Lab facilities

Visit	Clinical sputum sample for NHS labs		Research sputum sample
1* Screening	Sputum: • *P. aeruginosa* testing • Store excess at -80°C if possible	 Min 0.5 g	NA
2 Day 0	Sputum: • *P. aeruginosa* testing • Store excess at -80°C if possible	 Min 0.5 g	 Min 0.5 g
3 Week 2 (phone call)			
4 Week 12	Sputum: • *P. aeruginosa* testing • Store excess at -80°C if possible	 Min 0.5 g	 Min 0.5 g
5 Week 52	Sputum: • *P. aeruginosa* testing • Store excess at -80°C if possible	 Min 0.5 g	 Min 0.5 g
6 Week 104	Sputum: • *P. aeruginosa* testing • Store excess at -80°C if possible	 Min 0.5 g	 Min 0.5 g

* Visit 1 clinical sputum sample is not needed if the participant has met the inclusion criteria and had a new positive *P. aeruginosa* sputum or other respiratory tract sample (e.g. bronchoalveolar lavage samples) within the 6 months prior to randomisation. If a participant does not have a positive sputum sample within 6 months prior to randomisation, the investigator may send a sputum sample at the screening visit.

5. Sample Processing

COLLECT	LABEL	PROCESS
 Clinical sputum sample: collect excess sputum from NHS labs after *P. aeruginosa* testing (only if possible at each site)		 If possible, freeze any excess clinical sputum sample at -80°C at site until end of trial. This is not a site requirement.
 Research sputum sample		 If the participant is able to produce a second research sputum sample during the visit, the fresh sputum sample will be posted from site to the research team laboratory on the day of the visit. Put the research sputum sample in the postage box provided and affix the postage label. Post the sample on day of collection in the morning (Monday-Thursday). If the sample is collected after the last post of the day, refridgerate at 4-10°C and ship the next morning (max. 24h). If a participant is unable to produce two sputum samples during the visit, the research team will provide the participant with a labelled sputum collection pot, a postage box where the research team have stuck the address label on, the ESCAPE Sputum Postal Form (with the first section completed by the research team) and the ESCAPE Sputum Sample Postage Instructions. The participant will post their research sputum sample from home. Please see postage instuctions below.

6. Labelling Research Samples

- All study samples should be labelled with the sample labels provided.

ESCAPE Research Sputum

ES-_ _ - _ _ - _ _
Date: _ _ - _ _ - _ _ _ _

- Ensure labels are attached **prior** to posting, freezing or giving the sputum pot to the participant. Ensure that the label is **firmly rubbed** on to the tube to prevent it coming off.
- Complete each label with full sample ID and date. Ensure label is completed **prior** to freezing and appropriate cryo-marker pen is used.
- Date to be filled in day/month/year i.e. 30-05-2022
- All samples must be logged in the ESCAPE Research Sputum Sample Log.

7. Postage of Fresh Research Sputum Samples

Fresh research sputum sample from sites/participants home to research team

- Samples should be posted on the day of collection.
- Prepare the sputum by:
    - Wrap the sample pot in the absorbent material, place in the small grip seal bag and seal it.
    - Place the grip seal bag with the sample in it, in the larger PathoSeal bag. Seal the bag.
    - Put the sample in the box.
    - Label the box with the postage label provided.
    - Fill in the Sputum Postal Form, with the date and time the sputum was produced.
    - Put the Sputum Postal Form in the box with the sample.
    - Close the box, tuck the tab in.
- Visits should be arranged for Monday-Thursday to allow sputum to be posted before the last postage.
- All samples must be accompanied by:
    - Completed ESCAPE Sputum Postal Form (paper)
- Complete the ESCAPE Research Sputum Sample Log including the box number.

- If the participant does not provide a second sputum sample at the visit, the research team will provide the participant with:
    - A constructed postage box with the postal address attached during the visit
    - A pre-labelled sputum pot with their Sample ID
    - ESCAPE Sputum Sample Postage Instructions
    - ESCAPE Sputum Postal Form (part 1 completed)
    - Advise participant to post the sample within 7 days of the visit, on the day the sample is produced.
- The ESCAPE Research Sputum Sample Log, including the box number, will be completed before the end of the visit.

All research sputum samples must be recorded on the ESCAPE Research Sputum Sample Log. This will document whether the research sputum sample was collected during the trial visit or is to be collected post-visit by the participant.

8. Storing Excess Clinical Sputum Samples

If possible, freeze any excess clinical sputum sample at -80°C at site until end of trial.

Excess sputum sample must be stored without any additives.

Label the sputum collection pot provided with the excess sputum sample label provided. After collecting a clinical sputum sample, either:

- Remove the sample volume required for local NHS testing and then freeze the excess, unmodified sputum sample in the sputum pot provided.

Or

- If freezer space is an issue, aliquot any excess, unmodified clinical sputum sample into 2 ml tubes, label with a new excess sputum sample label and freeze aliquots. Additional 2 ml tubes and labels can be provided.

ESCAPE Excess Sputum

ES-_ _ - _ _ - _ _
Date: _ _ - _ _ - _ _ _ _

- The samples should be stored in the storage boxes provided.
- The storage boxes should be labelled with study title, site number, sample type and box number.
- Excess sputum sample collection and storage location must be recorded on the ESCAPE Excess Clinical Sputum Sample Log.

9. Shipping of Excess Clinical Sputum Samples

Frozen sputum samples from sites to research team

At the end of the study the Trial Manager, Tayside Clinical Trials Unit, will organise a courier to transport frozen excess sputum samples on dry ice to the research team laboratory.

- Collection will be arranged for Monday-Wednesday to arrive next day.
- All samples must be accompanied by:
    - Completed ESCAPE Excess Clinical Sputum Sample Log (paper)
    - The Sample Log (scanned copy) should also be emailed to ESCAPE-TM@dundee.ac.uk and rhull002@dundee.ac.uk