Validation of EBUS sampling

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1 Document History and Control

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1.1 Version History

Version	Date	Description
0.1	04/12/2017	First draft
0.2	18/12/2017	Sandi and Shirley's edits
0.3	15/1/2018	Final draft inc permission to sample
0.4	29/01/2018	Updating to include changes made to FNA pilot protocol
0.5	5/2/18	New title and checklist

2 Purpose & Scope

For many patients with lung cancer, endobronchial ultrasound guided fine needle aspiration sampling (EBUS) is the only diagnostic material available. To validate this method of sampling for whole genome sequencing (WGS) this protocol has been devised.

NHS GMCs can opt to take part in a pilot of this technique and the requirements for this are outlined in this document.

3 Roles & Responsibilities

Role	***Responsibility***
Clinical Lead for validation cohort	Nominated NHS GMC individual responsible for ensuring protocol is adhered to; samples are tracked and data is collected and submitted correctly.
Bronchoscopist	The NHS GMC Bronchoscopist is responsible for ensuring adequate material is sampled and all or some is kept fresh for genomic testing
Pathologist or Scientist	Tumour content assessment and ensuring material is appropriately prioritised to best answer the diagnostic questions for the patient.

4 Background to validation cohort

A proof of principle experiment (WGS of eight EBUS samples) had mixed results. Three of the four samples in the first batch showed artefact that made interpretation impossible which may have been due to extraction methods. The repeat experiment with a further

four cases showed that these EBUS samples had a high DNA yield and WGS data demonstrated high tumour purity; an evenness of sequencing coverage and comparable variant calling to fresh frozen solid, lung cancer tissue samples. Therefore a validation to analyse a pilot cohort has now been initiated and NHS GMCs are invited to participate.

Many centres currently put EBUS material into formalin at the bedside in preparation for both immunohistochemical testing and molecular testing. This reduces quantity and quality of DNA that can be extracted. By flushing the needle into PBS rather than formalin and keeping the sample fresh and refrigerated, DNA yield can be optimised. The sample can be split into material for cytological diagnosis including a cell block for immunocytochemistry, and material for molecular testing.

DNA yield from EBUS samples must be sufficient for PCR free Whole Genome Sequencing. Samples with only enough DNA for PCR based library preparation cannot be submitted as part of the validation cohort.

Validation of this sampling method will require approximately 50 cases. The number required may increase during the course of the pilot if initial data reveals unexpected findings that require further work to fully understand. If there are inadequate samples to validate a particular cell preservation medium or a particular extraction technique then the validation cohort may be extended to cover more samples of that particular type.

Samples will be submitted as part of the main programme with a Whole Genome Analysis report returned to the GMC and these samples will count towards the recruitment trajectory and be eligible for funding.

5 General Information

When a sample is submitted as part of this validation cohort then the way the tumour is sampled will be affected as well as how the data is collected and submitted. There is a requirement to track these cases through the Genomics England service desk as outlined below.

For all other aspects the usual guidance applies to how patients should be approached for consenting and how sampling should be carried out. The current sample handling guidance can be found here: https://www.genomicsengland.co.uk/information-for-gmc-staff/sample-handling-guidance/ and guidance on consenting can be found as part of the Genomics Education Programme here: https://www.genomicseducation.hee.nhs.uk/courses/courses/consent-ethics/

5.1 Consenting

This work is considered service development work. The samples are therefore diagnostic samples. The decision on whether there is sufficient material for WGS will take place after arrival of the specimen in the pathology department. The decision on the best use of any DNA retrieved will be taken once the diagnosis and yield of DNA are known. Sampling for DNA can take place in the patient's best interests prior to specific consent for participation in the 100,000 Genomes Project in accordance with the Consensus Statement which can be accessed here: https://www.genomicsengland.co.uk/information-for-gmc-staff/cancer-programme/cancer-mdt-engagement-pack/

5.2 Germline DNA sampling

A germline peripheral blood sample must be taken in an EDTA tube and the extracted germline DNA must be submitted with the tumour DNA sample to UKB for plating as set out in the current Sample Handling Guidance.

5.3 Tumour sampling

Only tumour that has been collected fresh can be submitted for WGS. The tumours must fulfil the eligibility criteria detailed here: https://www.genomicsengland.co.uk/information-for-gmc-staff/cancer-programme/eligibility/

5.4 Data entry

The patient must have a completed Registration file including consent information and a completed sample metadata file and Clinical Sample Test csv file (for tumour content assessment) prior to sample submission to UKB.

5.5 Sample submission

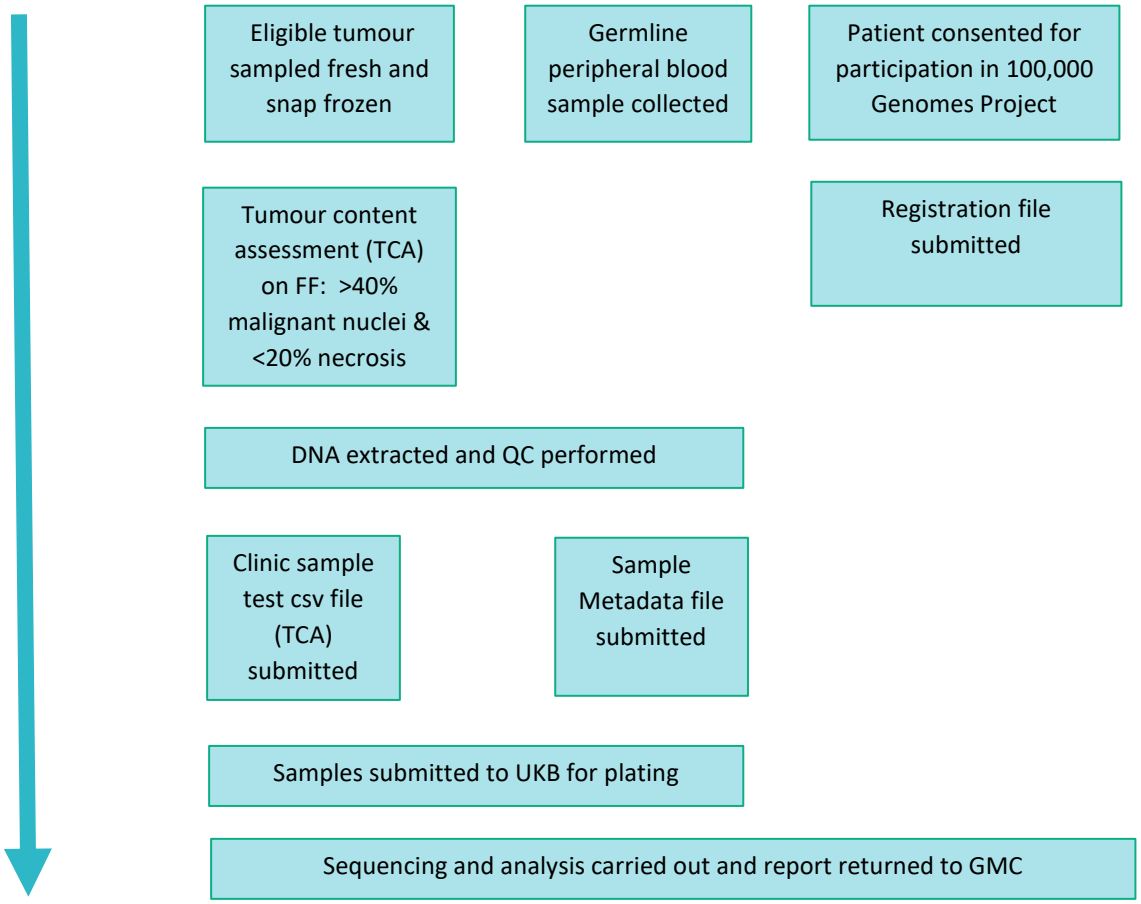
After DNA extraction, samples must be submitted to UKB following the recommendations of the current Sample Handling Guidance. Samples from this pilot should not be a clinical priority for fast or medium track slots, however, if they meet the volume and concentration requirements for fast track cases then they can be included on a fast track or medium track plate. Where possible samples submitted as part of the pilot should fulfil the fast track criteria in order to expedite the validation work.

5.6 Return of Results

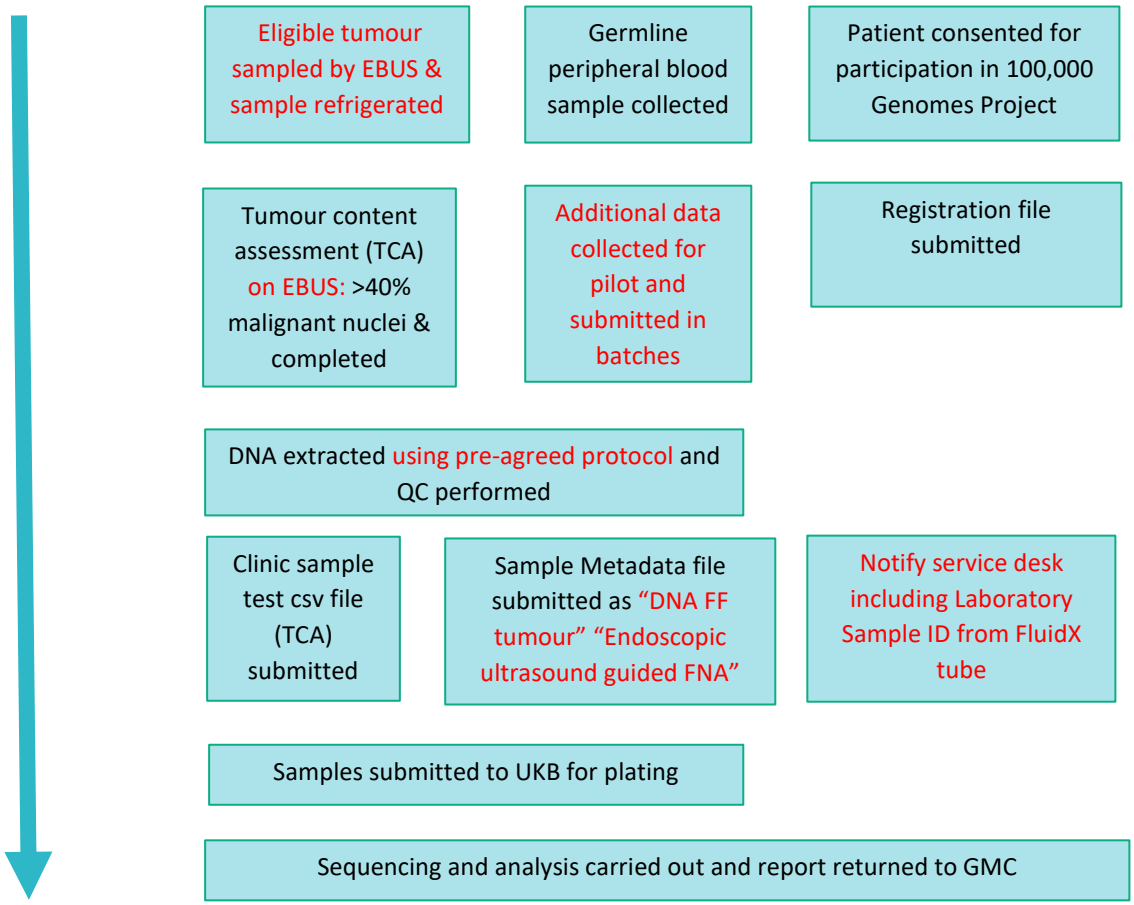
Samples submitted as part of the validation cohort will be processed through the main programme and results will be returned to NHS GMCs via the usual route (cancer interpretation portal). As with all 100,000 Genomes Project cancer cases results should be interpreted and reported in accordance with the NHSE Cancer Validation and Reporting working group guidance document (in preparation).

5.7 Where pilot cases differ from normal practice

Conventional process

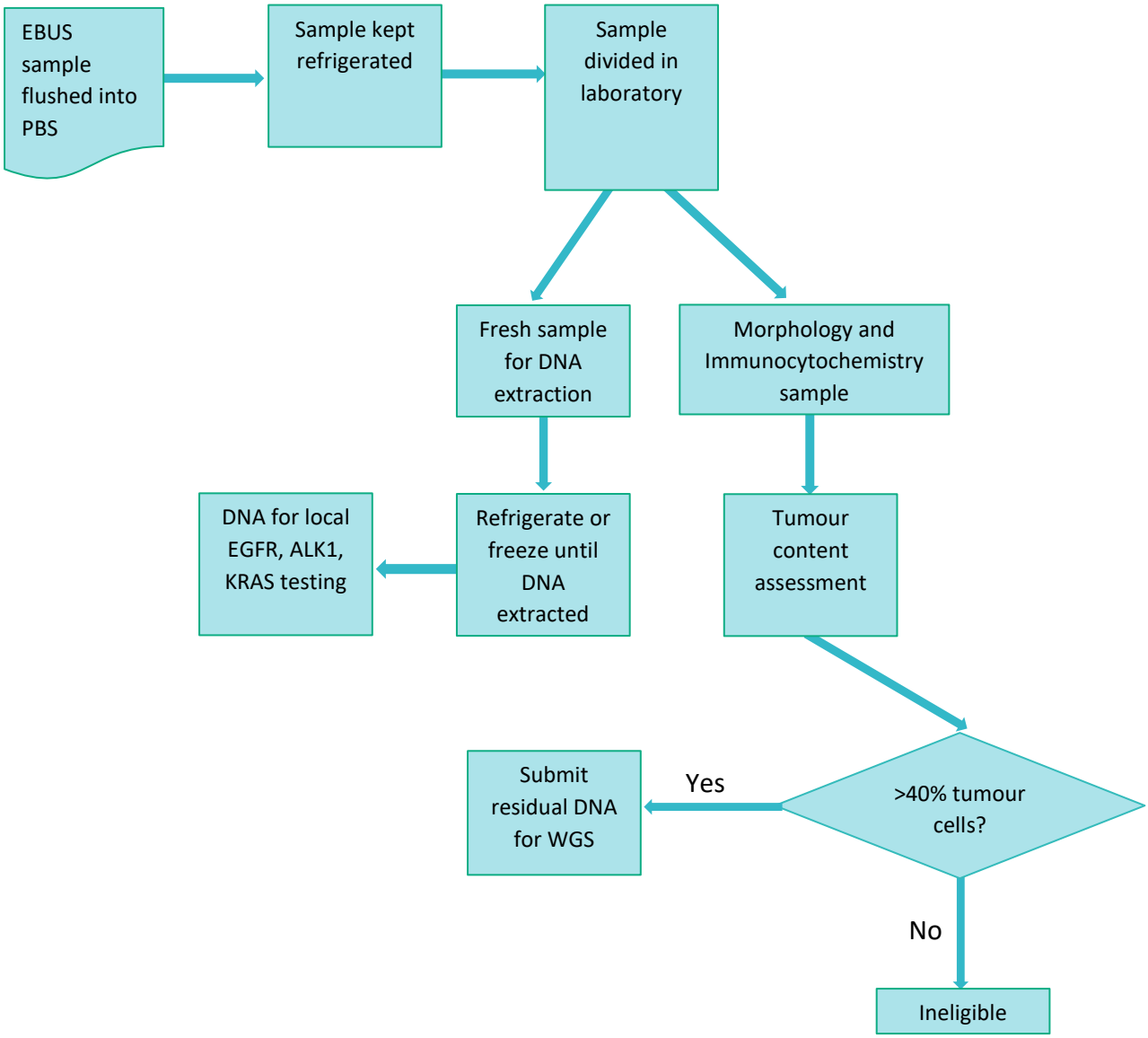


Validation pilot process



6 Procedure

6.1 Process Flow



6.2 Procedural Steps

1. EBUS sampling should be carried out with separate samples collected from each lymph node station.
2. Several passes should be taken from each lymph node station, wherever possible to ensure sufficient material for all diagnostic testing.
3. The needle should be flushed into PBS/RPMI /ThinPrep or equivalent
4. All samples should be kept refrigerated to avoid DNA contamination and to inactivate nucleases.
5. The sample should be split, either at the bedside or in the laboratory, into a genomic sample and a sample for local diagnostic workup.
6. The sample for local workup can be prepared in a variety of ways depending on the wishes of the local pathologist. This sample should be used for morphology, tumour content assessment and immunocytochemistry when required.
7. The genomic sample can be kept refrigerated for up to 72 hours before being frozen or have DNA extracted.

7 Outcomes

In addition to the submission of sample metadata the following data must be collected on each case within the pilot.

1. Participant ID
2. Disease subtype
3. Diagnostic morphology code
4. Collection media used: PBS / RPMI / ThinPrep etc
5. Time refrigerated before freezing / extraction
6. Was the sample frozen?
7. Tumour content assessment
8. DNA yield
9. Local DNA QC metrics

Any NHS GMCs participating in the pilot must submit Standard Operating Procedures for the DNA extraction method they propose to use for cytology samples prior to commencing the pilot. This method must be used for all cases in the pilot. These methods must be submitted to the Clinical Lead for the validation cohort when requesting permission to participate in the pilot.

This data must be sent to the Clinical Lead for the EBUS sampling validation cohort at Genomics England, or after every 10 cases have been sampled whichever is soonest. Genomics England will then assess the following outcomes for these samples:

1. DNA yield

2. Tumour purity
3. Coverage
4. Variant calling rate
5. Pick up rate for known mutations including smoking related genomic signatures

8 Definitions & Abbreviations

Abbreviation / Term	Description
FNA	Fine Needle Aspirate
DNA	Deoxyribonucleic Acid
PBS	Phosphate Buffered Saline
RPMI	Roswell Park Memorial Institute cell culture medium
QC	Quality Control
UKB	United Kingdom Biobank
WGS	Whole Genome Sequencing
NHS GMC	National Health Service Genomic Medicine Centre
FF	Fresh Frozen

9 Data Model Requirements

There are a number of specific requirements around entering sample metadata on these samples since the current data model does not fully support EBUS samples.

Clinic Sample Type should be entered as DNA FF Tumour since there is no DNA FNA Tumour enumeration in the current data model. Laboratory Sample ID from the FluidX tube for the FNA DNA sample should be documented.

Tissue Source should be entered as ‘endoscopic_ultrasound_guided_fna’.

10 DNA Extraction Protocol

EBUS samples can have cohesive sheets of cells so it is important that the homogenisation and lysate steps are completed as for any fresh tumour sample.

The DNA extraction technique used is critical to achieving adequate DNA yield and quality. Extraction must be performed using a kit and protocol which has been recommended by the manufacturer as appropriate for cells pelleted from suspension and using a suitable volume. QC requirements of the resulting DNA are the same as for a fresh frozen tissue sample.

11 Permission to Commence Collection of EBUS samples for the 100,000 genomes cancer programme

Prior to commencing collection of EBUS samples, NHS GMCs will be required submitted a completed checklist (see appendix) and reviewed procedural aspects of the EBUS sample collection and the details of the DNA extraction method they plan to use with Genomics England and NHS England (usually via a teleconference).

12 Notification

Whilst early experimental outcomes for this technique have been very encouraging, this has only been on a limited number of tumours. In order that we may carefully track and monitor the metadata and sequencing metrics on these samples the Genomics England Helpdesk must be notified of each case (ssd.genomics@hscic.gov.uk) with the subject title 'EBUS Sample', the extraction technique used and both the Participant ID and Laboratory Sample ID (for the FNA DNA sample) should be included in the text of the notification.

13 Related Documents, References & Procedures

Sampling of tumours with this technique should adhere to the guidance set out in the current Sample Handling Guidance.

14 Requirements for success

EBUS sampling allows genomic sequencing from cancer patients who otherwise could not get a sequence. The standard that these samples need to reach in order to be of diagnostic benefit therefore needn't be as high as the gold standard. For example, if purity or coverage were not as good as for fresh frozen samples but the samples were still able to pick up mutational signatures with an acceptable degree of sensitivity then there will still be diagnostic value in sequencing these samples.
Results of the validation from each GMC cohort will be shared with the participating GMC once they are available.

Once results are available for the validation cohort as a whole these will be shared with all GMCs.

15 Appendix

Checklist for participation in EBUS validation pilot	
Sample Handling	
Number of cases expected	
Confirmation of which laboratories will process the samples	
Storage and transportation requirements prior to DNA extraction	
Details of proposed DNA extraction method and SOP	
Data capture	
Confirmation of how Pathology Department will record cases including data on ineligible cases and those that fail QC i) Tissue sampling details ii) DNA extraction details	
Designated responsibility for collecting the meta-data	
Sample tracking	
Process for tracking when these patients have been diagnosed, consented to GEL and sample has been retrieved, including responsibility to complete this action	
Confirmed process to notify GEL service desk that a sample has been submitted and associated patient identifier, including responsibility to complete this action	
Teleconferences	
Proposed operators to talk through intended sampling methods	
Clinical Scientist to talk through DNA extraction methods proposed	