VitalBE Laboratory Manual

Title: *Value of inhaled treatment with Aztreonam lysine in bronchiectasis-VitalBE*

IRAS Ref: 252929

Version: V3

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Version No	Edited by (job title)	Effective Date	Details of Changes Made
1		30-05-19	First version
2	Clare Clarke, Trial Manager	28-10-20	Sputum induction removed from visits
3	James D Chalmers, Chief Investigator Holly Keir Research Fellow Fiona McLaren-Neil, Trial Manager	15-02-23	Removal of sample collection at visits 3 & 4 Changes/additional instructions to sample collection process

CONTENTS

See also VitalBE Trial Research Samples picture guide

1. Equipment

Ensure all equipment and sample collection tubes are within the expiry dates

- Blood collection tubes appropriate for each visit
- Sputum sample pot, if required for visit
- Pernasal (NP) swab, if required for visit
- Venepuncture equipment (e.g. needles, tourniquet, cotton wool etc.)
- When Research Samples are taken at visits 2,5, 6 & any unscheduled visits a collection set compatible with vacutainer tubes must be used. See Section 6 for details of obtaining blood samples where Monovette tubes are required for NHS samples.
- Centrifuge
- Pipettes
- Fluidx tubes and storage box
- Sample labels
- -80 sample storage boxes
- -80C freezer

2. Procedures

- Ensure all equipment used is within expiry date
- Obtain blood samples as per local venepuncture Standard Operating Procedure.
- Obtain sputum samples as per local Standard Operating Procedure
- Obtain nasopharyngeal swab as per Working Practice Guideline for Obtaining Nasopharyngeal Swab
- Dispose of all clinical equipment as per local policy.
- Deal with any needlestick injury or body fluid spillage as per local policies

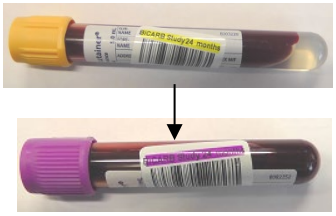
3. Blood samples

3.1. Local NHS lab samples visit 1

- See section 6 for samples to be collected.
- Collect and send to local lab as per local procedure.

3.2. Research samples visits 2, 5 and 6, and unscheduled visits

- Samples should be drawn from participant in the following order:
    - SST tube
    - EDTA tube



3.3. Inadequate Blood Sample Volume

- The NHS samples (visit 1, screening only) MUST be obtained as these are safety samples.
- If there is a problem obtaining blood then another member of staff should be asked to assist. If it is not possible to obtain a blood sample then the participant should be asked to return on another day, this should be as soon as possible after the day of visit.
- Where NHS samples (visit 1, screening only) are not obtained the participant will not be eligible to continue in the trial.
- If samples were not obtained then this should be noted in the appropriate section of the visit in the eCRF and documented in the Breach Log.

4. Sputum samples

- Where possible use a sputum sample provided spontaneously by the participant
- A sputum pot should be given to participants, labelled with participant ID, to bring a first in morning sample at their next visit. Participants should add the date and time obtained on the sample pot.
- Participants should also be asked to produce a spontaneous sputum sample at the time of the visit.

4.1. Local NHS lab samples for C&S visit 1

- A sputum sample is required for culture and sensitivity to determine if the participant has sputum that is culture positive for *Pseudomonas aeruginosa* or other Gram-negative respiratory pathogens.

- The sample must be obtained and a positive culture confirmed in time to allow visit 2/randomisation to occur within 35 days of Visit 1/screening.
- Sputum samples should be obtained using the following hierarchy:

  At visit

  Spontaneous sample
  If not obtained - arrange follow up visit and provide a sputum container for patients to bring a sample from home.

  Follow up visit

  At the repeat visit the following sputum should be used:
  Home sample brought in by participant
  If not provided - spontaneous sample produced at the visit

- The participant should be asked to bring a sputum sample produced at home on the morning of the follow up visit. This sample should be used if a sample is not obtained following sputum induction. Ideally this sample should be obtained within 2 hours of the trial visit. Samples collected outwith this period will be acceptable if this is the best available.
- The time the sputum sample was obtained should be written on the container.
- If no sputum sample is obtained prior to randomisation the participant should be withdrawn.

4.2. Local NHS lab samples for C&S visits 5 and 6 and any unscheduled visits

- The following sputum sample should be used:
  Home sample brought in by participant
  Spontaneous sample obtained at visit

- The best quality sputum sample between the sample brought in by the participant and the sample produced at the visit should be used.

4.3. Research samples visits 2, 5 and 6

- The following sputum sample should be used:
  Spontaneous sample obtained at visit
  If not obtained - home sample brought in by participant

- The sample should be frozen within one hour unless home sample used.
- The time of sample production and time of freezing should be entered on the Sample Log.

5. Nasopharyngeal swabs

- Collect as per Working Practice Guideline

6. NHS Laboratory Samples

- Send to local NHS lab via normal procedure for analysis:

Visit 1 Screening	**Visit, 5 & 6 (Months, 6 & 12)**
Bloods Sodium Potassium creatinine urea eGFR full blood count albumin alkaline phosphatise (Alk Phos) Alanine Aminotransferase (ALT) Sputum Culture & sensitivity	Bloods Nil Sputum Culture & sensitivity

- As soon as the blood and/or sputum results are available these should be reviewed by the Principal Investigator or delegated doctor.
- The results of all the blood and sputum tests should be entered in the appropriate pCRF Bloods/Sputum results page. Paper copies of the blood and sputum results should be filed in the participants' medical notes for source data verification or where held electronically these must be made available to the Sponsor Monitors for source data verification.
- The initials of the person who has reviewed the results and the date they were reviewed should be entered in the appropriate eCRF Blood/Sputum Results page. There should be evidence of this review either electronically or in paper format, noting Clinically Significant (CS) or Not Clinically Significant (NCS) and must be made available for source data verification during monitoring.
- When there are abnormal results the Principal Investigator/Investigator should use their own clinical judgement to determine if any action is required.
- Any action taken should be entered in the participant's medical notes as per normal local procedure.

7. Trial Research Samples

Samples required per visit.

Visit 1 screening	Visits 2, 6 & unscheduled Baseline, month 12, as required	Visits 5 Month 6
Nil	 SST tubes 4-5 mL blood X5 0.5 mL Fluidx tubes* *number of tubes filled will depend on volume of serum obtained, fill tubes to **max** 0.5mL	 SST tubes 4-5 mL blood X5 1.5 mL Fluidx tubes *number of tubes filled will depend on volume of serum obtained, fill tubes to **max** 0.5mL
	 EDTA tubes 4 mL blood	 EDTA tubes 4 mL blood
	Nasopharyngeal viral swab 	
	Sputum sample  **this is an optional sample and if not obtained sputum induction should **not** be carried out.*	Sputum sample  **this is an optional sample and if not obtained sputum induction should **not** be carried out.*

- SST tubes:
  - Invert 5 times
  - Label tube Record time taken on tube
  - Allow to stand for minimum 30 minutes, maximum 60 minutes.
  - Centrifuge at 1000 x g (relative centrifugal force rcg) for 15 mins as per local SOP ensuring adequate separation has taken place. Ensure centrifuge is set to rcf or x g NOT rpm. Advice can be provided to help convert 1000 x g to rpm if required.
  - Aliquot serum into the Fluidx tubes provided as per local SOP.
  - Fill each tube with 0.5ml max

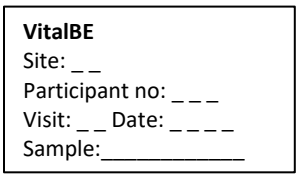
- The number of tubes filled will depend on the amount of serum obtained after centrifuging.
- Label Fluidx tubes
- Add details to Sample Log

- EDTA tubes:
  - Invert 8-10 times
  - Label tube
  - Freeze whole
  - Add details to Sample Log
- Nasopharyngeal swab
  - Ensure swab end is submerged in collection fluid
  - Label tube
  - Place swab tube **upright** in storage box
  - Add details to Sample Log
- Sputum pot
  - Label pot
  - Ensure pot lid is tightly screwed shut
  - Freeze whole
  - Add details to Sample Log

NOTE: Maximum of 1 hour from sample collection to freezing. If greater than one hour between collecting and freezing a sample this should be documented on the Sample Log.

7.1. Labelling Research Samples

- All trial samples should be labelled with the labels provided.

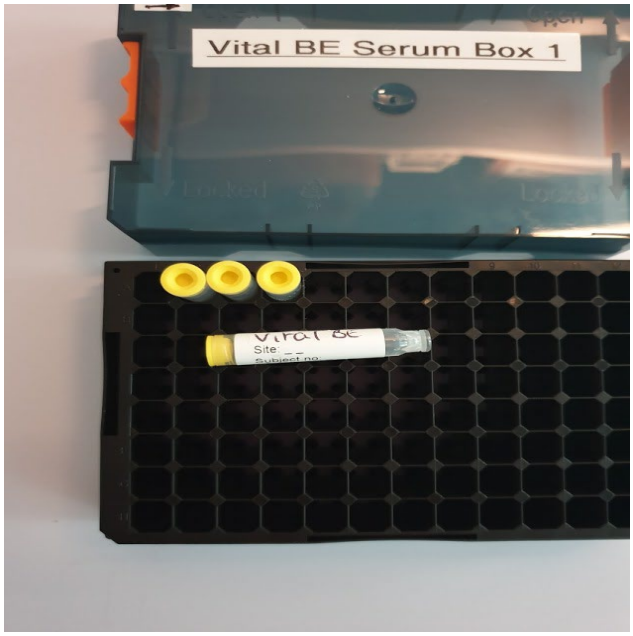


- Complete each label in a permanent marker pen with full sample ID and date as below.
- Site number is two digits long
- Participant number is three digits long (001, 002, 003 etc)
- Visit number is two digits long (02, 03, 04, 05, and 06. Unscheduled visits codes are 99, 98 etc)
- Date to be filled in day/month/year i.e 30/11/2022
- Place label on each tube ensuring that the label is completed correctly
- For Fluidx, blood and swab tubes ensure label is applied longitudinally, do not wrap round the tube and obscure information on the label
- Ensure that the label is **firmly rubbed** on to the tube to prevent it coming off.

7.2. Storing Research Samples

- The samples should be stored in the storage boxes provided.
- Trial samples should be stored in separate boxes according to sample type.
- The storage boxes should be labelled with trial title, site number, sample type

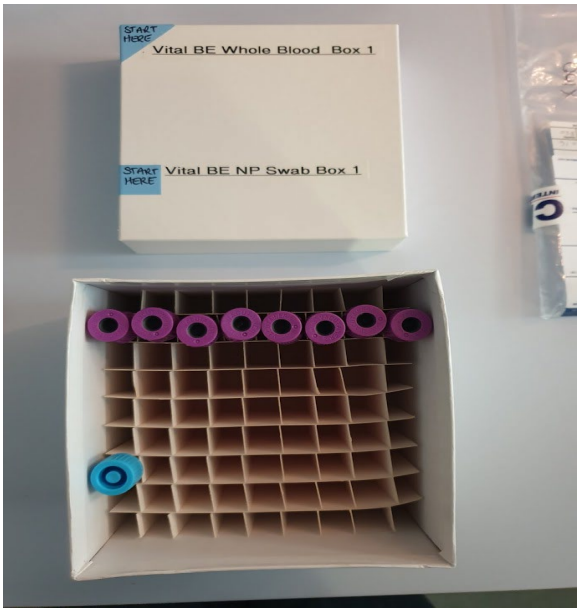
- Serum
  - Label orientation on the tube, the label should read from left to right beginning at the yellow capped end of the tube.
  - Ensure label is pressed on very firmly to ensure it sticks properly.
  - The Fludix tubes should be put in the box beginning at position ‘A1’ and continue from left to right.
  - Fludix tubes should be “clicked” into their storage box to prevent tubes falling out.



- Sputum
  - The sputum should be laid out as shown.

- Close sputum box with elastic band, provided, to ensure the lid stays secure. Label is placed at top of the box lid.

- Whole blood and NP swabs
  - There should be enough room in the box to fit both the whole blood and NP swabs.
  - Eight whole blood tubes/NP swabs can fit horizontally.
  - The first sample should be placed at the left of the box and continue to the right.
  - Ensure the whole blood samples at the top of the box and the NP swabs located from the middle of the box



- Complete the Trial Sample Log to record the location of where the samples will be stored. This should be updated if samples are moved at any time during the trial.
- There should be a separate page of the Trial Sample Log for each sample box, i.e. serum, sputum, whole blood & nasal swab.
- Place all samples in appropriate sample box and place box in allocated -80C freezer.

7.3. Transportation of Research Samples at End of Trial

- All research samples should be stored as above until requested by the Trial Manager or Laboratory Manager
- Batches of samples will then be transported to the appropriate laboratories for analysis.
- When all participants have completed the trial any remaining samples will be transported to the appropriate laboratories for analysis

Details of transportation to be confirmed.