UNIVERSAL

Understanding **I**nfection, **V**iral **E**xacerbation and **R**espiratory **S**ymptoms at **A**dmission- **L**ongitudinal (UNIVERSAL) Study

LABORATORY MANUAL

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Contents

1. CONTACT AND MAILING INFORMATION

For questions regarding this Laboratory Manual, including specimen collection, processing, storage and shipment, or the UNIVERSAL Protocol, please contact:

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Southampton, SO16 6YD, UK
Email: UNIVERSAL@soton.ac.uk

***** Hours of operation for SCTU are Monday to Friday, 9:00am to 5:00pm UK time.

For shipping details at the end of study contact the UNIVERSAL Study team on the study email: UNIVERSAL@soton.ac.uk

2. INTRODUCTION

UNIVERSAL is a prospective longitudinal observational study for phenotypic characterisation of the heterogeneous nature of acute respiratory viral infection utilising combined nose and throat swabs as well as blood samples taken from individuals admitted to a number of hospital sites with symptoms of a respiratory viral infection.

As laid out in the protocol, individuals admitted to hospital with a suspected acute respiratory viral infection will be approached by a member of the clinical team and asked if they are willing to take part in the study. If the individual lacks capacity to consent, an appropriate consultee may consent for the individual for as long as they lack capacity.

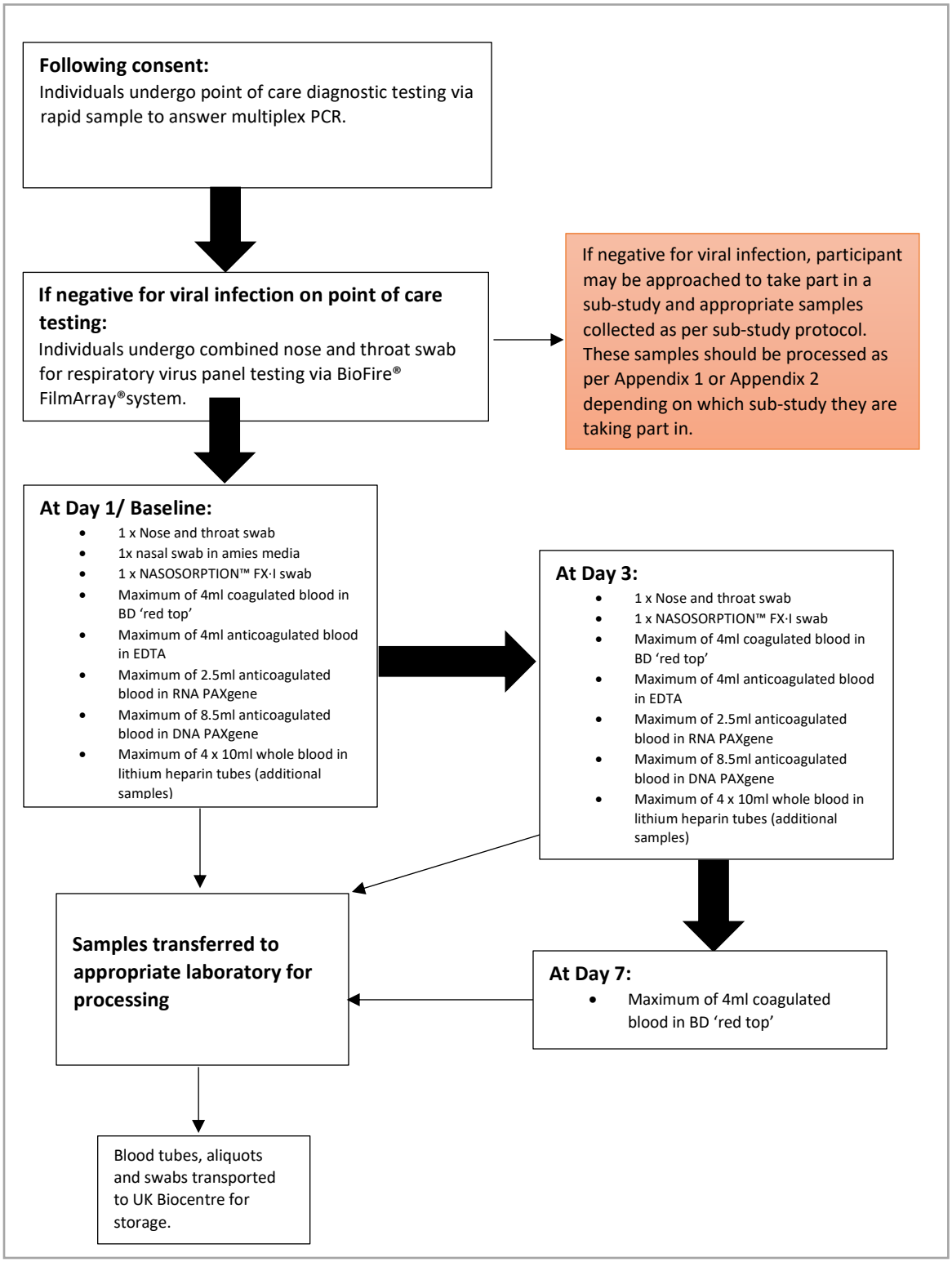
Following consent, respiratory samples will be obtained by a combined nose and throat swab. Those patients who test positive for a respiratory viral infection will continue into stage two of the study where two types of samples will be collected:

- Peripheral blood (122 ml) for analysis of DNA, RNA, serum, plasma and PBMC.
- Combined nose and throat swab sample for viral sequencing and PCR, nasorption and nasal swab for microbiome analysis.

The optional UNIVERSAL sub-studies may involve collection and processing of further samples. Details of these are included in Appendix 1.

The purpose of this laboratory manual is to provide all sites involved with detailed instructions on the collection, handling, processing and shipment of the biological specimens.

3.OVERVIEW OF SAMPLE FLOW



4. LABORATORY SUPPLIES, GLYCEROL PREPARATION AND CONTROLS

Stage 1 Nose and throat swabs:

Combined nose and throat swabs supplied from Copan (Regular Flocked swab and eNat® Medium Product code 6U073S01)

If re-supply of any of these are required **in Southampton or other sites** please e-mail: UNIVERSAL@soton.ac.uk detailing how many will be required and include the name and address to whom it will need to be sent.

Stage 2 Swabs:
Medical Wire Combined nose and throat swab (Product code MW951S) – Green top

MWE Sigma transwab – nasal swab in liquid amies media (Product code MW176S) – purple/orange top

Nasosorption™ FX·i devices supplied from Mucosal Diagnostics.

Blank swab controls:
A closed, unopened swab (within its original packaging) of both the VTM and amies media swabs should be collected from each new lot of swabs used in the study.

- These will be collected at Southampton only.
- The swabs should be stored at -70°C (+/-20°C) in a swab controls box/ziplock bag.
- Label the control swabs with date taken and lot number of swab tube.
- Log the details and date in the controls log and the identity of the sites that the swabs will be sent to.

Blood collection tubes:

DNA and RNA PAXgene tubes supplied from Fisher Scientific:

- 8.5ml DNA PAXgene tubes (BD Product code 761133)
- 2.5ml RNA PAXgene tubes (BD Product code 762165)

4ml BD (Red Top) tubes are supplied from SCTU (BD Product code 368975 **OR** equivalent alternative)

4ml BD EDTA tubes are supplied from SCTU (BD Product code 367839 **OR** equivalent alternative)

Blood aliquot tubes:

0.5ml Microtube: Sarstedt 72 730 005 **OR** Alpha Labs CP5933 **OR** equivalent alternative
Screw cap lids for microtubes: Alpha Labs CP5940N **OR** equivalent alternative (note Sarstedt tubes come with caps)

Glycerol and tubes for amies media nasal swab processing:
Glycerol – Scientific laboratory supplies, G5516-1L
2mL culture storage tubes.

Preparation of glycerol storage tubes at Southampton:
Glycerol tubes should be made in batches ahead of time and stored. Preparation should be carried out in a MSC class II hood.

- Add 500µl of glycerol into a sterile 2ml tube.
- Label each tube as described in 6.1.
- Store tubes upright until required at room temperature.
- Prepared tubes will then be shipped to other sites taking part in UNIVERSAL for storage of the amies media from nasal swab samples.

Glycerol Control sample:
For each batch of glycerol tubes created, a control tube is required to be prepared as follows:

- >1ml glycerol added to a sterile 2ml tube
- Control tube to be labelled with the date and the lot number of the glycerol and tubes. Also record location of preparation ie. If more than one lab space used.
- Store at -70°C (+/-20°C) in a glycerol controls box.
- Log the details (date, lot number of glycerol and tubes) in the controls log.
- Collected at Southampton only.

Collection of further swab control samples is described in detail in Section 12.1.5.

5. SAMPLE COLLECTION, STORAGE & SHIPPING SUMMARY

Day	Sample Type	Tube Type	Max volume collected	No. of Tubes	Storage conditions at laboratory	Conditions for long term storage	Fraction isolated	Transportation Destination
1 (stage 1)	Combined nose and throat swab	Copan eNAT tube	1 swab	1	2-8°C	N/A	DNA	**Samples collected in Wessex**: For shipping details at the end of study contact UNIVERSAL@soton.ac.uk
1 (stage 2)	Nasosorption	Tube containing 1ml viral transport medium	1 Synthetic absorptive matrix	1	Less than or equal to -70° C with a tolerance range of +/- 20° C (-50 to -90° C)	Less than or equal to -70° C with a tolerance range of +/- 20° C (-50 to -90° C)	DNA	
	Combined nose and throat swab	Copan eNAT tube	1 swab	1	2-8°C	Less than or equal to -70° C with a tolerance range of +/- 20° C (-50 to -90° C)	DNA sequencing	
	Nasal swab	Copan tube with amies media	1 swab	1	2-8°C for up to 48 hours before processing and freezing at -70° C	Less than or equal to -70° C with a tolerance range of +/- 20° C (-50 to -90° C)	DNA /microbiome	
	Coagulated Whole blood	BD Red Top	4ml	1 x 4ml	Room Temperature (18-25°C)	Less than or equal to -70° C with a tolerance range of +/- 20° C (-50 to -90° C)	Serum for biomarker assay	

	Anticoagulated Whole blood	EDTA	4ml	1 x 4ml	Room Temperature (18-25°C)	Less than or equal to -70°C with a tolerance range of +/-20°C (-50 to -90°C)	Plasma for cfDNA	
	Anticoagulated Whole blood	PAXgene RNA	2.5 ml	1 x 2.5 ml	2-8°C for a maximum of 5 days	Less than or equal to -70°C with a tolerance range of +/-20°C (-50 to -90°C)	RNA	
	Anticoagulated Whole blood	PAXgene DNA	8.5ml	1 x 8.5ml	2-8°C for a maximum of 28 days	Less than or equal to -70°C with a tolerance range of +/-20°C (-50 to -90°C)	DNA	
	Anticoagulated Whole blood	Lithium heparin	40ml	4 x 10ml	-70 to -80°C	Liquid Nitrogen (below -135°C)	PBMC	
3 (stage 2)	Nasosorption	Tube containing 1ml viral transport medium	1 Synthetic absorptive matrix	1	Less than or equal to -70°C with a tolerance range of +/-20°C (-50 to -90°C)	Less than or equal to -70°C with a tolerance range of +/-20°C (-50 to -90°C)	DNA	

	Combined nose and throat swab	Copan eNAT tube	1 swab	1	2-8°C	Less than or equal to -70°C with a tolerance range of +/-20°C (-50 to -90°C)	DNA	
	Coagulated Whole blood	BD Red Top	4ml	1 x 4ml	Room Temperature (18-25°C)	Less than or equal to -70°C with a tolerance range of +/-20°C (-50 to -90°C)	Serum for biomarker assay	
	Anticoagulated Whole blood	EDTA	4ml	1 x 4ml	Room Temperature (18-25°C)	Less than or equal to -70°C with a tolerance range of +/-20°C (-50 to -90°C)	Plasma for cfDNA	
	Anticoagulated Whole blood	PAXgene RNA	2.5 ml	1 x 2.5 ml	2-8°C for a maximum of 5 days	Less than or equal to -70°C with a tolerance range of +/-20°C (-50 to -90°C)	RNA	
	Anticoagulated Whole blood	PAXgene DNA	8.5ml	1 x 8.5ml	2-8°C for a maximum of 28 days	Less than or equal to -70°C with a tolerance range of +/-20°C (-50 to -90°C)	DNA	
	Anticoagulated Whole blood	Lithium heparin	40ml	4 x 10ml	-70 to -80°C	Liquid Nitrogen	PBMC	

						(below -135°C)	
7 **(stage 2)**	Coagulated Whole blood	BD Red Top	4ml	1 x 4ml	Room Temperature (18-25°C)	Less than or equal to -70°C with a tolerance range of +/-20°C (-50 to -90°C)	Serum for biomarker assay

6.SUBJECT ID AND LABELLING OF SAMPLES

To ensure robust sample tracking all samples collected must be labelled appropriately detailing the participant ID, date collected and sample type. Samples of the same type collected on different days will be labelled as D_ where D is the day number, in order to differentiate between them.

6.1 Samples collected

All consented patients will be logged and assigned a registration number until point-of-care testing via BioFire Film Array has been carried out. Those who test positive will be assigned a unique participant identification number. The unique participant identification number will consist of 4 digits made up of the site number (e.g. site 1 will be 1001) and the 4 digit participant number. The 4 digit participant number will be assigned sequentially to each patient within each site starting with 0001. For example, the first patient recruited at site 1 will have the unique 8-digit participant identification number 10010001.

For these participants ensure that you pre-label the blood collection tubes with the correct pre-printed labels supplied, **ensuring the label is applied vertically i.e not wrapping around the tube.**
An example of the label formats in the correct orientation are shown below, in the event of lost/damaged labels ensure that the details are written on the tube as shown with a minimum of Participant ID, sample type and date.
Labels will be provided to sites for collection of monthly environmental swab controls – this is further described in Section 12.1.5.

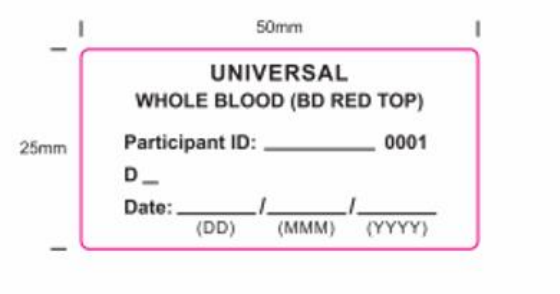


Figure 1: Example of label for collection tubes

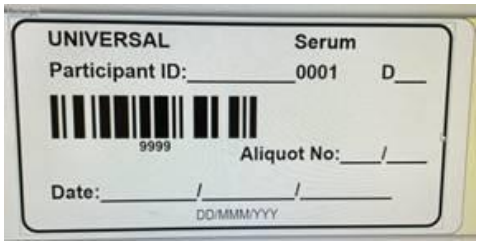


Figure 2: Example of label for aliquot tubes (post processing)

7. COLLECTING NASAL SPECIMENS VIA NASOSORPTION™ FX·i

- Pre-label collection tube containing 1ml viral transport medium (VTM) with correct subject ID number, applying label vertically.
- Ask participant to remove their mask and blow their nose to remove any debris.
- Take nasosorption device with synthetic absorptive matrix (SAM™) attached and insert into the nostril at the anterior part of the inferior turbinate (approximately 1.5cm inside the nostril.
- Gently press on the exterior part of the nostril to oppose the SAM against the nasal mucosa for a timed 60 second absorption.
- Remove the device and insert into the pre-labelled collection tube containing VTM. The participant may now replace their mask.
- Ensure the tube is sealed and store at -70°C with a tolerance range of +/- 20°C (-50 to -90°C).

8. COLLECTING NOSE AND THROAT SPECIMENS

- Select a swab kit ensuring the swabs are labelled with the correct subject ID number, apply label **vertically** to avoid any overlapping and ensure the barcode is readable.
- Write the collection date in the space provided on the collection tube containing 1ml of virus-inactivating media such as Copan eNAT tube. Remove cap just before you are ready to begin the collection.
- Ask participant to remove mask and blow their nose to remove any debris.
- Remove the swab from the packaging and ask the participant to open their mouth wide and to stick out their tongue.
- Advise the participant that they may gag however it should not be painful to lessen the anxiety. gently rub the swab along the back of the throat and around the tonsils, avoiding the tongue and the teeth of the participant.
- Next, using the same swab, insert the tip 1.5cm inside the participants nostril. No force should be needed. Gently rotate the swab for 10 seconds. Remove and repeat for the other nostril.
- Remove the swab and insert into the already opened collection tube containing 1ml inactivation media, the participant may now replace their mask.
- Break the swab at the groove, discard what remains of the swab and close the tube. Wipe the tube down with an alcohol wipe.
- At the end of the procedure, check caps are secure, and tubes are labelled appropriately.
- **Store vial upright in the fridge at 2-8°C** – *Samples should be placed at 2-8°C as soon as possible and within 2 hours of collection. Samples can be stored for 48 hours at 2-8°C before transfer to -70°C(+/-20°C) for long term storage. This should be noted on the sample log provided.*

9. COLLECTING NASAL SWAB IN AMIES MEDIA SPECIMENS

- Select a liquid amies media swab kit ensuring the swabs are labelled with the correct subject ID number, apply label vertically to avoid any overlapping and ensure the barcode is readable.
- Write the collection date in the space provided on the collection tube.
- Tilt patient's head back approximately 70 degrees.
- Nasal swab: insert the tip of the swab into the nostril. Using a gentle rotation push the swab approximately 3.5 cm into the nostril (the swab should be inserted about half the distance from the opening of the patient's nostril to the ear), until a slight internal resistance is met at the level of the turbinates.
- Gently rotate the swab several times.
- Remove from the nostril and perform the same insertion procedure into the other nostril with the same swab.
- Aseptically remove the cap from the amies media tube. Insert the swab into the tube and break the swab shaft at the scoreline so that the swab will fit in the transport medium container.
- Replace the cap onto the tube and transport to lab for processing.
  Store vial upright in the fridge at 2-8°C – *Samples should be placed at 2-8°C as soon as possible and within 2 hours of collection. Samples can be stored for 48 hours at 2-8°C before processing. This should be noted on the sample log provided.*

10. COLLECTING WHOLE BLOOD SPECIMENS

Blood collection must be performed by qualified personnel who are trained to draw blood according to your institution's requirements and procedures for venepuncture technique. Protective non-latex gloves should be worn when performing venepuncture.

After blood draw, remove the needle from participant's arm and apply gauze with adequate pressure to the site of venepuncture. Dispose of needles and supplies into appropriate medical waste container.

10.1 Collecting the BD 'red top' whole blood Samples

- Collect these samples first
- Pre-label one 4ml Becton Dickinson (BD) Serum tube (Red Cap) or equivalent with the labels provided, ensuring that you are using the correct participant ID number.
- Ensure tubes are gently inverted 5-10 times
- **Place the tube (labelled for UNIVERSAL) inside the clear specimen bag provided and keep at ROOM TEMERATURE (+18 to +25°C) ready for transportation.**

10.2 Collecting the EDTA whole blood sample

- Collect this sample after collecting BD 'red top' (serum).

- Pre-label one 4ml EDTA tube with the label provided, ensure that you use the correct participant ID number.

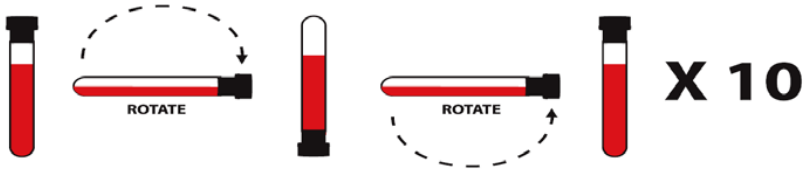
- Gently invert the tubes 5-10 times and place the filled tubes inside the clear specimen bag provided at **room temperature (+18 to +25°C)** ready for transportation.

10.3 Collecting DNA and RNA PAXgene whole blood samples

General Instructions

- Both DNA Paxgene and RNA PAXgene tubes must be collected after plasma collection. PAXgene tubes should be ideally drawn last in the phlebotomy procedure, unless additional samples for PBMC isolation are to be collected.
- Ensure PAXgene Blood DNA and RNA tubes are at room temperature (18-25°C) prior to use, and properly labelled with subject ID number with the provided labels.
- Volumes to be collected:
    a) DNA PAXgene: Max blood volume **8.5ml**
    b) RNA PAXgene: Max blood volume **2.5ml**

- Prevention of backflow: since PAXgene blood DNA and RNA tubes contain chemical additives, it is important to avoid possible backflow from the tube. To guard against backflow, observe the following precautions:
    a) Keep participant's arm in the downward position during the collection procedure.
    b) Hold the tube with the stopper in the uppermost position so that the tube contents do not touch the stopper or the end of the needle during sample collection.
    c) Release tourniquet once blood starts to flow in the tube.
    d) Allow at least 10 seconds for a complete blood draw.
    e) Make sure tube additives do not touch stopper or non-patient end of the needle during venepuncture.
    f) After the blood draw, gently invert PAXgene tubes 8 to 10 times as shown below. **Inadequate or delayed mixing may result in inaccurate test results**.



- Place the PAXgene DNA and RNA tubes inside the same bag as the BD red top tube.

For both DNA and RNA PAXgene tubes, the preferred storage conditions are as follows:

1. Room temperature (18°C-25°C) for 2 to 24 hours following blood draw.
2. Followed by freezing at -20°C for a minimum of 24 hours but up to 72 hours
3. After which, transferred to -70° C (+/- 20° C) for long term storage.

Note: PAXgene tubes must be frozen upright in a metal or plastic wire rack. Styrofoam trays should be avoided as this can cause the tubes to crack.

11. LOGGING AND PACKAGING OF SAMPLES COLLECTED

- When all blood samples have been collected, ensure they are packaged together per subject according to storage temperature.
- One copy per subject of the completed form [insert] should accompany the samples to the processing lab. If the samples are shipped in separate boxes this should be placed with the samples sent at room temperature.
- Samples should be transferred to the processing laboratory according to locally agreed procedures and processed within the timeframes specified.
- Samples collected in other sites can be logged according to local procedures.

12. SAMPLE PROCESSING AND SWAB CONTROLS

12.1 Nose and throat specimen (Medical Wire Swab)

12.1.1 BioFire® FilmArray® System

- If participant tests negative for a respiratory viral infection on a rapid multiplex PCR for point-of -care, a combined nose and throat swab is carried out to test on the BioFire® FilmArray® system against a larger panel of viruses.
- Following validation, BioFire® FilmArray® respiratory panel is run by clinical trial assistant or research nurse, as specified on the delegation log - see BioFire® Respiratory Panel 2.1 Quick Guide (APPENDIX 1).
- To avoid contamination, ensure clean gloves are always worn and work is carried out behind a protective shield.

- After each 10th run, a negative control is to be run to ensure no contamination has occurred.

12.1.2 NASOSORPTION™ FX·i

- Nasosorption™ FX·i to be store whole at -70°C (+/-20°C).

12.1.3 Nose and throat swab for storage for laboratory PCR and sequencing

- Following a positive respiratory viral infection highlighted by the BioFire® FilmArray® Respiratory Panel, an additional nose and throat swab will be collected.
- Upon receipt, swabs should be stored whole at -70°C (+/-20°C) awaiting laboratory PCR.

12.1.4 Nasal swab in amies media for storage for microbiome analysis

- Samples should be processed in a MSC class II cabinet.
- Upon receipt of swab sample, ensure the lid is secured and Vortex swabs for 10 seconds in their transport tube.
- Ensure a pre-prepared glycerol storage tube is correctly labelled with date and participant ID, if not prepared ahead of time (500µl glycerol storage tubes will be pre-prepared in Southampton as per Section 4).
- Any glycerol tube that is opened, damaged or shows evidence of leakage should be discarded.
- Use an XL 1000µl pipette or Pasteur pipette to avoid touching the inside of the transport tube with the pipette and transfer as much transport Amies media as possible (max. 1000µl) to the culture storage tube containing glycerol. This gives a final concentration of >/= 30% glycerol.
- Pipette up and down slowly to mix thoroughly, being careful not to touch the inside of the tube with the pipette. The arriving swab tubes can now be discarded.
- Store the culture storage tubes at -70°C (+/-20°C) awaiting transfer to Biocentre.
- Record the storage location including the box and slot within box.

12.1.5 Environmental swab control samples:

UNIVERSAL sites are required to collect monthly environmental swab controls:

2 x Nose and throat swab controls (Green top swab in viral transport media)

1 x Sampling environment control:

- An unused green top nose and throat swab is opened and exposed to the sampling environment for 5 seconds (E.g. open in the ward where participants are recruited) and then the swab is inserted into the media tube and closed tightly.

- Ensure the control swab tube is labelled with a control label, the site ID, date taken and the lot number of the swab tubes.
- Transport to lab and store at -70°C (+/-20°C) awaiting transfer to biocentre.
- Log the details (date, lot number of swab tube) in the controls log.

1 x Lab environment control:

- An unused green top nose and throat swab is opened and exposed to the laboratory environment for 5 seconds (E.g. the laboratory cabinet where samples are processed) and then the swab is inserted into the media tube and closed tightly.
- Ensure the control swab tube is labelled with the type of control, the site ID, date taken and the lot number of the swab tube.
- Transport to lab and store at -70°C (+/-20°C) awaiting transfer to biocentre.
- Log the details (type of control, date taken, lot number of swab tube) in the controls log.

2 x Nasal swab control (Purple/orange top swab in amies media):

1 x Sampling environment control:

- An unused purple/orange top amies media swab is opened and exposed to the sampling environment for 5 seconds (E.g. open in the ward where participants are recruited) and then the swab is inserted into the media tube and closed tightly.
- Ensure the control swab tube is labelled with the type of control, the site ID, date taken and the lot number of the swab tube.
- Log the details (type of control, date taken, lot number of swab tube) in the controls log.
- Transport to lab for processing as per section 12.1.4 awaiting transfer to biocentre.
- In the lab ensure the control sample is labelled with the type of control, the site ID, date taken and the lot number of the swab tubes.

1 x Lab environment control:

- An unused purple/orange top amies media swab is opened and exposed to the laboratory environment for 5 seconds (E.g. open in the laboratory cabinet where samples are processed) and then the swab is inserted into the media tube and closed tightly.
- Ensure the control swab tube is labelled with the type of control, the site ID, date taken and the lot number of the swab tube.
- Log the details (type of control, date taken, lot number of swab tube) in the controls log.
- Transport to lab for processing as per section 12.1.4 awaiting transfer to biocentre.
- In the lab ensure the control sample is labelled with the type of control, the site ID, date taken and the lot number of the swab tubes.

Control Log:
Each site should keep a brief log of the control samples taken detailing:

- The type of control sample taken – laboratory/sampling environment
- The date the controls were taken
- The lot numbers of swabs and glycerol.

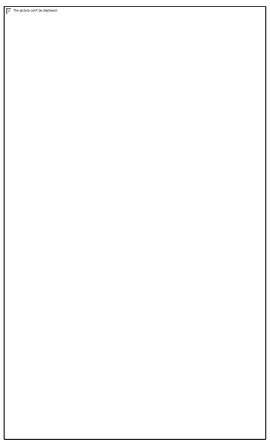
<u>Reagent control samples at Southampton only:</u>
Reagent sample controls of unopened swabs and glycerol will be taken and stored from each lot used in the study. These samples will be taken at the main site in Southampton and are detailed in section 4.

- **Swab controls:** Unopened swab packages from each lot of both the amies media swabs and viral nose and throat swabs in VTM.
- **Glycerol control:** sample of the glycerol used in processing amies media swabs taken from each lot of glycerol used.

12.2 Anticoagulated whole blood (EDTA):

12.2.1 Plasma isolation for cfDNA assay

- Ensure all aliquot tubes are labelled appropriately (apply any barcoded labels **vertically**).
- Ensuring the centrifuge is balanced, centrifuge the EDTA tube(s) in a 'swing-out' rotor at **1600 xg (+/- 10 xg) for 10 minutes at room temperature, with the brake off to avoid disrupting the cell layer.**
- Making sure you are working under a microbiological safety cabinet and using a new disposable transfer pipette, transfer the plasma layer to clean 2ml PP skirtless tubes *(Alpha Labs CP5932* ***OR*** *equivalent).* Remove as much plasma as possible but without disturbing the buffy coat layer by aspirating to 5mm above the buffy coat layer.



- Perform a second centrifugation of plasma aliquots at **16,000 xg* (+/- 1,000 xg) for 10 minutes at room temperature** (20-25°C) in a microfuge to pellet any remaining cellular debris. *Up to 20,000G is preferred if possible.

- Following completion of the second centrifugation step, carefully transfer **a maximum of 500µl plasma in up to 4 x** 500µl Alpha Labs Microtubes and freeze immediately at -70°C (+/- 20°C).

12.3 Coagulated whole blood tubes (BD red cap)

12.3.1 Serum isolation for protein biomarker analysis

- Store blood upright (for at least 30 minutes up to 2 hours after collection), process and freeze within a maximum of 24 hours.
- Ensure all aliquot tubes are labelled appropriately (apply any barcoded labels **vertically**).
- Centrifuge tube after 30 minutes when fully coagulated and ASAP at **1300g for 10 minutes at room temperature, brake on.**
- Collect the serum fraction and aliquot **a maximum of 500µl serum in up to 4 x** 500µl Alpha Labs Microtubes.
- Store aliquots -70°C (+/- 20°C)
- **Samples must be frozen within 24 hours of collection**

12.4 Sample collection and processing from Universal Sub-studies

Procedures for sub-study sample collection and processing are covered in Appendix 1.

13. SAMPLE STORAGE

All samples must be stored in a -70°C (+/-20°C) freezer unless otherwise stated in this laboratory manual.

Store all aliquots upright in boxes in an organised fashion, for example by aliquot type and by sequential participant ID.

13.1 Sample Destruction

If required, destruction of samples is allowed only with written agreement from SCTU team. Destruction should be carried out and recorded according to local policy.

14. DEVIATION REPORTING

In the event of any deviations from this laboratory manual these must be documented locally and notify UNIVERSAL@soton.ac.uk soon as you are made aware with a list of the affected samples and associated participant ID(s).

Examples of deviations may include but are not limited to:

- Incorrect storage temperature
- Incorrect processing parameters (i.e. centrifuge speed)
- Samples processed beyond time requirements
- Tubes received by biorepository/ for long term storage with patient identifiers

15. SAMPLE SHIPPING/TRANSFER DETAILS

For long term storage:

UK Biocentre
Units 2 & 3, Java Park
Bradbourne Drive
Tilbrook
MK7 8AT
For further shipping details or any queries please contact the UNIVERSAL Study team on the study email: UNIVERSAL@soton.ac.uk

16. Revision History

Date and version	Summary of significant changes
V2.0 12-Aug-2022	Revision of type of aliquot tubes used for serum and plasma post-processing in the Lab Manual. Change of Serum processing, blood does not have to be stored in the fridge prior to processing. Label mockups changed to account for new participant ID layout.
V3.0 20-Sep-2023	Change of dry swab to swab in Amies media. Addition of virus negative sub-study as appendix 1 to be carried out at the Southampton site only.
V4.0 07-Aug-2024	Addition of Bordetella pertussis sub-study as appendix 2.

17. Appendix 1

Virus negative sub-study

Sample collection and processing is identical to the sampling and processing for participants enrolled in Stage 2 of the main UNIVERSAL protocol. Procedures described in the main UNIVERSAL lab manual should be followed for samples collected a spart of this sub-study.

18. Appendix 2

Universal – Bordetella pertussis pilot-study

Equipment:

- Nasopharyngeal swab – MW172C Transport Swab Gel Media Amies
- Nasopharyngeal/Oropharyngeal swab – Medical Wire Combined nose and throat swab (Product code MW951S) – Green top
- Pertussis serology samples - Venous tube Serum Plastic 5mL gold with gel SST11

Collection of Nasopharyngeal swab:

- Tilt patient's head back approximately 70 degrees.
- Gently and slowly insert the swab through the nostril parallel to the plate (not upwards) until resistance is encountered or the distance is equivalent to that from the ear to the nostril of the patient, indicating contact with the nasopharynx.
- Gently rub and roll the swab for 5-6 rotations.
- Leave swab in place for several seconds to absorb secretions.
- Slowly remove swab while rotating it. Specimens can be collected from both sides using the same swab, but it is not necessary to collect specimens from both sides if the mini-tip is saturated with fluid from the first collection.
- If a deviated septum or blockage create difficulty in obtaining the specimen from one nostril, use the same swab to obtain the specimen from the other nostril.

Collection of Oropharyngeal swab (If unable to take nasophargyngeal):

- Open and remove the swab, taking care not to touch the tip of the swab or lay it down.
- Take an oropharyngeal swab. Insert into mouth and swab the posterior pharynx and tonsillar areas (avoid the tongue.)
- Aseptically remove the cap from the tube. Insert the swab into the tube and break the swab shaft at the scoreline so that the swab will fit in the transport medium container.
- Replace the cap onto the tube and close tightly to prevent leaks.

Blood samples for pertussis serology:

- Blood samples for pertussis serology are taken as per this lab manual section 10.

Sample labelling and processing:

- Samples are labelled with the local site NHS Trust labels. The request details will contain the study identifier number for the participant and state the name of the UNIVERSAL study.
- Nasopharyngeal swabs and blood samples will be maintained at room temperature and transported directly to the local site NHS laboratory for storage and processing as per the local site Bordetella pertussis PCR and serology SOPs.