ISARIC/WHO Clinical Characterisation Protocol UK

Lab Manual (Scotland)

Version 1

15 June 2021

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INTRODUCTION

The purpose of this lab manual is to outline the procedures that will be undertaken at sites for the collection, processing, storage and transport of trial samples for the ISARIC/WHO Clinical Characterisation Protocol.

This Laboratory Manual must be used alongside the current version of the ISARIC/WHO Clinical Characterisation Protocol. This document ONLY covers the sample collection aspects of the protocol.

GENERAL INSTRUCTIONS

- Great care must be exercised to ensure the safety of staff and others when dealing with novel respiratory pathogens where little is known about transmissibility and/or virulence. Strict adherence to sample collection, handling and biosafety protocols is essential.

- **THIS GUIDANCE IS SPECIFIC TO THE COVID-19 OUTBREAK and handling of material that is known or suspected to come from patients with SARS-CoV-2 infection.**

- Health boards should follow the usual sources of advice regarding laboratory containment of these pathogens. In an emerging infection this may include information from ACDP and PHE, which would support a local risk assessment and SOP covering the handling of such samples.

- Sample collection kits, pods and biobags will be supplied to sites. Sample collections kits can be requested from: sarah.mcdonald@glasgow.ac.uk

- Each kit will have a specific kit ID number, with each component within showing this kit ID and its own respective component ID for audit purposes.

- **Patient identifiable information must not be written on any of the labels or components.**

- **Sample Record Forms (also known as Transport Forms) included in the kits must be completed to ensure the samples can be linked to the correct patient.**

- All samples need to be processed as soon as they are received in the lab.

- **Ensure all sample containers are *tightly closed, not overfilled and not externally contaminated with any sample or other biological material.* Any samples that are leaking, contaminated or inappropriately closed need to be discarded upon receipt at the CVR.**

- Please note, ODS stands for Organisational Data Service on the Sample notification and information sheets.

SPECIMEN HANDLING

PHE have issued guidance on appropriate biosafety levels for handling specimens from people with COVID-19 or suspected SARS-CoV-2 infection. https://www.gov.uk/government/publications/wuhan-novel-coronavirus-guidance-for-clinical-diagnostic-laboratories/wuhan-novel-coronavirus-handling-and-processing-of-laboratory-specimens

- **Section 5.1:** Processing of respiratory tract specimens, faecal specimens, urine specimens, and tissue specimens in which virus has not been inactivated should be conducted at BSL3. No such processing is required for this protocol. These samples should remain double bagged and frozen pending transport to Glasgow
- **Section 5.2:** Accordingly, processing of whole blood, including aliquoting of serum and plasma can be conducted at BSL2, as long as it is consistent with the terms of the local risk assessment.
- **Section 6**: Manual centrifugation of specimens with infectious potential must be performed using sealed centrifuge rotors or sample cups. Rotors or cups should be loaded and unloaded in a Microbiology Safety Cabinet (MSC).

Thus, blood from COVID-19 patients participating in the CCP-UK study may be spun and aliquoted in usual BSL2 laboratories. Pathogens samples do not require any manipulation and should remain double bagged.

EQUIPMENT AND MATERIALS FOR SAMPLING

Each kit will have a specific kit ID number, with each component within showing this kit ID and its own respective component ID for audit purposes. Patient identifiable information should not be written on any of the labels or components.

Use one Sample collection kit for each patient at each timepoint

Kit contents

For Sample Collection		
Item	**Number**	
9ml Lavender topped vacutainer EDTA blood tubes	1	In recruitment kits only
3ml lavender topped vacutainer EDTA blood tubes	1	In follow-up kits only
3.5ml gold topped vacutainer SST serum tube	1	
3.5ml 9NC Coagulation sodium citrate 3.2 blood tube	1	
9ml Tempus RNA blood tube	1	
Universal container for urine	1	
Stool sample tube	1	
Nasorption SAM strip	1	
Oracol swab for oral fluid	1	
Swab in VTM	1	For throat swab
For laboratory processing		
Lavender topped cryovials	3	For plasma aliquots
Gold topped cryovials	3	For serum aliquots
Red topped cryovial	1	For cell pellet
Blue topped cryovials	2	For citrated plasma aliquots
Documentation & additional material		
Spare labels	3	For additional samples if required including ETA
Sample Record Form	1	
Secondary container (“pod”)	1	For transport

Additional secondary containers (if required) and bio-bags for transport will be supplied separately.

It is essential that sample record forms are fully completed, scanned and emailed to Sarah McDonald. This is the link between kit ID and patient.

BIOLOGICAL SAMPLES TO COLLECT

Weight	Samples at recruitment (R)	Serial samples (S)	Convalescent samples (C)
>40kg	• **9ml** EDTA blood • 3ml clotted blood • 3ml blood in RNA tube • 3ml blood in sodium citrate tube • Oral Fluid (Oracol swab) • Research pathogen samples	• **3ml** EDTA blood • 3ml clotted blood • 3ml blood in RNA tube • 3ml blood in sodium citrate tube • Oral Fluid (Oracol swab) • Research pathogen samples	• 3ml EDTA blood • 3ml clotted blood • 3ml blood in RNA tube • 3ml blood in sodium citrate tube • Oral Fluid (Oracol swab) • Research pathogen samples
20–40kg	• 6ml (3x2ml) EDTA blood • 3ml clotted blood • 3ml blood in RNA tube • Oral Fluid (Oracol swab) • Research pathogen samples	• 1ml EDTA blood • 2ml blood n RNA tube • Oral Fluid (Oracol swab) • Research pathogen samples	• 1ml EDTA blood • 3ml clotted blood • 2ml blood in RNA tube • Oral Fluid (Oracol swab) • Research pathogen samples
10–20kg	• 2ml (2x1ml) EDTA blood • 2ml clotted blood • 2ml blood in RNA tube • Oral Fluid (Oracol swab) • Research pathogen samples	• 1ml EDTA blood • 1ml blood in RNA tube • Oral Fluid (Oracol swab) • Research pathogen samples	• 1ml EDTA blood • 1ml clotted blood • 1ml blood in RNA tube • Oral Fluid (Oracol swab) • Research pathogen samples
<10kg	Refer to protocol. Table 3.		

Pathogen samples: *(double bag separately from blood samples)*

1. Throat swab in viral transport medium (VTM)
2. Nasal SAM strip*
3. Nasopharyngeal aspirate (NPA) in universal container OR (if NPA impossible) flocked throat swab in VTM
4. In all intubated patients with SARI or respiratory infection: endotracheal aspirate in universal container
5. Urine (~10ml in sterile universal container, if available; individually bagged). **Do not overfill.**
6. Rectal swab (in VTM) or stool (~10ml in sterile universal container/stool specimen container, if available)

BLOOD SAMPLE COLLECTION PROCEDURES

1. Blood is collected by venepuncture using the BD Vacutainer tubes, adapter and safety lok kits provided. Blood should be collected using the tube's vacuum.
2. Collect blood by vein puncture directly into the blood tubes.
3. Complete the Sample Information Sheet and Sample Notification Sheet with the relevant information.

BLOOD SAMPLE PROCESSING PROCEDURES

Blue topped Tempus Tube

- Immediately after collection, vigorously shake the Tempus Tube for 10 seconds.
- Ensure the outside of the tube is clean
- Freeze the tube immediately at -80°C (or at least at -20°C if a -80°C freezer is not available)
- Complete the Sample Information Sheet and Sample Notification Sheet with the relevant information.

Lavender EDTA vacutainer blood tubes

- In the absence of a refrigerated centrifuge, place samples in a 4°C fridge for 10 minutes prior to centrifugation.
- Centrifuge all three EDTA tubes at 1500 x g for 10 minutes at 4°C in a sealed rotor pod.
- In an MSC, inspect the rotor pod for signs of leakage, if none then open and inspect again.
- If there has been leakage, follow local disinfection guidance for the rotor pod.
- If the samples are intact, return the rotor pod and samples to the bench.
- For each EDTA tube, use a pipette with disposable sterile tips to make 3 aliquots of plasma supernatant of approximate equal volume in lavender topped cryovials
- **Avoid disturbing or touching the blood pellet with the pipette tip.**
- Following removal of the plasma from the EDTA blood tubes, aliquot 1ml of blood cell pellet from into the red topped cryovial.
- Do not overfill the vials and ensure that the lids are completely screwed down.
- Discard the primary purple topped EDTA tubes.
- Complete the Sample Information Sheet and Sample Notification Sheet with the relevant information.

Gold topped SST Serum vacutainer blood tube

- The clotted blood sample should be allowed to stand to clot for 30 minutes after sampling took place. This can be done in a refrigerator if convenient.
- In the absence of a refrigerated centrifuge, please place samples in a 4°C fridge for an additional 10 minutes.
- Centrifuge at 1500 x g for 10 minutes at 4°C in a sealed rotor pod.
- In an MSC, inspect the rotor pod for signs of leakage, if none then open and inspect again.
- If there has been leakage, follow local disinfection guidance for the rotor pod.
- If the samples are intact, return the rotor pod and samples to the bench.
- Using a pipette with disposable sterile tips, make 3 aliquots of serum supernatant of approx. equal volume in gold topped cryovials
- **Avoid disturbing or touching the blood pellet with the pipette tip, which will be below the gel separator.**
- Do not overfill the vials and ensure that the lids are completely screwed down
- Discard the primary gold topped SST tube

- Complete the Sample Information Sheet and Sample Notification Sheet with the relevant information.

Blue topped 9NC Coagulation sodium citrate 3.2 blood tube

- In the absence of a refrigerated centrifuge, please place samples in a 4°C fridge for an additional 10 minutes.
- Centrifuge at 2600 x g for 10 minutes at 4°C in a sealed rotor pod.
- In an MSC, inspect the rotor pod for signs of leakage, if none then open and inspect again.
- If there has been leakage, follow local disinfection guidance for the rotor pod.
- If the samples are intact, return the rotor pod and samples to the bench.
- Using a pipette with disposable sterile tips, make 3 aliquots of citrated plasma supernatant of approx. equal volume in blue topped cryovials
- **Avoid disturbing or touching the blood pellet with the pipette tip, only remove the top 2/3 of the plasma.**
- Do not overfill the vials and ensure that the lids are completely screwed down
- Dispose of the primary blue topped sodium citrate blood tube
- Complete the Sample Information Sheet and Sample Notification Sheet with the relevant information.

Double bag Tempus tube and all cryovial aliquots together and freeze at -80 (or at least at -20°C if a -80°C freezer is not available), ensuring samples are kept upright.

PATHOGENIC SAMPLE COLLECTION PROCEDURES

Urine

Please unsure the urine tube is only filled with ~15ml urine, ie half full. Following collection of urine sample**, double bag** and freeze the tube immediately at -80°C (or at least at -20°C if a -80°C freezer is not available). **Ensure the lid is completely secure and the tube remains upright at all times to prevent leaks.**

Stool

Collect stool sample in the container provided. Ensure the lid is tightly and correctly closed and that the outside of the container is clean.

A rectal swab may be obtained instead of a stool sample if necessary. Please ensure this is clearly labelled as a rectal swab.

Respiratory Samples

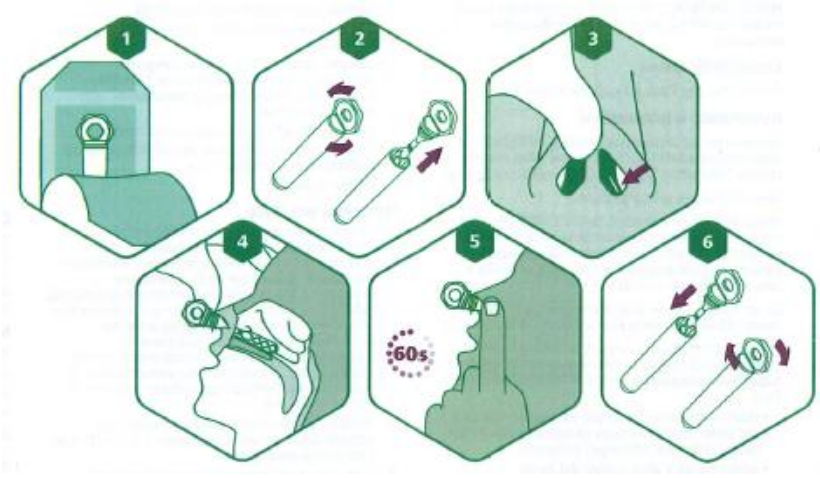
Small bag contains labels for the following:

- 1 x label for ORACOL tube
- 1 x label for SAM nasal strip
- 1 x label for VTM swab

When the ORACOL, Nasosorption SAM strip and swab have been unwrapped for use, place the relevant barcode label on the tube.

Nasosorption SAM Strips (Instructions from October 2016 Instruction leaflet – full version in the appendix)

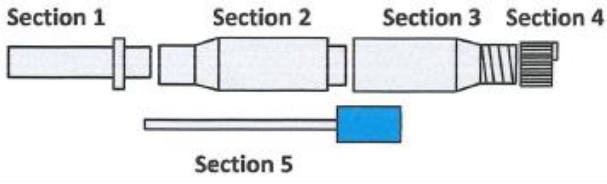
1. With washed and gloved hands, peel open the foil pack and remove the Nasosorption FX-i device.
2. Add the SAM Nasal Strip LIMS label to the Nasosorption tube.
3. Unscrew the applicator using the handle and remove the applicator from the tube, avoiding contact with the absorption end.
4. With one hand, hold the patients head and push back the tip of the nose with the thumb to provide a clear line of sight. With the aid of a light source, carefully insert the Nasosorption FX-I device into the nostril and gently locate the absorbent strip flat against the surface of the inferior turbinate.
5. Figure 4 shows the correct position of the strip against the inferior turbinate.
6. Hold the device in place in place by asking the patient to press a finger against the side of the nostril for 60 seconds.
7. Release the finger before removing the applicator from the nasal cavity. Return the divide to the tube and screw it back in.

8. 

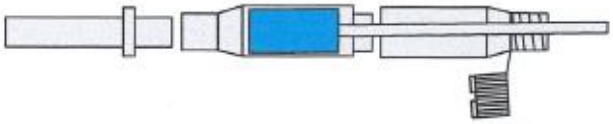
*A video demonstrating correct use of nasal SAM strips can be found here: **www.jove.com/video/56413**

ORACOL+ Saliva Collection (Instructions from Use S14 – full version in the appendix)

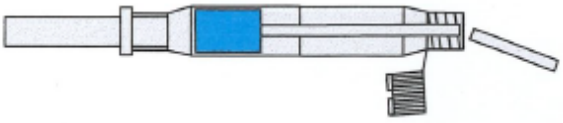
1. With washed and gloved hands, peel open the pack and remove the ORACOL+ Saliva device.
2. Add the ORACOL LIMS label to the ORACOL tube.
3. Remove the cap, unscrew section 2 from section 3 and remove the swab.



4. Collected the saliva by rubbing the sponge firmly along the gum; (at the base of the teeth if present) until the sponge swab is wet, this will take 1-2 minutes.
5. Put the swab sponge first in to section 2 and replace section 3.



6. Snap off the stick at the break point and replace cap.



A video demonstrating collection of oral fluid can be found here https://www.youtube.com/watch?v=6wDDLp_OaTc

Ensure the all parts of the ORACOL tube are tightly screwed together.

Throat swab
Swabs will be supplied with viral transport medium (VTM).

Label the sample tube with swab barcode label. Use the swab as a throat swab (not nasal), place in the liquid-filled tube and snap off the handle of the swab if necessary.
Ensure the lid is tightly and correctly closed.

Double bag the stool, ORACOL, Nasosorption (SAM) strip and throat swab together and freeze at -80°C (or at least at -20°C if a -80°C freezer is not available), ensuring samples are kept upright.

ADDITIONAL SAMPLES NOT INCLUDED IN KIT

If available, please collect additional samples such as swabs from infected sites, cerebrospinal fluid and nasopharyngeal aspirate. Label the additional samples with spare labels included in the kit.

Please complete the Sample Record Form and email a copy of this form to sarah.mcdonald@glasgow.ac.uk the same day the samples were taken.

PACKAGING AND TRANSPORTION OF SAMPLES TO CVR GLASGOW

PREPARATION OF SAMPLES FOR ONWARD TRANSPORT TO GLASGOW

Double bagged samples should be placed together into the sealable UN3373 compliant plastic pod and kept frozen. We suggest for convenience all samples are stored and frozen in this pod prior to collection. **The pod will already have a LIMS label, make sure that the labels on the pod and samples match. If an additional pod is used, attach one of the spare LIMS labels. Please also record the patient ID on the pod using an alcohol resistant marker.**

Contact Sarah McDonald (sarah.mcdonald@glasgow.ac.uk) to arrange transport to Glasgow. This will be booked with CryoPDP at a mutually agreed date & time. The courier will provide outer transport boxes half-filled with dry ice. When packing the secondary safety containers please decontaminate the outside of each container with 70% ethanol or IMS, place the container inside a biobag along with some absorbent material (eg tissue), and seal the bag. Following this please spray the outside of the biobag with 70% ETOH/IMS before placing in the dry ice boxes.

The courier should provide all labels required, but if needed the delivery address is as follows:

Dr Sarah McDonald
MRC-University of Glasgow Centre for Virus Research
Sir Michael Stoker Building
Garscube Campus
464 Bearsden Road
Glasgow
G61 1QH
Tel: 0141 330 2989

Please make sure that copies of all Sample Record Forms have been emailed to sarah.mcdonald@glasgow.ac.uk prior to shipping.

Each shipment must include the Sample Record Forms, a description of known pathogens contained and contact information as provided in Appendix 4, place these sheets between the secondary container and outer packing. This is a legal requirement under the Carriage of Dangerous Goods and Use of Transportable Pressure Equipment Regulations 2009.

APPENDIX 1 - Nasosorption SAM Strips Instructions

NASOsorption™ FX·i

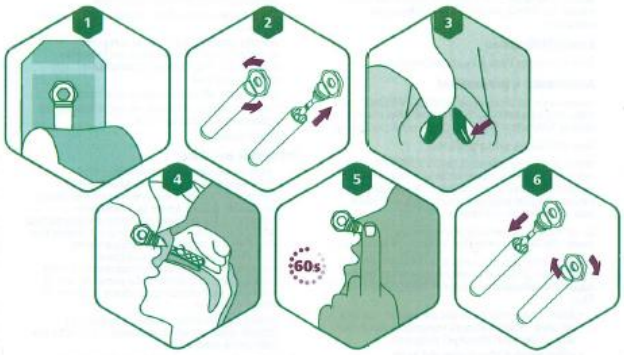
Hunt Developments (UK) Ltd
Units 15-18
Holmbush Industrial Estate
Midhurst, West Sussex
GU29 9HX
UNITED KINGDOM

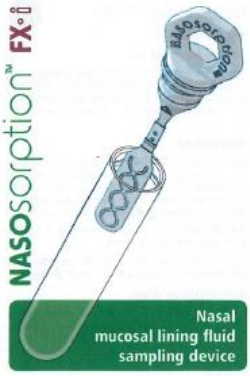
+44 (0) 1730 813020
www.nasosorption.com

General contact
info@huntdevelopments.co.uk
Complaint reporting
complaints@huntdevelopments.co.uk

HUNT

NSFL-FXI-IF10 rev 3





Nasosorption™ FX·i

en

Important

Please read before use.

Do not use the device if the packaging is opened, damaged or expired, or if there is physical contamination or discolouration of the device.

Description

NASOSORPTION™ FX·i is a non-sterile, single-use device consisting of a synthetic absorptive matrix (SAM) strip attached to an applicator handle for ease of nasal sampling.

NASOSORPTION™ FX·i is available in 3.0 mm, 4.5 mm and 7.0 mm widths.

NASOSORPTION™ FX·i is supplied housed in a cryo-transport tube for protection.

Intended Use

NASOSORPTION™ FX·i is intended to be inserted into the patient's nasal cavity by a trained healthcare professional. A sample of mucosal lining fluid is taken from the lowest nasal turbinate (inferior turbinate) as shown in figure 4.

Intended for use in a single nostril of a single patient.

NASOSORPTION™ FX·i is inserted into the nasal cavity for a period of 60±2 seconds in order to obtain a sample.

Contraindications

Not intended for infant use.

Warnings & Precautions

Select a NASOSORPTION™ FX·i device of a size appropriate to the patient. Do not insert SAM beyond the nasal cavity into the nasopharynx.

Do not sterilise before use.

Before applying NASOSORPTION™ FX·i to the nose, in all cases the anterior nasal cavity should be carefully examined by appropriately trained clinical staff using a light source, and a speculum where appropriate.

Nasal sensitivity may cause involuntary head movement, e.g. sneezing.

Caution should be employed in conditions where there is any risk of NASOSORPTION™ FX·i causing damage to the epithelial surface:

- local anatomical defects of the nasal and sinus passages: congenital malformations, septal defects, previous surgery
- nasal polyps and other luminal lesions
- nasal mucosal ulceration, including Wegener's granulomatosis (granulomatosis with polyangiitis, GPA) and Churg-Strauss syndrome
- nasal bleeding (epistaxis)
- local nasal vascular and/or capillary defects
- systemic bleeding disorders including coagulation defects (inherited or acquired), platelet insufficiency and thrombocytopaenia
- severe allergic rhinitis, chronic rhinosinusitis
- herpes simplex and herpes zoster (shingles), staphylococcal and streptococcal infection
- malignant lesions

Instructions for Use

1. Nasal examination should be performed as described in the Warnings & Precautions section above. With washed and gloved hands, peel open the foil pack and remove the NASOSORPTION™ FX·i device. Examine the device for signs of damage. If damage is found, do not use the device; contact the manufacturer. Keep the applicator housed in the tube until use to reduce the risk of contamination.
2. Unscrew the applicator using the handle and remove the applicator from the tube, avoiding contact with the absorbent end.
3. With one hand, hold the patient's head and push back the tip of the nose with the thumb to provide a clear line of sight. With the aid of a light source, carefully insert the NASOSORPTION™ FX·i device into the nostril and gently locate the absorbent strip flat against the surface of the inferior turbinate.
4. Figure 4 shows the correct position of the strip against the inferior turbinate.
5. Hold the device in place by asking the patient to press a finger against the side of the nostril for 60 seconds.
6. Release the finger before removing the applicator from the nasal cavity. Return the device to the tube and screw it back in.

Preservation of Sample

The sample should be refrigerated immediately but stored between -60° and -80°C as soon as practically possible.

Storage

Keep out of direct sunlight.

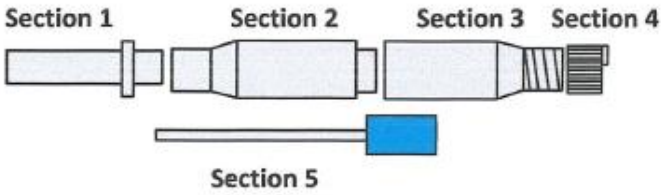
Until use, the device should be stored in its original packaging between 5°C and 25°C.

Revised October 2016

APPENDIX 2 – ORACOL Saliva Collection Instructions

Malvern Medical Developments Limited

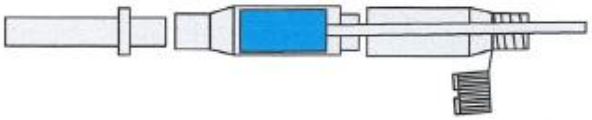
Instructions For Use – S14
ORACOL+ Saliva Collection System



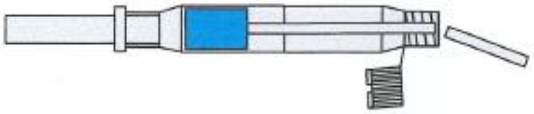
1. Remove the cap, unscrew Section 2 from Section 3 and remove the swab

2. Collected the saliva by rubbing the sponge firmly along the gum; (at the base of the teeth if present) until the sponge swab is wet, this takes about 1-2 minutes.

3. Put the swab sponge first in to section 2 and replace section 3



4. Snap off the stick at the break point and replace cap.



5. Return the sample to the lab for processing.

Unit 10, Northbrook Close, Worcester Worcs. U.K. WR3 8BP
Telephone: +44 (0)1905 731343 Fax: +44 (0)1905 731344

IFU-05 Issue 1, Issue Date 30-04-14

APPENDIX 3 – Sample Record Form

Site Name:			
Patient ID number:	**Site Code**		**Patient Code**
Visit:	Recruitment		Attach LIMS kit label here
	Day 3		
	Day 9		
	Convalescent		

Pathogen samples & Tempus – to be double bagged and frozen directly

Sample Collected	**Date Collected**	**Time Collected (estimate)**	**Comments**
Throat Swab			
SAM strip			
Oracol			
Urine			
Stool			
Rectal Swab*			
Tempus blood RNA tube			
Endotracheal Aspirate			
Other sample†			
Other sample†			

* Only collect rectal swab if not possible to obtain stool. Label with one of the spare LIMS labels.

† If other samples (eg ETA) are collected please indicate the sample type on the form and label with one of the spare LIMS labels.

All other blood samples should be sent to the lab for processing and recorded overleaf.

Blood Samples

To be completed by person collecting samples:					
EDTA		**SST**		**Sodium Citrate**	
Date Collected:		**Time Collected:**			
Comments:					

To be completed by person processing samples:				
Sample Type	**Number**	**Date Processed:**	**Time Processed (estimate):**	**Comments:**
EDTA Plasma (lavender)				
Cell pellet (red)				
Serum (gold)				
Citrated plasma (blue)				

APPENDIX 4 – Details of Contents of Package

A copy of this page must be included in every shipping box.

This package contains human samples infected with SARS-CoV-2, the virus causing COVID-19. These pose a potential risk of infection to humans and are classed as UN3373 Biological Substance Category B for transport.

These samples are being transported from:

<INSERT CONTACT NAME, ADDRESS AND PHONE NUMBER>

To:
Dr Sarah McDonald
MRC-University of Glasgow Centre for Virus Research
Sir Michael Stoker Building
Garscube Campus
464 Bearsden Road
Glasgow
G61 1QH
Tel: 0141 330 2989

In the event of a spillage or accident, the following guidelines must be adhered to:

First Aid and Reporting:

- Move exposed person(s) to a safe isolated area. Remove and isolate contaminated clothing and shoes
- CAUTION: exposed person(s) may be a source of contamination
- In case of contact with substance, immediately flush skin or eyes with running water for at least 20 minutes and seek medical attention
- Effects of exposure (inhalation, ingestion or skin contact) may be delayed. Ensure that medical personnel are aware of the substances involved and take precautions to protect themselves
- Seek medical help as soon as possible, stating clearly that individuals may have been exposed to SARS-CoV-2/COVID-19

Mitigation Procedures

- Isolate spill or leak area in all directions immediately and keep unauthorised personnel away
- Obtain identity of substance involved if possible and report the spill to the appropriate authorities
- Do not touch or walk through spilled material
- Do not touch damaged containers or spilled material unless wearing appropriate personal protective equipment
- Be particularly careful to avoid contact with broken glass or sharp objects that may cause cuts or abrasions that could increase the risk of exposure
- Absorb spilled materials with earth, sand or other non-combustible material (such as absorbable beads) while avoiding direct contact
- Cover damaged package or spilled material with damp towel or rag and keep wet with liquid bleach or other disinfectant
- Liquid bleach will generally inactivate the released substance