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Receipt, handling and storage of samples containing SARS-CoV-2

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# INTRODUCTION

## General

The correct handling and storage of samples from individuals infected with SARS-CoV-2 (COVID-19) is imperative in maintaining a safe working environment in the Biosafety level 3 (BSL3) laboratory.

The Centre for Clinical Microbiology (CCM) is acting as a supporting laboratory and it is processing samples on behalf of Royal Free NHS Foundation Trust Infection Diseases Unit as part of COVID-19 clinical trials.

This SOP outlines the correct procedures for the transfer of samples from the infectious disease ward to your department/laboratory name, and the disinfection and return of the specimen transport containers to the ward. It also outlines the correct procedures for the handling, storage and shipment of SARS-CoV-2 samples in your department/laboratory.

## Study overview

# HEALTH AND SAFETY

SARS-CoV-2 is classified as a Hazard Group 2 (HG) organism when using non-propagatory protocols (e.g. not culturing the virus) and a Hazard Group 3 (HG3) organism when using propogatory protocols (e.g. culturing the virus). As such all procedures relating to this SOP should be undertaken within a Biosafety level 3 laboratory, unless stated otherwise.

# RELATED DOCUMENTS

* RA024675- Safe Handling of *M. tuberculosis* and other HG3 organisms and materials containing them
* RA034368- Receipt, handling and Storage of SARS-CoV-2 samples
* RA023857- Decontamination of an individual from BSL3
* CCM\_POLICY\_006\_Overview of Biosafety level 3 laboratories
* CCM\_POLICY\_003\_Health & Safety Policy
* CCM\_POLICY\_011\_Procedure in the event of incident including inoculation
* CCM\_POLICY\_010\_Procedure in the event of a biological spillage or chemical event
* CCM\_POLICY\_009\_Procedure for disinfection
* CCM\_POLICY\_008\_Storage and removal of waste
* CCM\_SOP\_005\_ Decontamination of an individual from BSL3
* CCM\_SOP\_008\_Operation of microbiological safety cabinets
* CCM\_SOP\_026\_ Servicing, Maintenance and Calibration of Equipment SOP
* CCM\_SOP\_032\_Temperature monitoring SOP
* CCM\_SOP\_027\_Operation of centrifuges
* CCM\_SOP\_009\_Operation of the autoclaves
* CCM\_Form\_039\_Document Reading Log
* CCM\_CAF\_001\_BSL3 Decontamination Training CAF
* CCM\_CAF\_002\_Induction and Competency Assessment for Biosafety level 3 Laboratories
* CCM\_CAF\_009\_ Receipt, Handling and Storage of SARS-CoV-2 samples CAF

PHE Guidance- COVID-19: Safe handling and processing for samples in laboratories, updated 12 March 2020; https://www.gov.uk/government/publications/wuhan-novel-coronavirus- guidance-for-clinical-diagnostic-laboratories/wuhan-novel-coronavirus-handling- and-processing-of-laboratory-specimens

WHO Guidance on regulations for the transport of infectious substances 2019- 2020, applicable from 01 Jan 2019; https://apps.who.int/iris/bitstream/handle/10665/325884/WHO-WHE-CPI- 2019.20-eng.pdf?ua=1

ISARIC/WHO Clinical Characterisation Protocol UK, Lab Manual, updated 19 May 2020

ISARIC/WHO Clinical Characterisation Protocol for Severe Emerging Infections in the UK, updated 17 February 2020

# ABBREVIATIONS AND DEFINITIONS

|  |  |
| --- | --- |
| BSL | Biosafety level |
| CAF | Competency Assessment Form |
| CCM  | Centre for Clinical Microbiology |
| COVID-19 | Coronavirus disease 2019 |
| EDTA | Ethylenediaminetetraacetic acid |
| HG | Hazard Group |
| LRF | Laboratory Requisition Form |
| MGIT | Mycobacteria growth indicator tube |
| MRN | Medical Record Number (patient identifier) |
| MSC | Microbiological Safety Cabinet |
| NPS | Nasopharyngeal swab |
| RNA | Ribonucleic acid |
| SAM | Synthetic absorptive matrix |
| SARS-CoV-2 | Severe acute respiratory syndrome coronavirus 2 |
| SOP | Standard Operating Procedures |
| WHO | World Health Organisation |

# EQUIPMENT AND REAGENTS

## General

* Class 1 Microbiological Safety Cabinet (MSC)
* Centrifuge
* Centrifuge buckets with aerosol-resistant lids
* Vacutainer tubes to balance (red and purple lid)
* Microtube rack(s)
* MGIT rack
* Dispo Safe Jar. Supplier: Microbiological Supply. Product Code: DL64
* VernaGel (1 bag per dispo safe jar) Supplier: Verna Care, cat no 450SC100
* Tristel Disinfectant; Supplier: Tristel Solutions. Product Code: TSL010501
* -70°C to -80°C Freezer
* Micropipettes and Aerosol Resistant Tips (20-200μl and 100-1000μl)
* Safety Box: Supplier: Fisher Scientific Ltd. Cat no: 10561773.
* Sealable Polythene Bags: SLS BAG1208
* small specimen bags (4.5’x 4.5’)
* medium specimen bags (150 x 240 mm)
* big specimen bag (10’x 14’)
* Trolley (Clax Mobil): Product Number: 139-1107
* Marker pen

## Study name specific

Barcode-identified sample pack (the contents may vary):

* Serum vacutainer tubes
* EDTA vacutainer tubes (1-3 tubes)
* Tempus RNA vacutainer tubes
* Nasal synthetic absorptive matrix (SAM) strip
* Nasopharyngeal swab (NPS)
* Universal container (for urine, respiratory secretions and stool samples)
* 3 yellow top cryovials
* 9 purple top cryovials
* Pathoseal bag
* Sample information sheet (NOT to be taken into BSL3)

# PROCEDURE

## Transfer and receipt of samples from the infectious disease ward to the name of laboratory BSL3 laboratory

1. After sample collection in the infectious disease ward, samples must be wiped down with an appropriate viricidal disinfectant (e.g. chlorox wipes) before being placed into a sealable specimen bag, which must also be wiped down. All samples from the same patient must be placed in a second sealable specimen bag, which must also be wiped down. Ensure specimens and bags to dry following disinfection before placing into the next bag.

**NOTE:** Failure to ensure that all samples and bags are dry may result in the samples being misidentified as leaking and therefore not being processed by the name of laboratory BSL3 laboratory team.

1. Once double-bagged the samples (along with the corresponding barcoded cryovials and Pathoseal bags) must then be placed into a sealable canister (one per patient) in the ante-room of the infectious disease ward.

**NOTE:** please ensure that canisters are NOT taken into the contaminated areas. Canisters must be safe to handle in a non-contained environment.

1. The canister(s) must then be placed into a rigid, sealable transportation box before being transported to the name of laboratory. The sample processing sheet for each sample must accompany the samples.

**NOTE:** ALL canisters must be transported within the transportation box. If there are more canisters than will fit in the transportation box, make two (or more) journeys from the ward to name of laboratory.

1. Before bringing the transportation box to the name of laboratory, a message must be sent in the respective WhatsApp group. The time for delivery will be agreed to ensure that a member of the name of laboratory staff is available to receive the sample(s). A name of laboratory staff member will respond and advise how long they will take to arrive into name of laboratory, if not already present.
2. Once the transportation box arrives at name of laboratory, a BSL3 trained member of staff will place the transportation box on a trolley and transfer into the BSL3 laboratory. After opening the box, use gloved hands to remove the canisters and place in the fridge or cabinet. Spray the transportation box, internally and externally, with Tristel and wipe dry. The transportation box and trolley can then be removed from the BSL3 laboratory.
3. Return the transportation box to the infectious disease ward member of staff.
4. A sample information sheet, pre-filled and signed by the relevant nurse must also be provided for each patient. This is to be checked by the name of laboratory member of staff receiving the samples.

## Procedure for processing SARS-CoV-2 samples COVID-19 Rota

The sample processing requires the presence of at least two emergency response trained members of staff in name of laboratory, one or more BSL3 trained individuals to process the samples, and one BSL3 trained/emergency response trained individual to act as the ‘buddy’ to provide support in case of an incident. When more than two studies overlap collection and processing of samples, additional member(s) of staff will be present to assist with processing the samples. The rota can be found name the place the rota can be found (e.g. provide file address).

For study/clinical trial name samples in instances where only one staff member is available due to unforeseen circumstances, samples can be received by the staff member and stored overnight at 4°C inside the fridge in the BSL3 laboratory and processed the following day. Once samples have been received and stored in the fridge a ‘task completed’ message must be sent to the WhatsApp group allowing other members to ensure they are safe. If the ‘task completed’ message has not been received within 1 hour of sample receipt a member of the Group will attend the laboratory to ensure the safety of the worker.

## BSL3 Emergency response procedures

All BSL3 laboratory staff are trained to deal with an uncontrolled spillage incident in the BSL3 laboratory. Emergency response procedures for working in the BSL3 laboratory are outlined in CCM\_SOP\_005\_Decontamination of an individual from BSL3.

In the event of an incident on:

* Working days: routine procedures are followed as per CCM\_SOP\_005.
* Weekends and unusual working days (reduced staff numbers): the buddy will follow CCM\_SOP\_005, and at the first opportunity contact the Centre Director on provide a phone number/contact details and send an urgent request for support on the group on WhatsApp. Colleagues will attend. The priority is to attend to the safety of the laboratory worker.

## Labelling

### Tube labels

Each set of patient samples should arrive with a sample information sheet. All samples and cryogenic tubes are barcoded such that no further labels are to be printed by the name of laboratory team. Barcodes are provided for:

* Cryogenic tubes: yellow for serum aliquots and purple for plasma aliquots: serum #1, serum #2,
* Blood Vacutainer tubes: clotting, EDTA and Tempus RNA tube;
* Stool;
* Urine;
* Flocked swab samples (NPS);
* Nasal SAM sticks
* Extra labels for additional samples.
* Labelled, sealable, blood components and pathogen bags are also provided.

It is recommended upon opening the canisters, that the custody number (00## of the RAL01 subject number) is handwritten on the clotting (yellow) and EDTA (purple) vacutainers, as well as on top of the barcoded cryovials to identify samples correctly and avoid mix up errors.

### Bag labels for freezer storage and shipping

Although the samples will come double-bagged in a canister, they are shipped in triple bags (refer to Appendix 1).

Blank external bag labels (template stored on the name the file address/location of the template) will be printed and left in BSL3 ready to be filled in by the processing member of CCM. The bag label should contain the following information (if available):

* Study: name study
* Collection site: your site name
* Sample day (e.g. Day 1)
* Collection date
* Custody number: xxxx

The samples must be packed and labelled as described in the clinical trial manual.

## Sample processing

***In the event of any spillage (inside/outside the MSC) and any breakage/leakage inside the centrifuge please refer to CCM\_POLICY\_010 (Procedure in the Event of a Biological or Chemical Spillage) for instructions.***

### Preparation

1. Inside the MSC open the canisters and check what samples are in the canisters and there is no sign of leakage. If there are any signs of leakage proceed as described in section ‘6.2.6 How to deal with leaking samples’.
2. The samples may include the following:
	* Stool
	* Urine
	* Swab (15ml tubes) or nasal SAM strip (5ml tube)
	* Clotted blood vacutainer tube (yellow lid)
	* 1-3 EDTA blood vacutainer tubes (purple lid)
	* Tempus RNA tube (blue lid)
	* Sputum (occasionally)

**NOTE:** sometimes not all the sample types listed above will be present. Please record in the hard copy logs and on the S drive sample tracker spreadsheet (name the file address here) after finishing the processing. The samples may arrive at different times, so will need to be added to the rest of the samples for that patient and collection time point, then noted on the tracker spreadsheet.

1. If stool, urine and/or swab sample are received these should not be unpacked from the specimen bags. Check these are correctly labelled and place them, in their original specimen bag, back in the canisters.
2. Unpack the blood samples from the inner sample bags, wipe them with a paper towel soaked in Tristel and place in a rack.
3. Using a marker pen, label the vacutainer tubes with the corresponding custody number.
4. Unpack the cryovials and using a marker pen label them with the corresponding custody number.
5. After processing the clotting and EDTA blood, place the Tempus RNA tube in the same bag as the blood vacutainer tubes.

### Clotting blood vacutainer tube (yellow lid)

1. Take the centrifuge buckets into the MSC, ensure there are four blue bucket inserts, place the tubes in the buckets balancing them with balance vacutainer tubes filled with water if necessary.
2. Close the bucket lids, wipe down the buckets and the lids with a paper towel soaked in Tristel before removing from the MSC.
3. Place the buckets in the centrifuge and centrifuge the blood tubes at 1500 x *g* for 10 minutes.
4. Once these tubes have finished spinning, set the centrifuge to 4°C.
5. Take the centrifuge buckets to the MSC. Open the buckets and transfer the tubes to a rack.
6. Wipe down the centrifuge buckets and the lids with a paper towel soaked in Tristel before removing from the MSC.
7. Using a piece of paper towel, carefully open the vacutainer tube.
8. Collect all of the serum (the top, yellow layer) and place equally into the three barcoded yellow top cryovials. The average volume for each cryovial is approximately 500 μl.
9. Wipe the blood vacutainer tube and the cryovials with a paper towel soaked in Tristel.

### EDTA vacutainer tubes

1. Take the centrifuge buckets into the MSC, ensure there are three blue bucket inserts. Place the tubes, balancing them with balance vacutainer tubes filled with water if necessary.
2. Close the bucket lids, wipe down the buckets and the lids with a paper towel soaked in Tristel before removing from the MSC.
3. Place the buckets in the centrifuge and centrifuge tubes at 1500 x *g* for 10 minutes at 4°C.
4. Following centrifugation take the centrifuge buckets to the MSC.
5. Open the buckets and transfer the tubes to a rack. If the separated layers are mixed, a second centrifugation should be performed before processing the samples.
6. Wipe down the centrifuge buckets and the lids with a paper towel soaked in Tristel before removing from the MSC.
7. Using a piece of paper towel, carefully open the vacutainer tube.
8. Collect all of the plasma (the top, yellow layer) and place equally into three barcoded purple cryovials. The average volume for each cryovial is approximately 500 μl. Repeat for each EDTA tube received *e.g.* if three EDTA tubes are received then nine purple cryovial tubes will be aliquoted into.
9. Wipe the blood vacutainer tube and the cryovials with a paper towel soaked in Tristel.

### Sample packaging and storage

1. All equipment and consumables to be removed from the MSC **MUST** be wiped with a paper towel soaked in Tristel before removal.
2. Package the samples into the bags labelled in section 6.5.4 as follows (refer to Appendix 1):
3. Ensure all the vacutainer and cryovials are wiped with a paper towel soaked in Tristel and allowed to dry before placing them in the bags.
4. Take the inner small bags into the MSC and place the vacutainer tubes and the cryovials into their respective bags.
5. Ensure that air is expelled from inside the bags and seal them correctly, wipe with a paper towel soaked in Tristel.
6. Place the inner small bags into the provided respective intermediate Pathoseal bags or a sealable medium bag, expelling the air and sealing the bags.
7. If stool, urine and/or swab samples are received these should be removed from the canisters and wiped with a paper towel soaked in Tristel.
8. Roll the bags and place in the respective Pathoseal bag.
9. Wipe the two Pathoseal bags with Tristel and remove them from the MSC. Place them inside the external big bag along with the bag label. Expel air and seal the bag.
10. Place the external big bags inside the appropriate box in the -70°C freezer. Late samples such as stool samples can be double bagged and placed inside the associated external big bag.

## Waste management

1. After sample processing is complete ensure all the original sample packaging (including sample bags and bubble wrap) is placed into the dispo jar and waste is removed as described in CCM\_POLICY\_008 (Storage and Removal of Waste).
2. Remove and discard or void any labels affixed to the canisters. Spray the inside and outside of the canisters and wipe down with a paper towel soaked in Tristel and place them open and the lids in a double autoclave bag.
3. Autoclave the canisters, and any full autoclave tins, using the BSL3 waste cycle appropriate for the autoclave used.
4. Following autoclaving, the canisters can be returned to the research nurses to re-use. **How to deal with leaking samples**
5. When taking the samples from the canister, observe if there is any sign of leakage inside or outside the bag containing the sample.
6. If there is leakage inside the bag, make sure the bag is sealed and discard the sealed bag in the dispo jar.
7. If the leakage is present outside the bag and/or the leaking bag is open, follow the steps below:
8. Place the bag inside the dispo jar (containing 1-3 VernaGel) inside the MSC, any other contaminated bags and any liquid present in the canister must also be discarded into the dispo jar (taking care to minimise aerosol generation).
9. Wipe the gloved hands with the paper towel soaked in Tristel.
10. Change your gloves.
11. Spray the outside of the canister, and the outside & inside of the lid with Tristel.
12. Fill the canister with Tristel, replace the lid and leave inside the MSC to soak for **at least 30 minutes**.
13. Discard the waste disinfectant into a dispo jar with sufficient VernaGel.
14. Place the lid on the canister *loosely*.
15. Spray the outside of the canister and lid before removal from the MSC and double bag the open canister and the lid for autoclaving as per CCM\_POLICY\_008 (Storage and Removal of Waste).

## Logs and records

There are two record forms to be completed: one is the sample information sheet that

comes with the samples, and the other is a name your laboratory record form for internal track purposes.

Archive the hard copy forms in the COVID-19 folder on the shelf above the laboratory manager’s desk in research office 1 (state location). The folders ‘High consequence pathogen group’ and COVID-19 2020 were created on S drive (add file address) to store digital records and any documentation related to this protocol.

The sample information sheet requires the following information:

* Which samples were collected and the time and date of collection, to be filled and signed by the nurse
* Record the date and time the samples were processed
* Record the time original samples and aliquots were placed in the -70°C freezer
* Signature of the person that processed the samples
* Internal record:
* Identification of the patient: custody number (RAL01 xxxx), MRN and initials (if available)
* Day and date of collection
* Colour in green the samples that arrived and in red those which were not collected.

Once all required information has been recorded, scan and take three photocopies of the original hard copy log. Place the digital copy in the appropriate folder on S drive (add file address). The original log and each of the three copies have a dedicated wallet in the hard copy folder: one for the shipment, one for the study name team and one for name of laboratory’s records. There is a log file to check the hard copy records in the same folder.

If a sample is delivered on a different day to the collection day, process it and store it with the previous samples (for that patient for that collection time point) at -70°C.

Amend the study and internal logs, make three copies and scan the amended log. Attach the copies of the original and amended logs together.

## Shipment

The shipment of the samples to other laboratory is arranged by the study name team and is pre-scheduled for every Tuesday at 10 am. The courier will bring the appropriate polystyrene boxes (45 L each) and packs of dry ice (5 kg each). Please advise the other laboratory team in advance on the appropriate number of boxes. A form recording which samples are being shipped must be pre-filled before the collection, printed and signed and sent to other laboratory every Monday. An example of the form is archived on the S drive (name of file address).

The courier will send one, or more, appropriate boxes and dry ice to accommodate the samples. The printed form, original logs and one copy of the logs should be prepared and placed in a big specimen bag (A4 size) and sent along with the shipment boxes.

1. On the day before the scheduled shipment (Mondays) complete the shipment form, print, sign and scan it.
2. Send the scanned form and the scanned logs for each sample being shipped to other laboratory (laboratory contact details).
3. On the scheduled date and time (Tuesdays at 10 am), the courier will bring the boxes and dry ice.
4. The courier boxes, dry ice and a sealable plastic bag (containing the original shipment form, original logs and copies) must be placed in a trolley and taken to the BSL3 laboratory.
5. In the BSL3, keep the box on the trolley, open the box and add a layer of dry ice.
6. Pack the samples leaving 10 cm for a second layer of dry ice. If the box is not filled with samples, fill it with dry ice to keep the appropriate temperature. Close the lid.
7. Before sealing the external cardboard box, place the documentation bag inside it. Seal the box with tape. If more than one box is necessary, identify the boxes (e.g. 1 of 2, 2 of 2) and place the documentation in the first box (e.g. 1 of 2). Transport the trolley outside the BSL3 and handle it to the courier staff who will label the boxes with the appropriate collection and destination information.

# TRAINING AND COMPETENCE ASSESSMENT

Staff involved in the processing of COVID-19 samples must be deemed competent to work in BSL3. All BSL3 procedures are described in CCM\_POLICY\_006\_Overview of Biosafety level 3 laboratories.

Training on the procedure for handling and processing COVID-19 will be provided by a trained member of staff. The newly trained member of staff will be observed by a competent member of staff to assess competency before being permitted to process COVID-19 samples unsupervised.

Once a staff member is trained and deemed competent, they must be added to the study delegation log for COVID-19 sample processing. The trainer will inform the study coordinator that the trainee is competent to be added to the delegation log.

All training and competency assessments are to be documented on the appropriate Training and Competency Forms (CAFs).

# QUALITY CONTROL PROCEDURES

N/A

# MAINTENANCE

All equipment is maintained and calibrated as per the Servicing, Maintenance and Calibration of Equipment SOP (CCM\_SOP\_026).

Temperature sensitive equipment and room temperatures are monitored via a continuous temperature monitoring system as per the Temperature monitoring SOP (CCM\_SOP\_032).

# VALIDATION DOCUMENTS

N/A

# APPENDICES