REMAP-CAP Laboratory SOP  
(INTENSIVE SAMPLING)  

COVID-19 IMMUNOGLOBULIN THERAPY  
DOMAIN
Summary
In this domain of the REMAP-CAP trial, participants meeting the platform-entry criteria for REMAPCAP will be randomised to receive interventions using immunoglobulins as the basis for treatment:

- No immunoglobulin against COVID-19 (no placebo)
- Convalescent Plasma

This laboratory manual includes information on sample processing, storage, recording, and transport of samples in the UK. There are two sampling structures for this clinical trial. **Standard sampling** will include serum and virology samples taken at baseline only (Baseline (Day-1) is defined as day patient is consented to be randomised into the trial). **Intensive sampling** includes up to 9 timepoints of repeated research blood sampling.

Samples should be taken from all patients randomised into the Immunoglobulin Therapy Domain.

Your site has opted for **INTENSIVE SAMPLING** (Table 1)

Contacts

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### Table 1: Schedule of intensive research sampling (includes blood and virology samples)

<table>
<thead>
<tr>
<th></th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
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<tr>
<td>Blood (EDTA)</td>
<td>*</td>
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<tr>
<td>2ml</td>
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<tr>
<td>Blood (EDTA)</td>
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<tr>
<td>4ml</td>
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<tr>
<td>Blood (serum)</td>
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<tr>
<td>6ml</td>
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<tr>
<td>PAXgene</td>
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<td>*</td>
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<tr>
<td>2.5ml</td>
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<tr>
<td>Nasopharyngeal or Oropharyngeal swab</td>
<td></td>
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<td>*</td>
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</tbody>
</table>

Samples taken from admission up to hospital discharge.

For patients randomised to plasma, the Day 1 and Day 2 samples should be taken prior to first and second units of plasma, respectively.

Follow-up samples at Day 3, Day 4, Day 6, Day 9, Day 15 and Day 28 are recommended. Samples can be taken +/- 12 hours of the defined time within the sampling protocol. Additional samples on Day 12 can be submitted.
1. Intensive sampling
A total of 400 patients will be included in this sampling sub-study (across all of the participating sites). Samples should be taken from all patients randomised into the Immunoglobulin Therapy Domain.

Labelling samples
Labels are provided by the core laboratory:

- Label each type of sample with corresponding sticker
- RN = REMAP-CAP Randomisation Number
- Day of sample collection (‘Day’ as per Table 1 Schedule of intensive sampling)
- Sample type is printed:
  - PLASMA
  - SERUM
  - CELLS
  - RNA = PAXgene Tube
  - VIRUS = Respiratory virology samples

- Number 01 to 36 after Sample type refers to the ‘aliquot’ number. We expect you to use 4 aliquots per day of sample collection.

- Number P001 refers to the LABEL number, which is the consecutive number of patients recruited at your site. Please include this number in the intensive sampling log.
  For example, your stickers may start from P020. Then the next patient at your site will be P021.
  NOTE: This number is DIFFERENT from the randomisation number (RN).
The supplies will be provided by the core Research Laboratory, with the exception of:
- Locally provided PPE as per local guideline
- Locally provided
  - Nasopharyngeal or oropharyngeal swabs
  - yellow top or red top or equivalent tube for serum
  - EDTA Blood Collection Tubes for plasma
  - 2ml EDTA tube for aliquoting whole blood

Details of samples and processing

1.1. Equipment required
   Nasopharyngeal or oropharyngeal swabs

1.2. Virology samples collection
   Collect either an oropharyngeal or nasopharyngeal swab, as per local ICU practice.
   Double bag the respiratory tract sample, after collection.
   Store in minus 80°C in the double bag.

1.3. Virology samples processing
   Double bag the respiratory tract samples, after collection.
   Store at minus 80°C in the double bag.
   All specimens should remain at -80°C prior to shipping.
   The samples should NOT be thawed prior to shipping.
1.4. Serum samples

1.4.1. Equipment required
Collect one yellow top or red top or equivalent tube for serum.
Centrifuge with swinging bucket rotor
Sterile cryovials with writing surface
Pipettes (250µL; 500µL; 1000µL)
Gloves and other PPE as per local guidelines

1.4.2. Serum separation
Keep the filled blood collection tubes (vacutainers) upright, after the blood is drawn, at room temperature (18°C-25°C) for 30 - 60 minutes to allow the clot to form.
Centrifuge the blood samples at the end of the clotting time as follows
- 10 minutes
- 1100-1300 g NOTE: DO NOT EXCEED centrifuge speed over 1500 g
- Room temperature

1.4.3. Serum labelling and aliquots
Label 4 cryovials using the stickers provided. Use a 500µL pipette to transfer the serum into the labelled cryovials, filling the vials in sequential order.
Please ensure that each aliquot volume ranges between 250 microlitres and 500 microlitres. Close the caps on the vials tightly. If there are additional aliquots, label additional cryovials using permanent marker provided with the randomisation number, date, and aliquot number. Do NOT use additional stickers. This process must be completed within 1 hour of centrifugation.

1.4.4. Serum storage
Immediately place all aliquots upright in a labelled specimen box in an -80°C or colder freezer.
All specimens should remain at -80°C prior to shipping.
The samples should NOT be thawed prior to shipping.

Additional notes
Be careful not to pick up red blood cells when aliquoting. This can be avoided by keeping the pipette above the red blood cell layer and leaving a small amount of serum in the tube. If immediate centrifugation is not possible, refrigerate the red top tubes at 2-80C and centrifuge within 4 hours and follow above steps.
CHECK: That all aliquot vial caps are secure and that all vials are labelled.
1.5. Plasma samples

1.5.1. Equipment required
Collect one EDTA Blood Collection Tubes for plasma (4mls of blood)
Centrifuge with swinging bucket rotor
Sterile cryovials with writing surface
Pipettes (250µL; 500µL; 1000µL)
Gloves and other PPE as per local guidelines

1.5.2. Plasma separation
After blood collection, mix the blood gently by inverting the collection tubes 8 to 10 times.
Centrifuge ideally within 2 hours* of collection
  - 10 minutes
  - 1100-1300 g NOTE: DO NOT EXCEED centrifuge speed over 1500 g
  - Room temperature

1.5.3. Plasma labelling and aliquots
Label 4 cryovials using the stickers provided. Use a 500µL pipette to transfer the plasma into the labelled cryovials, filling the vials in sequential order.
Please ensure that each aliquot volume ranges between 250 microlitres and 500 microlitres.
If there are additional aliquots, label additional cryovials using permanent marker provided with the randomisation number, date, and aliquot number. Do NOT use additional stickers.
Close the caps on the vials tightly. This process must be completed within 1 hour of centrifugation.

1.5.4. Plasma labelling and storage
Immediately place all aliquots upright in a labelled specimen box in an -80°C or colder freezer.
All specimens should remain at -80°C prior to shipping.
The samples should NOT be thawed prior to shipping.

Additional notes
Be careful not to pick up red blood cells when aliquoting. This can be avoided by keeping the pipette above the red blood cell layer and leaving a small amount of plasma in the tube.
*Plasma collection and storage should ideally be done within 2 hours and acceptable to done within 4 hours.
CHECK: That all aliquot vial caps are secure and that all vials are labelled.
1.6. Cell aliquots from 2ml EDTA samples

1.6.1. Equipment required
Collect one EDTA Blood Collection Tubes for plasma (2mls of blood)  
Pipettes (250µL; 500µL; 1000µL)  
Gloves and other PPE as per local guidelines  
Cytodelics blood stabiliser will be provided by the central lab and it is stored in a Fridge at 2-4C.

1.6.2. Cell labelling and aliquots
• Preparation BEFORE blood collection (includes labelling)  
Label 4 cryovials with stickers provided.  
Aliquot 500ul of cytodelics blood stabiliser to each (cryovials can be pre-prepared and kept in the fridge)  
Allow 10 minutes to equilibrate to room temperature.

• Steps AFTER blood collection  
Invert the 2ml EDTA tube 6-8 times.  
Transfer 500ul into each cryovial (within 2 hours of collection).  
Mix well blood with the cytodelics reagent by inverting 15 times (DO NOT vortex).  
Incubate at room temperature for 10 minutes.  
If there are additional aliquots, label additional cryovials using permanent marker provided with the randomisation number, date, and aliquot number.  
Do NOT use additional stickers.

1.6.3. Cell aliquots storage
Place in Eppendorf box and store in -80 freezer.  
All specimens should remain at -80℃ prior to shipping.  
The samples should NOT be thawed prior to shipping.

Additional notes
The aliquoting of blood into cytodelics reagent filled cryovials should be completed as soon as possible, ideally within 2 hours and no later than 4 hours.  
If -80 freezer is not available a -20 freezer can be used. Samples do not last as long so ensure transport to central laboratory.  
CHECK: That all aliquot vial caps are secure and that all vials are labelled.
1.7. PAXgene samples

1.7.1. Equipment required
Ensure that the PAXgene Blood RNA Tubes are stored at room temperature (18°C-25°C) prior to use.
Gloves and other PPE as per local guidelines

1.7.2. PAXgene Tube collection
Label one PAXgene Tube with stickers provided
Collect one PAXgene Blood RNA Tubes at pre-defined time points.
Hold the PAXgene Blood RNA Tube vertically
Allow at least 10 seconds for a complete blood draw to take place. Ensure that the blood has stopped flowing into the tube before removing the PAXgene Blood RNA Tube from the holder.
Gently invert the PAXgene Blood RNA Tubes 8 to 10 times.

1.7.3. PAXgene Tube processing
Store the PAXgene Blood RNA Tubes upright at room temperature (18°C-25°C) for a minimum of 2 and a maximum of 6 hours.

1.7.4. PAXgene Tube storage
Transfer the tubes to a -80°C freezer for storage until shipping.
All specimens should remain at -80°C prior to shipping.
The samples should NOT be thawed prior to shipping.

Additional notes
If -80 freezer is not available a -20 freezer can be used. Samples do not last as long so ensure transport to central laboratory.

PAXgene tubes can be transported via pod system as per local hospital policy.
1.8. Transfer of samples to core laboratory

Samples should be sent weekly to the core laboratory. Sites will be responsible for arranging the courier collections. Collections must take place between Monday – Thursday.

The courier will provide dry ice.

Copies of the relevant ‘Intensive Sampling Logs’ must be included with each shipment, and the originals retained on site.

Please notify the core laboratory of planned shipments (include number and type of samples):

remap-plasma@kcl.ac.uk
(cc matthew.fish@kcl.ac.uk)

Core laboratory Address:
MSH Group (REMAP Trial), Department of Immunobiology, 2nd Floor Borough Wing, Admin Office, Guy’s Hospital, SE1 9RT

1.9. Courier Details

City Sprint
# Intensive Sampling Log

## For Intensive sampling sites

**SITE:**______ **RANDOMISATION NUMBER:** ________________  **Label Number** _____________ **SHEET NUMBER** 1/2

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<tr>
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<td><strong>no. aliquots</strong></td>
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<td><strong>Time frozen</strong></td>
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<tr>
<td>6ml Blood (serum)</td>
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<tr>
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<tr>
<td><strong>Comments</strong></td>
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For Intensive sampling sites.

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**SITE:**______ **RANDOMISATION NUMBER:** ________________  **Label Number** _____________ **SHEET NUMBER** 1/2
## For Intensive sampling sites

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<td>2ml Blood (EDTA)</td>
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<td>4ml Blood (EDTA)</td>
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<td>6ml Blood (serum)</td>
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**Comments**

**SITE:**

**RANDOMISATION NUMBER:**

**Label Number**

**SHEET NUMBER 2/2**