**General**

* 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.
* Well-established protocols can be used to handle samples from patents with suspected or confirmed pathogens of interest (currently novel coronavirus MERS-CoV and influenza A/H7N9 & A/H5N1).
* Trusts 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.
* All samples need to be processed as soon as they are received in the lab.
* All samples should have been received marked with a minimum data set of name, date of birth, date of sample and sample type. Please ensure this data is present and then recorded on the ISARIC laboratory record form.
* Red ISARIC RESEARCH labels, if provided, should be attached to all samples and secondary packaging before forwarding to PHE or HPRU labs. When labels are not available please ensure secondary containers are distinctly marked “ISARIC RESEARCH” preferably using a thick red marker pen.

**Expected blood samples on Day 1 (D1) Refer to tables 2 & 3**

* Up to 3 x 2 ml (varies with weight of child) EDTA blood tubes (purple-top)
* 1 x 3ml plain clotted blood (red-top)
* 1 x 9 ml Tempus tube (blue-top)

**EDTA tube**

* Centrifuge EDTA tubes at 1500 x g for 10 minutes at 4°C
* For each EDTA tube, use a pipette with disposable sterile tips to make 3 aliquots of plasma supernatant of approximate equal volume (total max 9 aliquots of plasma for all EDTA tubes, number will vary per number of EDTA tubes received)
* **Avoid disturbing or touching the blood pellet with the pipette tip**
* **DO NOT discard** **the primary EDTA tubes** containing blood pellets and **DO NOT discard** **the EDTA tube caps**
* It is expected that a maximum of 3 x 500ul plasma supernatant aliquots can be made from each EDTA tube
* Each aliquot should be in an individual, screw-top (with silicone o-ring) Cryovial (or similar container)
* Do not overfill the vials and ensure that the lids are completely screwed down.
* Ensure secondary containers for these aliquots clearly identifies them as **PLASMA** and ISARIC RESEARCH, as once made they are indistinguishable from serum aliquots.
* Replace and secure the caps on each of the four EDTA tubes containing pellets and ensure that each tube has been labelled (labelling should have taken place at the time of sampling)
* Freeze all aliquots and the 3 primary EDTA tubes containing packed cells at -80°C (or at -20°C if a -80°C freezer is not available)

Continued over.

**Plain clotted blood (red-top) tube**

* The clotted blood sample should have been allowed to stand for 30 minutes after sampling took place
* Centrifuge at 1500 x g for 10 minutes at 4°C
* Using a pipette with disposable sterile tips, make 3 aliquots of serum supernatant of approx. equal volume
* **Avoid disturbing or touching the blood pellet with the pipette tip**
* It is expected that a maximum of 3 x 500ul serum supernatant aliquots can be made from a 3ml clotted tube
* Each aliquot should be in an individual screw-top (with silicone o-ring) Cryovial
* Do not overfill the vials and ensure that the lids are completely screwed down
* Ensure secondary containers for these aliquots clearly identifies them as **SERUM** and ISARIC RESEARCH, as once made they are indistinguishable from plasma aliquots.
* Freeze all aliquots at -80°C (or at -20°C if a -80°C freezer is not available)
* The original red-top blood tube, its cap and its remaining clot should be disposed

**Tempus Tube (note glass tube – FRAGILE)**

* No processing of the contents is required, but ensure that the tube has been labelled at source with name, date of birth and sample date, and secondary container is clearly marked ISARIC RESEARCH.
* Freeze the tube immediately at -80°C (or at least at -20°C if a -80°C freezer is not available)

Table 2. Sampling pattern - In Patient Recruitment

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Serial samples. | | | | | | | | | | | | | | |  |
|  | Recruitment | Week 1 | | | | | | Week 2 | | | | | | |  | Further samples | Convalescent samples |
| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | Weekly until max 100 days | 3 months and 6 months after recruitment |
| 20 to 40kg | R |  | S |  | S |  | S |  | S |  | S |  |  |  |  | S | C |
| 10 to 20kg | R |  | S |  | S |  | S |  | S |  | S |  |  |  |  | S | C |
| 4 to 10kg | R |  | S |  | S |  | P |  | S |  | P |  |  |  |  | S | C |
| >4kg | R |  | S |  | S |  | P |  | S |  | P |  |  |  |  | S | C |
| Sample priority | 1 |  | 2 |  | 5 |  | 7 |  | 3 |  | 8 |  |  |  |  | 6 | 4 |

R = recruitment samples. S = serial samples including pathogen samples; P = research pathogen samples only; C = convalescent samples (see Table 3). In the event that local resource limitations require sampling frequency to decrease, samples will be prioritised as shown (1=highest priority). Serial sampling will stop when acute illness resolves or a patient is discharged from hospital: next samples taken will be the blood sample at 3 months and 6 months post recruitment.

Table 3. Samples

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Weight | Samples at recruitment (R) | Serial samples (S) | Convalescent samples | Total Volumes of blood taken |
| 20 to 40kg | 6ml (3x2ml) EDTA blood  3ml blood in serum(clotted) tube  3ml blood in blood RNA tube  Research pathogen samples | 1ml EDTA blood  2ml blood in blood RNA tube  Up to 3 additional 0.5ml samples in EDTA or fluoride oxalate tubes spread throughout dosing schedule for pharmacokinetic/pharmacodynamic studies.  Research pathogen samples | 1ml EDTA blood  3ml blood in serum(clotted) tube  2ml blood in blood RNA tube  Research pathogen samples | Maximum any day: 12ml (0.6ml/kg)  Maximum any 4 weeks: 42ml (maximum 2.1ml/kg) |
| 10 to 20kg | 2ml EDTA blood  2ml blood in serum(clotted) tube  2ml blood in blood RNA tube  Research pathogen samples | 1ml EDTA blood  1ml blood in blood RNA tube  Up to 3 additional 0.2ml samples in EDTA or fluoride oxalate tubes spread throughout dosing schedule for pharmacokinetic/pharmacodynamic studies.  Research pathogen samples | 1ml EDTA blood  1ml blood in serum(clotted) tube  1ml blood in blood RNA tube  Research pathogen samples | Maximum any day: 6ml (0.6ml/kg)  Maximum any 4 weeks: 23.6ml (maximum 2.36ml/kg) |
| 4 to 10kg | 1ml EDTA blood  1ml blood in serum(clotted) tube  ml blood in blood RNA tube  Research pathogen samples | 1ml EDTA blood  Up to 3 additional 0.2ml samples in EDTA or fluoride oxalate tubes spread throughout dosing schedule for pharmacokinetic/pharmacodynamic studies.  Research pathogen samples | 1ml EDTA blood  1ml blood in serum(clotted) tube  Research pathogen samples | Maximum any day: 2ml (0.5ml/kg)  Maximum any 4 weeks: 9.4ml (maximum 2.35ml/kg) |
| < 4kg | 0.5ml EDTA blood  0.1ml blood in serum(clotted) tube  ml blood in blood RNA tube  Research pathogen samples | 0.2ml EDTA blood  Up to 3 additional 0.1ml samples in EDTA or fluoride oxalate tubes spread throughout dosing schedule for pharmacokinetic/pharmacodynamic studies.  Research pathogen samples | 0.2ml EDTA blood  0.2ml blood in serum(clotted) tube  Research pathogen samples | Maximum any day: 0.8ml (~0.27ml/kg)  Maximum any 4 weeks: 2.4ml (maximum 2.4ml/kg) |
| Research pathogen samples (all patients) | Pathogen samples taken solely for research purposes:   1. In all patients: combined nose and throat swab 2. In all intubated patients: endotracheal aspirate   also where resources permit:   * 1. Nasopharyngeal aspirate (NPA) OR (if NPA impossible) flocked nose and throat swab   2. Urine (up to 10ml in sterile universal container, if available)   3. Rectal swab or stool (up to 10ml in sterile universal container or stool specimen container, if available)   4. Samples/swabs from infected sites or sores. | | | No patient will give more than 0.6ml/kg (>1% blood volume) on any one day, or more than 2.4ml/kg (approx 3% blood volume) in any four week period (MCRN recommendations). |
| Clinician-requested pathogen samples (all patients) | Where possible, we will obtain an aliquot of any residual and unwanted sample volume from specimens that have been sent by clinicians for pathogen detection, including those obtained before recruitment to the study: urine; stool; respiratory tract samples (NPA, ETA, BAL, sputum, ENT swabs); cerebrospinal fluid. | | |  |