A Phase III, Multicentre, Randomised Trial Comparing SARS-CoV-2 Re-Boost Vaccine Strategies in Immunocompromised Patients

Laboratory Processing Manual

Sponsor:	University of Birmingham
EudraCT Number:	2021-003632-87
Sponsor Number:	RG_21-112
CRCTU Protocol Number:	MX3010
IRAS Number:	302634

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Ensure all laboratory trial documentation references the identifiers and protocol version it relates to	
Full trial title	A Phase III, Multicentre, Randomised Trial Comparing SARS-CoV-2 Re-Boost Vaccine Strategies in Immunocompromised Patients
Short trial title	OCTAVE-DUO
IRAS ID	302634
R&D number	<Recruiting site's reference number>
EudraCT	2021-003632-87

GOOD CLINICAL PRACTICE IN THE LABORATORY COMPLIANCE STATEMENT
The analysis or evaluation of samples collected from subjects participating in clinical trials forms a key part of the clinical trials process. There is an expectation, as detailed in the European Medical Agency - Reflection paper for laboratories that perform the analyses or evaluation of clinical trial samples, that clinical trial samples collection and analysis is managed to Good Clinical Practice (GCP) in the laboratory standard, therefore ensuring that patient safety is not compromised, that data is reliable and accurately reported, and in accordance with accepted principles of GCP and applicable law.

Version History		
Version	**Date**	**Reason for change**
1.0	27 July 2021	***

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Section 1: Contact Information

Trial Contacts	
Role	**Name & contact information**
Chief Investigator (CI)	
Sponsor	
Trial Office Email	
CRCTU Director of Operations	
Trial Management Team, Leader	
Senior Manager	
Trial Coordinator(s)	
Laboratory Lead for Trial	
Processing Laboratory Site	
See separate document: **"OCTAVE DUO Study: Contacts for Laboratory Processing Manual"**	

Section 2: Introduction

In the UK, the government's Vaccine Taskforce, has secured early access to over 500 million doses of either of the most promising vaccine candidates, including: BioNTech/Pfizer, Oxford/AstraZeneca, Moderna, Janssen, Novavax, GSK/Sanofi, Valneva and CureVac. Four vaccines are already approved by the Medicines and Healthcare products and Regulatory Agency (MHRA); the COVID-19 mRNA Vaccine BNT162b2 (Pfizer/BioNTech), COVID-19 Vaccine AstraZeneca (formerly AZD1222), the mRNA vaccine developed by Moderna (COVID-19 Vaccine Moderna), and the peptide vaccine developed by Janssen (COVID-19 Vaccine Janssen). To date, in the UK, over 75 million doses of SARS-CoV vaccines have been given with nearly 32 million people (over 45%) of the population fully vaccinated with 2 doses.

Participants in OCTAVE-DUO will represent patients who are clinically vulnerable to COVID-19 infection and have immune system status that could impair their response to SARS-CoV-2 vaccines, namely immune mediated inflammatory diseases, hepatic and intestinal disease, renal failure, breast cancers, lymphoid malignancies, haematopoietic stem cell transplant and chimeric antigen receptor T-cell therapy recipients and patients with primary immune deficiency. Their potential for altered immune responses to SARS-CoV-2 vaccines are either a function of their underlying disease and associated immune dysregulation or due to their requisite management with immune modifying medications, including cytotoxic chemotherapies biologics, disease-modifying anti-rheumatic drugs (DMARDs), broad spectrum immune suppressants and glucocorticoids. The OCTAVE-DUO trial will recruit patients with known inadequate SARS-CoV-2 vaccines responses and will determine whether a SARS-CoV-2 re-boost vaccination strategy can induce an adequate immune response and whether this is affected by disease phenotype.

The majority of SARS-CoV2 vaccinations performed in the UK used the mRNA vaccine BNT162b2 (Pfizer) or chimpanzee adenovirus vector vaccine ChAdOx1-nCov19 (Astra Zeneca). It is not known whether re-vaccination with the same type of vaccine technology or an alternative vaccine technology is more likely to boost the immune response. There are trials on-going examining these questions in healthy volunteers (the COM-COV trial, IRAS Project ID: 291055 EudraCT Number: 2020-005085-33 and COV-BOOST IRAS Project ID: 299180 EudraCT Number: 2021-002175-19). OCTAVE-DUO will address this question in the immunocompromised patient and evaluate whether the third vaccine immune response can be induced by the mRNA vaccine BNT162b2 or a nano-particle vaccine Novavax. The comparative response between participants receiving 3 doses of BNT162b2, 2 doses of ChAdOx1-nCov19 and a booster with either BNT162b2 or Novavax will be investigated. State-of-the-art immune technologies on common assay platforms will be used, so that vaccine responsiveness between different disease cohorts can be directly assessed. This knowledge is urgently required to guide re-vaccination strategies and to inform government policies in the UK and globally.

Preliminary data from the COM-COV trial suggests that healthy participants that have received either ChAdOx1 nCOV-19 vaccine or mRNA vaccine BNT162b2, who then subsequently receive a dose of BNT162b2 generate a superior immunological response compared to those who received a dose of ChAdOx1 nCOV-19 vaccine [personal communication]. However, it should be appreciated that ChAdOx1 nCOV-19 vaccine followed by ChAdOx1 nCOV-19 vaccine does generate an effective immune response in healthy individuals. Given the scale of the increased response with BNT162b2 boost (approximately 5 times higher) and the fact that we need to sufficiently enhance the sub-optimal immunological response in clinically vulnerable patients, the decision was made to include BNT162b2 as one of the vaccines for the OCTAVE DUO study. The second vaccine chosen is the Moderna mRNA vaccine. In a sub-set of patients, the Novavax nano-particle vaccine will also be evaluated. The rationale for inclusion of the Novavax nano-particle vaccine is based on the unique formulation compared to the ChAdOx1 and mRNA vaccines. It is important that we determine whether this type of vaccine formulation will provide added benefits in clinically vulnerable patients. Finally, the use of the Novavax nano-particle vaccine aligns with the COM-COV2 trial, which is comparing boosting of healthy individuals that have received either ChAdOx1 nCOV-19 vaccine or mRNA vaccine BNT162b2 with a dose of an mRNA vaccine (BNT162b2 or COVID-19 Vaccine Moderna) or the Novavax nano-particle vaccine.

OCTAVE-DUO will address the re-boost question in the immunocompromised patient and evaluate whether the third vaccine immune response can be induced by the mRNA vaccines Pfizer BNT162b2 or Moderna. In a sub-set of patients, immune response will also be evaluated using the unlicensed nano-particle vaccine Novavax. The comparative response between participants receiving 3 doses of BNT162b2, 2 doses of ChAdOx1-nCov19 and a booster with BNT162b2 or Moderna, (and in a sub-set Novavax) will be investigated. State-of-the-art immune technologies on common assay platforms will be used, so that vaccine responsiveness between different disease cohorts can be directly assessed. This knowledge is urgently required to guide re-vaccination strategies and to inform government policies in the UK and globally.

Section 3: Purpose

This purpose of this standard operating procedure (SOP) laboratory processing manual is to describe the procedures to take receipt of and process blood samples for the OCTAVE-DUO trial, sponsored by the University of Birmingham.

Section 4: Sample handling

The analysis or evaluation of OCTAVE-DUO trial samples in accordance with this laboratory processing manual should be overseen by the Chief Investigator (CI) who assumes responsibility for its various components. Oversight of the sample processing is delegated by the CI to the Academic Lead (or equivalent) for each local on-site/off-site processing laboratory.

Trial sample collection, handling at the participating sites and sample transport to the local on-site/off-site processing laboratory is described in the current version of the OCTAVE-DUO trial Sample Handling Manual document.

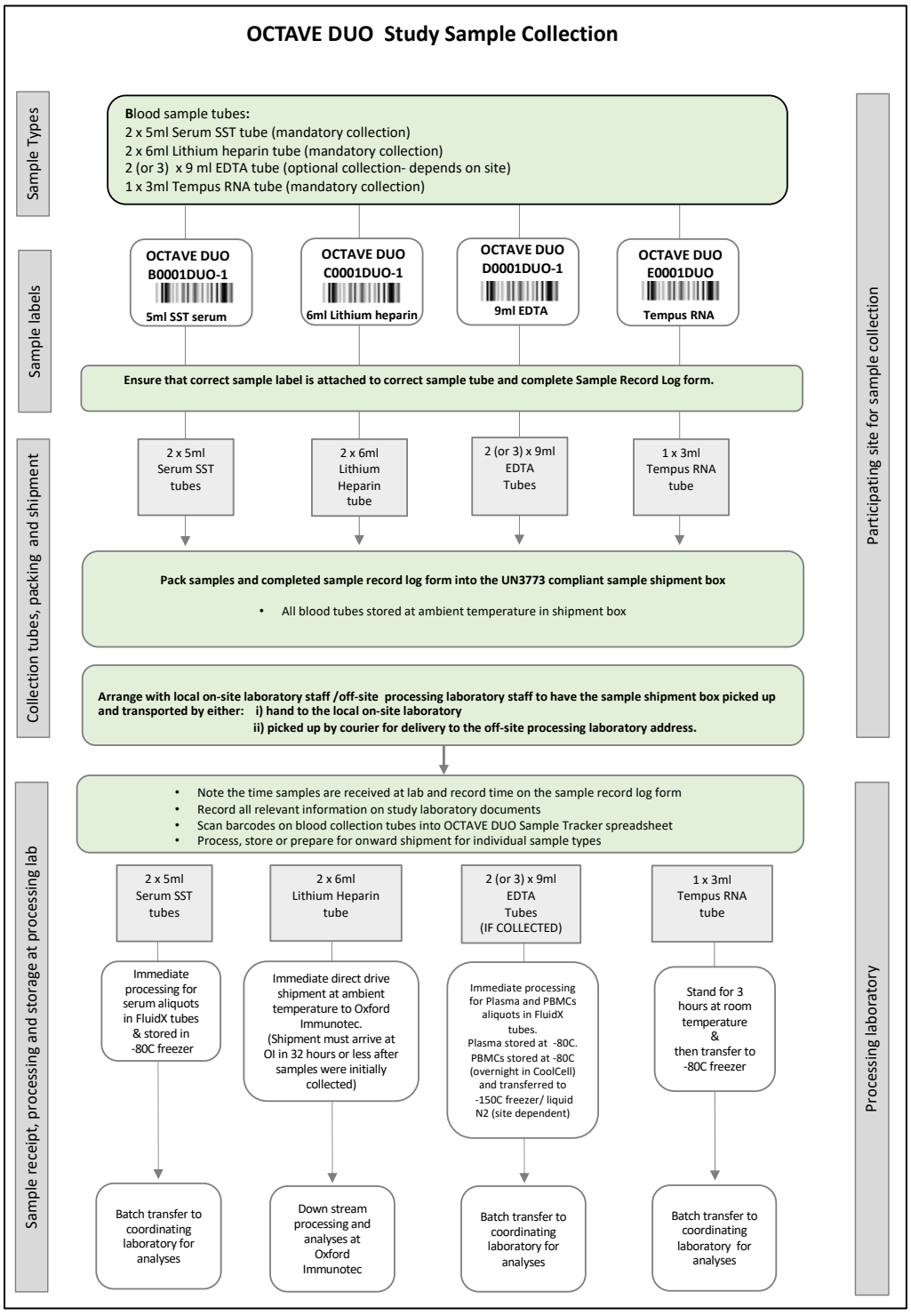
All trial samples, except for the 2 x 6ml lithium heparin tubes will be processed and stored on laboratory site, followed by batch transfer during the recruitment phase to coordinating laboratories carrying out analyses following the schedule and instructions described in section 7.4 of this laboratory manual. The 2 x 6ml Lithium Heparin tubes will be immediately packed and shipped at ambient temperature to Oxford Immunotec for processing and analyses.

Sample receipt, reconciliation and tracking will be conducted using the OCTAVE-DUO Sample Tracker spreadsheet which links the unique sample barcodes to the participant trial ID, participant initials and visit type, alongside other relevant laboratory documentation.

4.1 Sample handling and chain of custody from participating site to processing laboratory

The chain of custody of the samples from the participating site to the processing laboratory, along with a summary of the workflow at the processing laboratory is shown in **Figure 1.**

Figure 1: OCTAVE DUO Study Sample collection and Chain of Custody Diagram



After blood sample collection is complete for each participant at the trial visit, samples will either transported by hand to the local on-site laboratory area for processing or picked up and transported by courier to the off-site processing laboratory. Samples are transported in UN3373 compliant packaging at ambient temperature.

The schedule of events as described in the protocol is outlined below in **Table 1**. The details of blood sample collection and storage are shown in **Table 2**.

These tables will be amended to reflect any relevant protocol amendments. The Trial Office will notify the processing laboratory sites of any protocol amendments that are related to the sample scheduling and provide updated relevant documentation to the processing laboratory site.

Table 1: Schedule of events

	Screening	Trial Entry	Baseline *Prior to re-vaccination*	Re-vaccination[1]	Post-re-vaccination[2] *21 days post re-vaccination*	3-month follow-up *Seen in accordance with clinical practice[3]*
Eligibility assessment (including dip stick pregnancy test)	x					
Consent	x					
Randomisation		x				
Re-vaccination				x		
Data collection		x	x		x	x
Assessment of adverse events					x	
Research blood samples[4]			x[5]		x	
Participant Diary Booklet[6]			x		x	

Key

[1] At least 14 days after receipt of the second dose of vaccine

[2] Minimum 21 days + 14 days post re-boost vaccination

[3] Three months after re-vaccination, where possible data collected retrospectively from participants medical records, telephone follow-up permissible

[4] Research blood samples include: Whole blood, serum, plasma, and where possible peripheral blood mononuclear cells (PBMC)

[5] Research blood sample to be collected -14 to 0 days before re-boost vaccination

[6] Participant Diary Booklet handed out at baseline and collected at post re-vaccination appointment to aid in collection of Adverse Event (AE) data

Table 2: Details of Sample Collection and Storage

Sample Type	Collection Tube	Volume	Laboratory Analysis	Aliquots	Sample Storage	
Whole Blood	Tempus RNA	1 x 3ml	RNA	None	-80°C	
Whole Blood	EDTA	2 (or 3) x 9ml	Cellular immunoassays & DNA	4 x 500ul (as PBMCs) 5 x 700ul (as plasma)	-80°C for plasma. PBMCs in CoolCell overnight at -80°C (short-term), then liquid nitrogen or -150 °C; depends on site (long-term)	**NOTE: Optional – depends on local site.**
Serum	SST	2 x 5ml	Immunoassay; ELISA and virus neutralisation assays	10 x 400ul	-80°C	
Whole Blood	Lithium Heparin*	2 x 6ml	ELISpot assay	None	None	

*Two 6ml lithium heparin tubes will be shipped to Oxford Immunotec at ambient temperature. Samples must be received by Oxford Immunotec within 32h of collection.

4.2 Sample labelling

The trial samples will be labelled in such a way as to allow their unequivocal identification at all times during processing and analyses.

At time of sample collection, the samples will be labelled in accordance with the guidance set out in the current version of the OCTAVE-DUO Sample Handling Manual. Labels must be applied directly onto the sample as soon as they are taken.

Participating sites will also apply a label to the appropriate Sample Record Log Form that will accompany the samples to the processing laboratory site. A second copy of the Sample Record Log label is provided to attach to any relevant corresponding participating site documentation for each participant.

The appropriate Sample Record Log Form will be completed by the designated individual at the participating site and will record details of:

- Site name
- Trial Number

- Participant initials
- Sample time point (baseline or day 21 post re-vaccination)
- Date sample collected
- Time samples were collected
- Sample ID (e.g. B0001DUO)
- Type of samples collected
- Name of person who collected the samples for onward transport to processing laboratory site

When samples are received at the processing laboratory site the designated individual from the laboratory will record the following on the appropriate Sample Record Log Form:

- Name of person receiving samples
- Date and time samples are received

Figure 2: Sample labelling schema example
(Using alphanumeric sample identifier "0001DUO")

Alphanumeric sample identifier on label	**Label purpose/tube type**	
A0001DUO-1	Affixed to Sample Record Log form	
A0001DUO-2	Affixed to participating site paperwork	
B0001DUO-1	5ml SST Serum	Mandatory collection of these sample tubes
B0001DUO-2	5ml SST Serum	
C0001DUO-1	6ml Lithium Heparin	
C0001DUO-2	6ml Lithium Heparin	
D0001DUO-1	9ml EDTA	Collection of these tubes depends on feasibility of local laboratory to process this sample type
D0001DUO-2	9ml EDTA	
D0001DUO-3	9ml EDTA	
E0001DUO	Tempus RNA tube	Mandatory collection of this sample tube

4.3 Receipt and storage of samples at local on-site/off-site processing laboratory

4.3.1 Sample receipt

a) Samples will be processed, stored and shipped by the participating site as stipulated in the current version of the OCTAVE-DUO Sample Handling Manual.
b) The dispatching participating site is instructed to liaise with the designated individuals of the laboratory and administrative team about the sample shipment date and time.
c) Samples will be delivered by hand to the local on-site laboratory or collected the approved courier company to the off-site processing laboratory site; see separate document "OCTAVE-DUO Study: Contacts for Laboratory Processing Manual" for details.

4.3.2 Sample Condition Assessment and Logging

a) On receipt, laboratory staff should ensure that the OCTAVE-DUO Sample Record Log Form has accompanied each set of samples. If the Sample Record Log Form is missing inform the Laboratory Manger/other designated individual, who will contact the participating site to investigate and resolve the issue.
b) The name of the person, plus the date and time of receipt of the samples should be recorded in the appropriate section of the Sample Record Log Form.
c) The laboratory staff will check the appropriate number of samples has been received against the Sample Record Log Form and ensure that labelling is per the Sample Handling Manual and protocol.
d) If samples have been compromised in transit this should be recorded on the OCTAVE-DUO Sample Receipt Form and the Laboratory Manger/other designated individual should be promptly notified and the inform the OCTAVE-DUO Trial Office
e) If samples are poorly labelled, missing or if unexpected samples are receipted, this should be recorded on the OCTAVE-DUO Sample Receipt Form and the Laboratory Manger/other designated individual should be promptly notified.
f) The Laboratory Manager/other designated individual should contact the participating site to investigate and reconcile errors and inform the OCTAVE-DUO Trial Office
g) Receipting staff should sign and date the OCTAVE-DUO Sample Receipt Form as confirmation of receipt.
h) The OCTAVE-DUO Sample Receipt Form should be stored together with the Sample Record Log Forms and filed with other trial specific laboratory site documentation in a dedicated trial specific ring binder, in a timely manner. The trial ring binder should be stored in a secure part of the processing laboratory or site office in a locked filing cabinet or equivalent.

SST tubes (mandatory collection and processing)

i) SST tubes will be scanned onto the OCTAVE-DUO Sample Tracker spreadsheet using the parent barcodes for each sample placed on each tube at the collection site. This barcode will link the participant Trial Number to the sample ID; sample type, sample time point (baseline or day 21 post re-vaccination); along with the date of sample collection.

j) After processing to obtain the downstream derivatives for each sample type, each aliquot will be placed into barcoded FluidX storage tubes as shown in figure 1.

k) The FluidX tube barcodes will be scanned into the OCTAVE-DUO Sample Tracker spreadsheet, which will link it to the sample parent barcode, Trial Number and sample time point.

l) The FluidX tubes will then be stored at the correct temperature conditions for the derivative type and the time and number of aliquots for each derivative type noted on the relevant OCTAVE-DUO Blood Processing QC sheet.

EDTA tubes (optional collection and processing – depends on site)

m) EDTA tubes will be scanned onto the OCTAVE-DUO Sample Tracker spreadsheet using the parent barcodes for each sample placed on each tube at the collection site. This barcode will link the participant Trial Number to the sample ID; sample type, sample time point (baseline or day 21 post re-vaccination); along with the date of sample collection.

n) After processing to obtain the downstream derivatives for each sample type, each aliquot will be placed into barcoded FluidX storage tubes as shown in figure 1.

o) The FluidX tube barcodes will be scanned into the OCTAVE-DUO Sample Tracker spreadsheet, which will link it to the sample parent barcode, Trial Number and sample time point.

p) The FluidX tubes will then be stored at the correct temperature conditions for the derivative type and the time and number of aliquots for each derivative type noted on the relevant OCTAVE-DUO Blood Processing QC sheet.

3ml Tempus Blood RNA tube (mandatory collection and processing)

q) 3ml Tempus Blood RNA tube will be scanned into the OCTAVE-DUO Sample Tracker spreadsheet using the parent identification barcode placed on the tube at the collection site, which will link the participant Trial Number, to the sample ID; sample type, sample time point along with the date of sample collection and then the tube is stored at -80°C (labels are temperature resistant to -196°C and are suitable for storage at -80°C).

6ml lithium heparin tubes (mandatory collection and processing)

r) 2 x 6ml lithium heparin tubes will be scanned into the OCTAVE-DUO Sample Tracker spreadsheet using the parent identification barcode placed on the tube at the

collection site, which will link the participant Trial Number to the sample ID; sample type, sample time point along with the date of sample collection and then the samples are immediately shipped to Oxford Immunotec at ambient temperature. Samples must be received by Oxford Immunotec within 32h of blood sample collection.

*4.3.*3 Sample Reconciliation

a) ***Unexpected samples should not be analysed until their identity is confirmed.***
b) Any sample which cannot be identified using the OCTAVE-DUO Sample Record Log Form should be scanned into a temporary storage location on the OCTAVE-DUO Sample Tracker spreadsheet; the position documented, and the samples transferred to the correct temperature controlled, temporary storage place until sample identity/error has been reconciled between the processing lab and participating site.
c) The Laboratory Manager or other designated individual should be promptly notified to investigate the issue.
d) Once the sample identity/error has been reconciled, the sample barcode will then be rescanned into the permanent position in the OCTAVE-DUO Sample Tracker spreadsheet either downstream processing or storage.
e) Any sample identity/error that cannot be reconciled must immediately be reported to the OCTAVE-DUO Trial Office
f) Reconciled OCTAVE-DUO Sample Receipt Forms should be stored with the reconciled Sample Record Log Forms as described above.

4.4 Blood sample storage conditions at participating site and during transport by hand to on-site laboratory/off-site laboratory by approved courier.

Blood samples will be collected from the participant and stored at room temperature at the participating site; packaged in UN3373 compliant packaging and then either delivered by hand to the local on-site laboratory for processing or collected by approved courier for onward transportation at ambient temperature to the off-site processing laboratory.

4.5 Identity of participants

All trial samples should be labelled by the participating site as per the instructions in the OCTAVE-DUO Sample Handling Manual. All samples and accompanying paperwork received at the processing laboratory site must not contain any patient identifiable details other than patient initials, which has been approved by the Research Ethics Committee and each participant consents to the use of their initials on relevant paperwork. The Laboratory

Manager/other designated individual must contact the OCTAVE-DUO Trial Office if patient identifiable details are present on sample or accompanying paperwork. The Laboratory Manager/other designated individual must follow instructions given by the Trial Office for rectifying this and how to report the incident.

4.6 Processing of laboratory samples

IMPORTANT: Collection of SST tubes; Lithium Heparin tubes and the Tempus RNA tube is MANDATORY for all sites. Collection of the 9ml EDTA tubes is optional and depends on feasibility of local laboratory to process this sample type.

Please check with your local site cohort lead/co-lead or other designated local study team individual for confirmation of the sample tube types to be collected

All trial samples receipted and processed at the laboratory will be held in the correct storage conditions described below in the sample processing procedures.

Summary

Mandatory collection and processing

a) 6ml Lithium Heparin tubes (x 2) will be shipped directly to Oxford Immunotec to arrive no later than 32 hours after initial collection from trial participant.
b) SST tubes (x 2) will be processed at room temperature and derived serum aliquots stored in a -80°C freezer.
c) Tempus RNA tube will be stored at room temperature for 3 hours from collection time and then transferred to a -80°C freezer.

Optional collection and processing – depends on local laboratory site

d) EDTA tubes (x 2 minimum, 3 maximum) will be processed at room temperature and derived plasma aliquots will be stored in -80°C freezer; derived PBMCs will be temporarily stored in -80°C freezer (overnight in CoolCell) and then transferred to -150°C freezer or liquid nitrogen storage (depending on processing laboratory).

The designated fridges, -20°C, -80°C and -150°C freezer/liquid nitrogen storage vessels in the processing laboratory should have their operating temperature recorded at designated intervals.

Evidence of monitoring and action taken in the event of any deviation from the specified ranges should be documented and retained in either trial specific documentation or centrally

held documentation within the laboratory. and must immediately be reported to the OCTAVE-DUO Trial Office

The laboratory should also keep a record of certificates of calibration/maintenance with trial specific documentation or centrally held documentation.

IMPORTANT Health and Safety Instructions:

a) *All samples are taken from participants will have a general population risk of containing material considered potentially infective with HBV, HCV (hepatitis), HIV (AIDS), or other infective agents.*
b) *All staff should wear appropriate PPE for handling human blood and saliva samples according to local Health and Safety rules and all relevant local COSHH/ BioCOSHH risk assessment documentation.*
c) *All processing should be carried out in a CL2 facility, within a Class II microbiological safety cabinet until otherwise instructed in this laboratory manual.*
d) *All centrifugation steps must be carried out using centrifuge buckets with sealable lids to prevent the generation of aerosols.*
e) *All used consumable waste should be discarded into the correct containers with appropriate disinfection before disposal using the correct locally approved clinical waste stream.*
f) *All other solid and liquid biological waste should be discarded into the correct containers with appropriate disinfection, before disposal using the correct locally approved clinical waste stream.*

4.6.1 Receipt of 2 x 6ml Lithium Heparin vacutainer and shipping to Oxford Immunotec

NOTE: Oxford Immunotec are only able to analyse 30 samples for the OCTAVE-DUO trial per-day. Therefore, sites will be provided with a timetable for collection of baseline and day 21 post re-vaccination samples by the Trial Office. The Processing Laboratory must process and ship samples promptly to avoid missing their allocated time slot with Oxford Immunotec.

Items required

- UN3373 compliant shipment box and packing materials/ Royal Mail Safe Box (depends on laboratory site)
- Printed out copy of completed electronic manifest excel spreadsheet

Procedure

1) Immediately upon receipt of the 2 x 6ml Lithium Heparin tubes, scan each barcoded 6ml Lithium Heparin tube into the OCTAVE-DUO Sample Tracker spreadsheet.
2) Transfer the scanned barcode for each sample into the Oxford Immunotec manifest spreadsheet; complete all other sections of the spreadsheet; and print out a paper copy of the completed manifest form.
3) Pack samples in accordance with UN3373 regulations for shipping Category B Biological Substances into the shipment box (or Royal Mail Safe Box).
4) Place the printed copy of manifest into an approved UN3373 shipment box labelled with consignee and receivers address and contact details (name and telephone number); and appropriate UN3373 hazard labels and seal box.
5) Use locally approved courier (or Royal Mail, if using Safe Box) to ship the samples at room temperature using a service that will arrive at Oxford Immunotec within 32 hours of sample collection at the participating site.
6) Send email to designated individual at Oxford Immunotec containing an electronic copy of shipment manifest; plus, courier/Royal Mail safe box details; shipment reference number and tracking information.
7) Instruct site to e-mail you when the shipment has arrived at Oxford Immunotec with a copy of the electronic manifest detailing sample reconciliation against the original manifest and notification of any discrepancies for investigation and resolution.

4.6.2 Tempus RNA tube processing

NOTE: **mandatory sample collection and processing using procedure described below**

Equipment

- Barcode scanner
- -80˚C freezer
- Wire tube rack
- OCTAVE DUO blood processing QC sheet

Procedure

a) All processing should be carried out in a Class II microbiological safety cabinet (sterile environment) until transferred to -80°C freezer for storage.
b) Note arrival time of sample in the Sample Record Log Form.
c) Complete all relevant sections of the OCTAVE-DUO blood processing QC sheet.
d) Place Tempus tube into the Class II microbiological safety cabinet and invert the tempus tube 10 times.
e) Sit Tempus RNA tube upright in a tube rack, at room temperature inside the Class II microbiological safety cabinet for 3 hours from when blood was collected (blood sample collection time is recorded on the Sample Record Log)
f) Once 3 hours has elapsed, transfer the Tempus tube to the correct tube storage box and position location identified by OCTAVE-DUO Sample Tracker spreadsheet.
g) Place storage box into specified location in the -80˚C freezer.

4.6.3 Processing of 2 x 5ml Serum SST tubes for isolation of serum

NOTE: **mandatory sample collection and processing using procedure outlined below**

Equipment

- 1200ul pipette and sterile filter tips
- 10 x pre-barcoded FluidX storage tube with lid (#68-1002-11N or FluidX equivalent)
- FluidX storage box
- Barcode scanner
- -80 °C Freezer
- OCTAVE DUO blood processing QC sheet

Procedure

1) All processing should be carried out in a Class II microbiological safety cabinet (sterile environment) unless indicated in the laboratory processing manual; and until transferred to -80°C freezer for storage.
2) The serum SST tube must be allowed to sit for a minimum of 30 minutes from the time of blood collection (as recorded in the Sample Record Log form) before processing.
3) If 30 minutes or more has not elapsed by the time the sample is received in the laboratory, sit tubes upright in the Class II MSC and do not process until 30 minutes have elapsed from the time of blood collection.
4) Place both serum SST tubes into the centrifuge ensuring that the bucket lids are sealed securely, and the centrifuge is balanced.
5) Centrifuge blood sample at 1200g at room temperature for 10 minutes.
6) Remove the centrifuge bucket lid and return the serum SST tubes to the MSC class II hood.
7) Place the tubes upright in an appropriate stand and carefully remove the SST tube lid.
8) Using a sterile tip, pipette 400ul of serum into 10 pre-barcoded FluidX tubes.
9) Seal the FluidX tubes and scan each barcode into the OCTAVE DUO Sample Tracker spreadsheet.
10) Place FluidX tubes into the correct storage box and position as specified by the OCTAVE DUO Sample Tracker spreadsheet, then return the box to its specified position in the -80°C freezer.
11) **Batch shipment of stored serum aliquots will take place during the recruitment phase of this trial to the relevant co-ordinating laboratories according to the procedures described in section 4.7.**

4.6.4 Preparation of freezing buffer

NOTE: Procedure described below is only applicable if laboratory is processing EDTA tubes to obtain PBMCs

Reagents

- Fetal Bovine Serum (FBS) (Invitrogen, #10270-106)
- Dimethyl Sulfoxide (DMSO) (Sigma #D2650-100ML)

Equipment

- 200ul pipette and sterile filter tips
- Sterile 7ml bijou tube or equivalent

Procedure

In advance of receiving the first blood samples into the laboratory, heat inactivate the FBS, aliquot and freeze at -20°C as follows:

a) Heat inactivate the 500ml bottle of FBS by incubating at 56°C for 30 minutes in a water bath.
b) Allow the FBS to cool down and then transfer to a Class II MSC hood to aliquot into 2ml volumes in a bijou tube or equivalent.
c) Freeze at -20°C for long-term storage until required to prepare the freezing buffer.

On day of sample processing

NOTE: Make up fresh FBS/20% DMSO solution for the day that the samples are processed and discard any unused freezing medium at the end of processing.

a) Remove a 2ml aliquot of heat inactivated FBS from the -20°C freezer and thaw at room temperature.
b) Once the solution has thawed completely, remove 800ul into a new sterile bijou tube (or equivalent) and label "freezing buffer".
c) Add 200ul DMSO to the heat inactivated FBS to the labelled tube and clearly mark that the DMSO has been added.
d) Place the lid back on the tube and invert the tube 10 times to ensure that the FBS/DMSO solution is well mixed.
e) Place the freezing buffer tube on ice to use when required (keep extra heat inactivated FBS on ice in case you need to make up more freezing buffer)

NOTE: *the quantities of heat inactivated FBS and DMSO described above are for processing one sample only. If multiple samples are being batch processed scale up amounts of FBS and DMSO required.*

Heat inactivated FBS - volume required *= [Number of samples] x 800ul*
DMSO - volume required = [Number of samples] x 200ul

4.6.5 Processing of 2-3 x 9ml EDTA vacutainer for isolation of plasma and PBMCs

NOTE: **Procedure described below only applicable if laboratory is processing EDTA tubes to obtain plasma and PBMCs**

NOTE: Either use the method described below or follow one of the alternative methods for Processing of 2-3 x 9ml EDTA vacutainer for isolation of plasma and PBMCs described in:

Appendix 1: Use of SepMate-50 PBMC Isolation tubes (manufacturer – StemCell Technologies) plus density gradient medium.

Appendix 2: Use of generic 50ml centrifuge tube plus density gradient medium.

The method used should be recorded by adding an extra column to the "PBMC (from EDTA)" tab on the OCTAVE-DUO Sample Tracker spreadsheet.

For this method use either abbreviation A, B or C (highlighted in bold) to indicate the tube type and reagent used:

Method	**Abbreviation for spreadsheet**
Prefilled Leucosep tube	**A**
Empty Sterile Leucosep and Ficoll-Paque Plus	**B**
Empty Sterile Leucosep and LymphoPrep	**C**
SepMate and Ficoll-Paque Plus	D
SepMate and Lymphoprep	E
50ml centrifuge tube and Ficoll-Paque Plus	F
50ml centrifuge tube and Lymphoprep	G

Reagents

- Prefilled Leucosep tube with LymphoPrep (Greiner; # 227 288)
  OR
- Empty sterile Leucosep tubes (Greiner; #227 290)
- Density gradient medium:
  - Ficoll-Paque PLUS (Cytiva [Formerly GE Healthcare Life Sciences] #17-1440-03)
    OR
  - LymphoPrep (StemCell Technologies 500ml; # 07851)

- Dulbecco's Phosphate Buffered Saline (dPBS; Ca++ and Mg++ free) (Gibco; #14190-094)
- Heat inactivated Fetal Bovine Serum (FBS) (Invitrogen, #10270-106)
- Freezing buffer: 20% DMSO in heat inactivated FBS (pre-made in section 4.6.4)

Equipment

- 1200ul pipette and sterile filter tips
- 200ul pipette and sterile filter tips
- 20ul pipette and sterile filter tips
- Sterile Pasteur pipette
- 15ml conical tube
- 50ml conical tube
- Centrifuge
- CoolCell
- 9 x FluidX pre-barcoded storage tubes with lid (#68-1002-11N or FluidX equivalent)
- FluidX storage box
- Barcode scanner
- -150 ˚C freezer or liquid nitrogen storage (depends on processing site)
- OCTAVE-DUO blood processing QC sheet

Procedure

1) All processing should be carried out in a Class II microbiological safety cabinet (sterile environment) until transferred to -80°C for temporary overnight storage and then moved to permanent storage in -150°C freezer/liquid nitrogen storage (site dependant)
2) Complete all relevant sections of the OCTAVE-DUO blood processing QC sheet.
3) Warm one Leucosep tube pre-filled with LymphoPrep separation medium and a bottle of dPBS to room temperature.
4) Pulse the Leucosep tubes in centrifuge for 30 seconds at 1000 x g at room temperature to move all the separation medium below the filter and then:

   Proceed to instruction 8)
   OR
   If using empty sterile Leucosep tubes and separate density gradient medium (Ficoll-Paque PLUS or* LymphoPrep); *follow instructions 5,6 and 7.

5) Warm bottle of density separation medium up to room temperature protecting it from light.
6) In Class II microbiological safety cabinet aliquot gently mix the density separation medium by inverting several times and then aliquot 15ml of separation medium into an empty sterile Leucosep tube.

7) Close the tube containing the separation medium with the screw cap and centrifuge at 1000g for 30 seconds at room temperature to locate the separation medium below the porous barrier.
8) Label the Leucosep tube cap and side with the <participant Trial Number>_PBMC.
9) Transfer the blood from the EDTA tubes directly into the Leucosep tube and securely close cap.
10) Record the total blood volume from the EDTA tubes on the OCTAVE-DUO blood processing QC sheet.
11) Place the Leucosep tube in the centrifuge ensuring that the centrifuge bucket lid is securely sealed, and the centrifuge is balanced.
12) Centrifuge at 800g for 15 minutes at room temperature, with **NO CENTRIFUGE BRAKE APPLIED**.
13) Remove the centrifuge bucket lid and return the Leucosep tube to the class II MSC hood.
14) Place the tube upright in an appropriate stand and carefully remove the lid of the tube.
15) Using a sterile tip, carefully pipette 700ul of plasma into 5 pre-barcoded FluidX tubes with lids.
    NOTE: Ensure that you only take plasma from the top fraction of the Leucosep tube and do not disrupt the buffy layer.
16) Seal the FluidX tubes and scan each barcode into the OCTAVE-DUO sample Tracker spreadsheet.
17) Place FluidX tubes into the correct storage box and position as specified by the OCTAVE-DUO-Sample Tracker spreadsheet, then return the box to its specified position in the -80°C freezer.
18) Continue with PBMC isolation by harvesting the enriched cell fraction (containing PBMCs) from the Leucosep tube by pouring the remaining supernatant above the porous barrier from the Leucosep tube into another 50ml centrifugation tube labelled on the cap and side with < Participant Trial Number>_PBMC.
19) Top up the enriched cell fraction to 50ml with sterile room temperature dPBS and secure lid.
20) As before, place the sample in the centrifuge bucket and fasten lid, ensuring the centrifuge is balanced.
21) Centrifuge at 300g for 10 minutes at room temperature.
22) Remove the centrifuge bucket lid and return the tube to the class II MSC hood.
23) Carefully remove the supernatant and resuspend the cell pellet by gently flicking the bottom of the tube with a gloved hand.
24) Top up to 50ml with sterile room temperature dPBS.
25) Repeat the centrifugation procedure, centrifuging the tube at 200g for 10 mins at room temperature.
26) Remove the centrifuge bucket lid and return the tube to the class II MSC hood.
27) Carefully remove the supernatant and resuspend the cell pellet by gently flicking the bottom of the tube with a gloved hand and top up to 50ml with dPBS.
28) Centrifuge the tube at 600g for 10 mins at room temperature.

29) Remove the centrifuge bucket lid and return the tube to the class II MSC hood.
30) Carefully remove the supernatant completely using a Pasteur pipette being careful not to disturb the cell pellet. When you have approximately 5ml of supernatant left, use a pipette with 1000ul filter tip to carefully remove the remaining supernatant and leave a dry cell pellet.
31) Using a pipette and sterile tip immediately slowly and gently resuspend the cell pellet in 1000ul of heat inactivated FBS.
32) Slowly, in a dropwise manner, add 1000ul of the freezing buffer (20%DMSO/heat inactivated FBS; prepared in Section 4.6.4) into the cells, gently swirling the tube after each drop to mix.

NOTE: The freezing buffer must be added slowly to prevent cell death and the tube must be swirled after each drop is added to ensure thorough mixing.

33) Aliquot the cell suspension into 4 pre-barcoded FluidX tubes (regardless of whether prepping two or three 9ml EDTA tubes) with lids, placing 500ul of the cell suspension into each tube.
34) Seal the FluidX tube and scan into the OCTAVE-DUO Sample Tracker spreadsheet for temporary storage in a CoolCell.
35) Place the FluidX tubes into a CoolCell, then place the CoolCell in the -80˚C freezer for overnight storage.
36) Within 3 days, transfer the samples on dry ice to the minus 150˚C freezer/ liquid nitrogen storage container, placing each tube in the sample location in the -150^{o}C freezer/ liquid nitrogen storage container indicated by OCTAVE-DUO Sample Tracker Spreadsheet.

4.7 Batch shipment of study samples to Public Health England laboratory for analysis

Batch shipment of stored serum aliquots to Public Health England laboratory will take place during the recruitment phase of this trial using the schedule determined by the OCTAVE-DUO Trial Office.

Public Health England (PHE) – shipment of 1 x 400ul serum aliquot.

- Contact PHE to notify PHE of your intended shipment date.
- Send the electronic copy of the completed shipment manifest (current template version is obtained from OCTAVE -DUO Trial Office) and notify PHE of the courier tracking details for the shipment.

4.8 Blinding

No blinding – OCTAVE-DUO is a Phase III, multi-centre, multi-disease, open-label, randomised trial of immune response to a SARS-CoV-2 re-boost vaccination different vaccination strategies. Participants may receive:

- Pfizer SARS-CoV-2 Vaccine; or
- Moderna SARS-CoV-2 Vaccine; or
- Novavax SARS-CoV-2 Vaccine Pfizer

4.9 Equipment

All new and current processing laboratory equipment will be maintained in accordance with the laboratories standard practice and added to the respective laboratory equipment inventory. Calibration and maintenance documentation should be stored in the trial specific laboratory files.

4.10 Method validation

Initially samples will only be received for processing and storage at the processing laboratory site using standard procedures hence method validation is not applicable. During trial recruitment, the relevant samples will be batch shipped at designated intervals to the relevant coordinating laboratory for analyses (see section 4.7), with the exception of the 2 x 6ml Lithium Heparin Vacutainers collected in participants, which will be shipped immediately after each trial visit to Oxford Immunotec. Future analyses carried out by the relevant coordinating laboratories (Oxford Immunotec and Public Health England) will be detailed in local standard operating procedures and other relevant documentation and will also describe appropriate method validation of the intended assays and analyses.

Laboratory data quality assurance should form part of the audit plan for the trial and where possible the audit plan for the coordinating laboratory.

4.11 Data recording

All laboratory data will be recorded directly, promptly, accurately and legibly, using the OCTAVE-DUO Sample Tracker spreadsheet, Sample Record Log Forms, OCTAVE-DUO blood QC sheets, and other relevant laboratory-based documentation. The identity of the person recording the data will also be documented.

CRF data will be captured at the participating sample collection site using the electronic Case Report Form (CRF) system described in Section 9.0 of the protocol.

4.12 Data reporting

Trial samples are processed, and samples/derivatives stored at the coordinating processing laboratories until trial recruitment is complete, apart from the Lithium Heparin samples collected from participants, which are shipped immediately to Oxford Immunotec once they are received at the processing laboratory.

Stored samples and derivatives will be batch shipped to the co-ordinating laboratory sites for planned sample analyses and data reporting (see protocol); during trial recruitment phase at designated intervals as described in section 4.7. Sample analysis using validated assays will be outlined and performed in accordance with coordinating laboratory standard operating procedures (SOPs) for carrying out the analyses (see protocol) and will include instructions for data reporting. Data reporting will be recorded using appropriate paper documents and electronic records, which will be described in the coordinating laboratory SOPs.

4.13 Data transfer

Trial samples are processed, and samples/derivatives stored at the coordinating processing laboratories and then batch transferred during the trial recruitment phase to co-ordinating laboratories for analyses and described in section 4.7 of this manual, or otherwise stated in protocol and/or in agreement with Trial Office. Transfer of any corresponding sample data with the sample shipments (i.e. sample shipment manifests) will be agreed in advance of the sample shipments to the co-ordinating sites and will be in line with REC permissions and informed consent.

Sample analyses performed at the coordinating laboratories, data recording; reporting of data and the transfer of data will be described in the coordinating sites SOPs; and will include instructions for data transfer to a data management centre; other co-investigators or other authorised stakeholders. This will be agreed ahead of the data transfer by the stakeholders and will include details of:

a) the processes for data transfer from the laboratory to the data management centre; other co-investigators or other authorised stakeholders
b) the analysis results output format; and
c) method for protection of data integrity.

A copy of the results of all analyses will be held by the Trial Office.

4.13.1 Scheduled transfer of each site's OCTAVE-DUO Sample Tracker Spreadsheet to Trial Office

Each site/processing laboratory must send an electronic copy of the completed OCTAVE-DUO Sample Tracker Spreadsheet to the OCTAVE-DUO Trial mailbox at the end of each week. If no samples have been collected that week the Trial Office should be informed via email. This is imperative to allow linkage of the laboratory data to a participant. This will also be reconciled against the sample collection data recorded in the CRF.

a) File name format

Save the file name in the following format:
<Insert Site Name>_OCTDUOsampletracker_WC_<Insert date for the Monday of the week in the format DDMMYYYY>. Where "WC" stands for week commencing.

For example: samples processed from Monday-Friday, week commencing 05 04 2021 until Friday 09 04 2021 will be labelled *"GLASGOW_OCTDUOsampletracker_WC_05042021"* and sent to the OCTAVE-DUO Trial mailbox at the end of the working week.

Note: The fully completed tracker including all participants recruited at that site should be provided each week.

b) Data Integrity - Password Protection and file compression

Each file must be password protected with the site-specific password issued by the OCTAVE-DUO trials team. Passwords are case sensitive so make sure you enter it carefully.

For Mac users:

- From Excel top drop down menu select File > Passwords…
- Enter the password issued to you by the OCTAVE-DUO Trial Office, to open and modify the excel workbook > press OK
- It will ask you to "re-enter password to proceed" > press OK
- It will then ask you to "re-enter password to modify" > press OK
- Save the workbook changes before exiting spreadsheet.

For PC users:

- From Excel top drop down menu select > File
- Select >Info from sidebar
- Select > Protect Workbook button from main screen
- Select > encrypt with Password from drop down menu
- Enter password issued to you by the OCTAVE DUO Trial Office and select OK
- Re-enter password as instructed and select OK
- Save workbook before exiting to save encryption.

Zipping files:
You may have need to compress the Excel file into a Zip format before sending due to size of the data contained in the spreadsheet. To do so use the appropriate method for either Mac or PC users.

c) Mailbox
E-mail: with the subject line indicated in d) below.

d) Subject line of e-mail
Use the subject line**:** <Insert Site Name> OCTAVE-DUO Sample Tracker spreadsheet; week commencing <Insert Date for the Monday in the format DD MM YYYY>"

For example:
"GLASGOW OCTAVE DUO Sample Tracker spreadsheet < insert spreadsheet number if required>; week commencing 05 04 2021"

This will allow the Trial Office to use a smart mailbox to filter spreadsheets by site name.

e) Storage of password protected Excel workbooks by site
Store each password protected version of the Excel workbook on a secure encrypted centrally managed Institution network server that is backed up on a regular basis.

4.14 Computerised systems

All computerised systems used at all coordinating laboratories for the capture, processing, reporting and storage of data will be developed, maintained, and appropriately backed-up in order to ensure the validity, integrity, and security of the data.

4.15 Retention of trial data

Trial specific documents should be retained in accordance with the requirements of GCP and national legislation and archived in accordance with the protocol and instructions from the OCTAVE-DUO Trial Office.

Non trial specific documents should be retained in accordance with the laboratory policies.

All copies of shipping logs, biobanking logs, and other relevant laboratory documentation should be retained in trial specific laboratory files prior to archive.

4.16 Withdrawal of participant consent

Participants may withdraw consent at any time during the trial. For the purposes of this trial two types of withdrawal are defined:

- The participants would like to withdraw from further sample collection but is willing to be followed up as standard (i.e. the patient has agreed that data can be collected at standard clinic visits and used in the analyses).
- The participants would like to withdraw from the trial entirely and is not willing to be followed up for the purposes of the trial (i.e. only data and samples collected prior to the withdrawal of consent can be used in the trial analysis) – withdrawal of consent.

The participating site eCRF will be updated by the site trial team to reflect withdrawal of consent.

Participants are informed that any samples and data collected prior to withdrawal will be retained for analysis, thus the Trial Office will not notify processing laboratories of a participant withdraw unless it becomes apparent the site have collected samples after withdrawal has taken place.

No copies of the consent form will be sent with the clinical trial sample shipment to the processing laboratory, these should be kept with the investigator site file at the participating site.

3.17 Retention and destruction of trial samples.

Any samples remaining at the end of the trial will be banked in a Human Tissue Authority (HTA) licenced biobank. The samples and data will be made available for future research in other ethically approved studies (see protocol: section 7.5.3).

If the participant withdraws consent, and samples are subsequently collected in error the Trial Office will notify the processing laboratory, regarding the destruction of the samples. The appropriate laboratory site documentation will be completed to note destruction of trial samples and filed with the other relevant laboratory site documentation in trial specific laboratory files.

4.18 Non compliances and potential serious breaches in GCP

Any incidents which occur at the processing laboratory that are considered to be a non-compliance with GCP and/or potential serious breaches of the protocol or GCP should be reported to the Trial Office immediately in accordance with the protocol.

4.19 Deviations

The Laboratory Manager/other designated individual at the processing laboratories must notify the Trial Office of all deviations from the protocol; processing manual, or GCP as soon as possible. A deviation form should be completed to document the incident. This should be completed by the site on the eCRF as soon as possible. If laboratory site staff are unsure whether a certain occurrence constitutes a deviation from the protocol or GCP, the Trial Office can advise. The Trial Office will assess all incidents with respect to the criteria of a "serious breach".

4.20 Communications related to safety

The Laboratory Manager/other designated individual at the coordinating processing laboratories must notify the Trial Office of all deviations from the protocol; laboratory processing manual or GCP related to safety immediately.

The Laboratory Manager/other designated individual at each processing laboratory should contact the Trial Office to discuss whether an issue constitutes a deviation related to safety. The Trial Office will advise the Laboratory Manager/designated individual and instruct them on how to proceed and complete the relevant deviation form.

4.21 Serious Breach

Events that match the criteria of a "serious breach" will be reported to the MHRA and REC within 7 days of the matter coming to the attention of the Trial Office. The report must include details of when the breach occurred, the location, who was involved, the outcome, the route cause, the corrective and preventative action plan including any information given to the participants. The MHRA must also be informed of any further corrective or preventative action the Sponsor plan to take.

In addition to the definition of a serious breach in GCP, systematic or persistent violation by a laboratory site of GCP and/or the protocol, including failure to report incidents occurring on trial within the specified timeframe, may be deemed a serious breach.

4.22 Protocol amendments

Any change to the trial protocol will require an amendment. Any proposed, non-administrative, protocol amendments will be initiated by the Chief Investigator following discussion with the Trial Management Group and any required amendment documentation will be submitted to the MHRA, REC and Health Research Authority (HRA) by the Trial Office. Before the amended protocol can be implemented favourable approval must be sought from the original reviewing REC, HRA and MHRA and participating site R &D offices.

Participating sites will be notified of any amendments and supplied with all relevant documentation. It is anticipated that the site will notify the processing laboratory where this is relevant to the collection of the samples.

Section 5: Inter-Lab Transfer of Trial Samples

Procedures for sample selection/generation of pick lists of required samples/manifest generation; identification of the designated individual who is responsible for overseeing the participating laboratory Sample Tracker spreadsheet; and the process for rescanning and preparing samples for shipment by approved couriers, will be documented in participating sites local SOPs and other relevant documentation.

Section 6: Transporting dangerous goods

For transportation of dangerous goods: ensure the person(s) preparing the "dangerous goods" for shipping is/are appropriately trained and responsible for ensuring that the package, when shipped, meets the requirements of all applicable laws.

The technical information presented in this manual is not intended to be, and should not be considered as, regulatory training in the handling of "dangerous goods". Any questions you may have about requirements for shipping dangerous goods must be directed to appropriate consultants, counsel, or your appropriate regulatory authorities.

- For the dry ice shipment, the packaging must be marked "UN3373 Biological Substance, Category B substance packed in UN1845, Dry Ice Class 9"
- For shipment of samples at ambient or +4°C temperature; the packaging must be marked with "UN3373 Biological Substance, Category B substance"

IATA Note: *Diagnostic specimens shipped in carbon dioxide, solid (dry ice), or liquid nitrogen must comply with the provisions of the DGR applicable to those substances in addition to the requirements of Packing Instruction 650.*

APPENDIX 1

NOTE: **Procedure described below is only applicable if laboratory is processing EDTA tubes to obtain plasma and PBMCs**

Use of SepMate-50 PBMC Isolation tubes (StemCell Technologies) plus density gradient medium.

The method used should be recorded by adding an extra column to the "PBMC (from EDTA)" tab on the OCTAVE-DUO Sample Tracker spreadsheet.

For this method use abbreviation D or E (highlighted in bold) to indicate the tube type and reagent used:

Method	**Abbreviation for spreadsheet**
Prefilled Leucosep tube	A
Empty Sterile Leucosep and Ficoll-Paque Plus	B
Empty Sterile Leucosep and LymphoPrep	C
SepMate and Ficoll-Paque Plus	**D**
SepMate and LymphoPrep	**E**
50ml centrifuge tube and Ficoll-Paque Plus	F
50ml centrifuge tube and LymphoPrep	G

Reagents

- 2 x SepMate-50 PBMC Isolation tubes (StemCell Technologies; 100 Tubes, # 85450 or 500 Tubes; # 85460)
- Ficoll-Paque PLUS (Cytiva [Formerly GE Healthcare Life Sciences] #17-1440-03)
  OR
- LymphoPrep (StemCell Technologies 500ml; # 07851)
- Dulbecco's Phosphate Buffered Saline (dPBS; Ca++ and Mg++ free) (Gibco; #14190-094)
- Heat inactivated Fetal Bovine Serum (FBS) (Invitrogen, #10270-106)
- Freezing buffer: 20% DMSO in heat inactivated FBS (pre-made in section 4.6.4)

Equipment

- 1200ul pipette and sterile filter tips
- 200ul pipette and sterile filter tips
- 20ul pipette and sterile filter tips
- Sterile Pasteur pipette
- 15ml conical tube

- 50ml conical tube
- Centrifuge
- CoolCell
- 9 x FluidX pre-barcoded storage tubes with lid (#68-1002-11N or FluidX equivalent)
- FluidX storage box
- Barcode scanner
- -150˚C freezer or liquid nitrogen storage (depends on processing site)
- OCTAVE-DUO blood processing QC sheet

Procedure

1) All processing should be carried out in a Class II microbiological safety cabinet (sterile environment), except centrifugation steps and transferring aliquots to -80°C for temporary overnight storage and then moved to permanent storage in -150°C freezer/liquid nitrogen storage (site dependant)
2) Complete all relevant sections of the OCTAVE DUO blood processing QC sheet.

Isolation of Plasma

3) Record the total blood volume from the EDTA tubes on the OCTAVE-DUO blood processing QC sheet.
4) Isolate plasma from EDTA tubes by centrifuging the EDTA tubes at 1200g for 10 minutes, with brake on; at room temperature.
5) Remove EDTA tubes from centrifuge and place in Class II microbiological safety cabinet.
6) Remove cap lids and using a sterile tip, carefully pipette 700ul of plasma into 5 pre-barcoded FluidX tubes with lids.
7) Re-cap each EDTA tube and set aside for isolation of the PBMCs (see below)
8) Seal the FluidX tubes and scan each barcode into the OCTAVE-DUO sample Tracker spreadsheet.
9) Place FluidX tubes into the correct storage box and position as specified by the OCTAVE-DUO Sample Tracker spreadsheet, then return the box to its specified position in the -80°C freezer.

Isolation of PBMCs

10) Warm the separation medium (Ficoll-Paque PLUS or LymphoPrep; protect both from light) and a bottle of dPBS to room temperature.
11) In class II microbiological safety cabinet label the cap and side of a fresh 50ml tube with Participant Trial Number>_PBMC

12) Transfer the remaining blood from the EDTA tubes into the 50ml centrifuge tube; add an equal volume of dPBS, cap and mix gently.
13) Label the tube caps and sides of 2 SepMate tubes with the < Participant Trial Number >_PBMC.
14) Aliquot 15ml of separation medium into each of the 2 SepMate tubes. The top of the density centrifugation medium will be above the insert.

NOTE: Small bubbles may be present in the density gradient medium after pipetting. These bubbles will not affect performance.

15) Keeping the SepMate tubes vertical, split the diluted sample evenly between the 2 tubes, by pipetting it slowly down the side of the tube. The sample will mix with the density gradient medium above the insert.

NOTE: The sample can be poured down the side of the tube. Take care not to pour the diluted sample directly through the central hole.

16) Cap the tubes and place the SepMate tubes in the centrifuge ensuring that the centrifuge bucket lid is securely sealed, and the centrifuge is balanced.
17) Centrifuge at 1200g for 10 minutes at room temperature, with the **CENTRIFUGE BRAKE ON.**
18) Remove the centrifuge bucket lid and return the SepMate tubes to the class II MSC hood.
19) Place the tube upright in an appropriate stand and carefully remove the lid of the tubes.
20) Pour off the top layer, which contains the enriched PBMCs from both tubes and combine into one new 50ml centrifuge tube labelled on the cap and side with <Participant Trial Number >_PBMC.

NOTE: Do not hold the SepMate tube in the inverted position for longer than 2 seconds.

21) Top up the enriched cell fraction to 50ml with sterile room temperature dPBS and secure lid.
22) As before, place the sample in the centrifuge bucket and fasten lid, ensuring the centrifuge is balanced.
23) Centrifuge at 300g for 10 minutes at room temperature.
24) Remove the centrifuge bucket lid and return the tube to the class II MSC hood.
25) Carefully remove the supernatant and resuspend the cell pellet by gently flicking the bottom of the tube with a gloved hand.
26) Top up to 50ml with sterile room temperature dPBS.
27) Repeat the centrifugation procedure, centrifuging the tube at 200g for 10 mins at room temperature.
28) Remove the centrifuge bucket lid and return the tube to the class II MSC hood.

29) Carefully remove the supernatant and resuspend the cell pellet by gently flicking the bottom of the tube with a gloved hand and top up to 50ml with dPBS.
30) Centrifuge the tube at 600g for 10 mins at room temperature.
31) Remove the centrifuge bucket lid and return the tube to the class II MSC hood.
32) Carefully remove the supernatant completely using a Pasteur pipette being careful not to disturb the cell pellet. When you have approximately 5ml of supernatant left, use a pipette with 1000ul filter tip to carefully remove the remaining supernatant and leave a dry cell pellet.
33) Using a pipette and sterile tip immediately slowly and gently resuspend the cell pellet in 1000ul of heat inactivated FBS.
34) Slowly, in a dropwise manner, add 1000ul of the freezing buffer (20%DMSO/heat inactivated FBS; prepared in Section 4.6.3) into the cells, gently swirling the tube after each drop to mix.

    NOTE: The freezing buffer must be added slowly to prevent cell death and the tube must be swirled after each drop is added to ensure thorough mixing.

35) Aliquot the cell suspension into 4 pre-barcoded FluidX tubes (regardless of whether prepping two or three 9ml EDTA tubes) with lids, placing 500ul of the cell suspension into each tube.
36) Seal the FluidX tube and scan into the OCTAVE-DUO Sample Tracker spreadsheet for temporary storage in a CoolCell.
37) Place the FluidX tubes into a CoolCell, then place the CoolCell in the -80˚C freezer for overnight storage.
38) Within 3 days, transfer the samples on dry ice to the minus 150˚C freezer/ liquid nitrogen storage container, placing each tube in the sample location in the -150ºC freezer/ liquid nitrogen storage container indicated by OCTAVE-DUO Sample Tracker Spreadsheet.

APPENDIX 2

NOTE: **Procedure described below is only applicable if laboratory is processing EDTA tubes to obtain plasma and PBMCs**

Use of generic 50ml centrifuge tube plus density gradient medium

The method used should be recorded by adding an extra column to the "PBMC (from EDTA)" tab on the OCTAVE-DUO Sample Tracker spreadsheet.

For this method use abbreviation F or G (highlighted in bold) to indicate the tube type and reagent used:

Method	**Abbreviation for spreadsheet**
Prefilled Leucosep tube	A
Empty Sterile Leucosep and Ficoll-Paque Plus	B
Empty Sterile Leucosep and LymphoPrep	C
SepMate and Ficoll-Paque Plus	D
SepMate and LymphoPrep	E
50ml centrifuge tube and Ficoll-Paque Plus	**F**
50ml centrifuge tube and LymphoPrep	**G**

Reagents

- Ficoll-Paque PLUS (Cytiva [Formerly GE Healthcare Life Sciences] #17-1440-03)
  OR
- LymphoPrep (StemCell Technologies 500ml; # 07851)
- Dulbecco's Phosphate Buffered Saline (dPBS; Ca++ and Mg++ free) (Gibco; #14190-094)
- Heat inactivated Fetal Bovine Serum (FBS) (Invitrogen, #10270-106)
- Freezing buffer: 20% DMSO in heat inactivated FBS (pre-made in section 4.6.4)

Equipment

- 1200ul pipette and sterile filter tips
- 200ul pipette and sterile filter tips
- 20ul pipette and sterile filter tips
- Sterile Pasteur pipette
- 15ml conical tube

- 50ml conical tube
- Centrifuge
- CoolCell
- 9 x FluidX pre-barcoded storage tubes with lid (#68-1002-11N or FluidX equivalent)
- FluidX storage box
- Barcode scanner
- -150˚C freezer or liquid nitrogen storage (depends on processing site)
- OCTAVE-DUO blood processing QC sheet

Procedure

1) All processing should be carried out in a Class II microbiological safety cabinet (sterile environment), except centrifugation steps and transferring aliquots to -80°C for temporary overnight storage and then moved to permanent storage in -150°C freezer/liquid nitrogen storage (site dependant)
2) Complete all relevant sections of the OCTAVE-DUO blood processing QC sheet.

Isolation of Plasma

3) Record the total blood volume from the EDTA tubes on the OCTAVE-DUO blood processing QC sheet.
4) Isolate plasma from EDTA tubes by centrifuging the EDTA tubes at 1200g for 10 minutes, with brake on; at room temperature.
5) Remove EDTA tubes from centrifuge and place in Class II microbiological safety cabinet.
6) Remove cap lids and using a sterile tip, carefully pipette 700ul of plasma into 5 pre-barcoded FluidX tubes with lids.
7) Re-cap each EDTA tube and set aside for isolation of the PBMCs (see below)
8) Seal the FluidX tubes and scan each barcode into the OCTAVE-DUO sample Tracker spreadsheet.
9) Place FluidX tubes into the correct storage box and position as specified by the OCTAVE-DUO Sample Tracker spreadsheet, then return the box to its specified position in the -80°C freezer.

Isolation of PBMCs

10) Warm the density separation medium (Ficoll-Paque PLUS **or** LymphoPrep; protect both from light) and a bottle of dPBS to room temperature.
11) In class II microbiological safety cabinet, label the cap and side of a fresh 50ml tube with <Participant Trial Number >_PBMC.
12) Transfer the remaining blood from the EDTA tubes into a 50ml centrifuge tube; add an equal volume of dPBS, cap and mix gently.

13) Label the tube caps and sides of two more 50ml centrifuge tubes with the < Participant Trial Number PBMC.
14) Gently mix the density separation medium by inverting several times and then remove the lid and aliquot 15ml into each of the two 50ml centrifuge tubes.
15) Keeping the 50ml centrifuge tubes vertical, split the diluted sample evenly between the 2 tubes, by pipetting it carefully and slowly down the side of the tube to ensure that the diluted sample does not mix with the density gradient medium.
16) Cap the tubes and carefully place the tubes in the centrifuge ensuring that the centrifuge bucket lid is securely sealed and the centrifuge is balanced.
17) Centrifuge at 800g for 20 minutes at room temperature, with the **CENTRIFUGE BRAKE OFF.**
18) Remove the centrifuge bucket lid and carefully return the 50ml centrifuge tubes to the class II MSC hood.
19) Place the tubes upright in an appropriate stand and carefully remove the lid of the tubes.
20) Remove and discard the upper plasma layer without disturbing the plasma: density separation medium interface.
21) Remove and retain the PBMC layer at the plasma: density separation medium interface from both tubes and combine into one new 50ml centrifuge tube labelled on the cap and side with < Participant Trial Number >_PBMC; being careful not to disturb the erythrocyte/granulocyte pellet.
22) Top up the PBMC cell fraction to 50ml with sterile room temperature dPBS and secure lid.
23) As before, place the sample in the centrifuge bucket and fasten lid, ensuring the centrifuge is balanced.
24) Centrifuge at 300g for 10 minutes at room temperature.
25) Remove the centrifuge bucket lid and return the tube to the class II MSC hood.
26) Carefully remove the supernatant and resuspend the cell pellet by gently flicking the bottom of the tube with a gloved hand.
27) Top up to 50ml with sterile room temperature dPBS.
28) Repeat the centrifugation procedure, centrifuging the tube at 200g for 10 mins at room temperature.
29) Remove the centrifuge bucket lid and return the tube to the class II MSC hood.
30) Carefully remove the supernatant and resuspend the cell pellet by gently flicking the bottom of the tube with a gloved hand and top up to 50ml with dPBS.
31) Centrifuge the tube at 600g for 10 mins at room temperature.
32) Remove the centrifuge bucket lid and return the tube to the class II MSC hood.
33) Carefully remove the supernatant completely using a Pasteur pipette being careful not to disturb the cell pellet. When you have approximately 5ml of supernatant left, use a pipette with 1000ul filter tip to carefully remove the remaining supernatant and leave a dry cell pellet.

34) Using a pipette and sterile tip immediately slowly and gently resuspend the cell pellet in 1000ul of heat inactivated FBS.
35) Slowly, in a dropwise manner, add 1000ul of the freezing buffer (20%DMSO/heat inactivated FBS; prepared in Section 4.6.3) into the cells, gently swirling the tube after each drop to mix.

    NOTE: The freezing buffer must be added slowly to prevent cell death and the tube must be swirled after each drop is added to ensure thorough mixing.

36) Aliquot the cell suspension into 4 pre-barcoded FluidX tubes (regardless of whether prepping two or three 9ml EDTA tubes) with lids, placing 500ul of the cell suspension into each tube.
37) Seal the FluidX tube and scan into the OCTAVE-DUO Sample Tracker spreadsheet for temporary storage in a CoolCell.
38) Place the FluidX tubes into a CoolCell, then place the CoolCell in the -80˚C freezer for overnight storage.
39) Within 3 days, transfer the samples on dry ice to the minus 150˚C freezer/ liquid nitrogen storage container, placing each tube in the sample location in the -150ºC freezer/ liquid nitrogen storage container indicated by OCTAVE-DUO Sample Tracker Spreadsheet.