The UK ME/CFS Biobank for biomedical research on Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) and Multiple Sclerosis

Eliana M Lacerda1, Erinna W Bowman1, Jacqueline M Cliff2, Caroline C Kingdon1, Elizabeth C King2, Ji-Sook Lee2, Taane G Clark3, Hazel M Dockrell2, Eleanor M Riley2, Hayley Curran1 and Luis Nacul1

1 CureME Research Team, International Centre for Evidence in Disability (ICED), Department of Clinical Research (CRD), London School of Hygiene and Tropical Medicine, GB
2 Department of Immunology & Infection, London School of Hygiene and Tropical Medicine, GB
3 Department of Pathogen Molecular Biology, London School of Hygiene and Tropical Medicine, GB

Corresponding author: Eliana M Lacerda (eliana.lacerda@lshtm.ac.uk)

The UK ME/CFS Biobank was launched in August 2011 following extensive consultation with professionals and patient representatives. The bioresource aims to enhance research on myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), related to pathophysiology, biomarkers and therapeutic approaches. The cohort includes 18–60 year olds, encompassing 284 clinically-confirmed ME/CFS cases, 60 neurologist-diagnosed multiple sclerosis (MS) cases, and 135 healthy individuals. The Biobank contains blood samples, aliquoted into serum, plasma, peripheral blood mononuclear cells (PBMC), red blood cells/granulocyte pellet, whole blood, and RNA (totalling 29,863 aliquots). Extensive dataset (700 clinical and socio-demographic variables/participant) enables comprehensive phenotyping. Potential reuse is conditional to ethical approval.

Keywords: Myalgic Encephalomyelitis/Chronic Fatigue Syndrome; multiple sclerosis; biobank; biospecimen; database

Funding statement: The UK charities Action for M.E., ME Association Ramsay Research Fund, ME Research UK, and a private donor funded the initial phase of the UK ME/CFS Biobank, which ran from 2011 to 2014. The project is currently funded by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (NIH) under Award Number R01AI103629, which was granted in 2013 for the longitudinal study of immunological, virological and genetic aspects of ME/CFS; and by the ME Association Ramsay Research Fund under a grant issued in 2016 to open the UK ME/CFS Biobank to external researchers, following a Big Give Christmas Challenge crowdfunding campaign. The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of any of the funders.

(1) Bioresource Overview

Project description

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a disabling illness characterised by incapacitating fatigue and a range of symptoms leading to substantial reductions in activity levels and quality of life. We estimated UK ME/CFS prevalence rates of approximately 1 in 500, with female-to-male ratio at 3:1 [1]; similar statistics have been identified in the US [2] and other European countries [3], variations being likely due to the clinical criteria used.

Despite significant research advances, ME/CFS aetiology and pathophysiology remains elusive. To enhance biomedical studies in this area, we conducted a participatory study to assess the feasibility of establishing a cost-effective disease-specific biobank. Discussions with ME/CFS patients and inputs from professionals with expertise in human tissue banks, law, ethics and ME/CFS guided our UK ME/CFS Biobank proposal. Funds from UK ME charities (see Funding statement) enabled the development and establishment of the bioresource protocol in August 2011; participant recruitment started in March 2012, after ethical clearance (see Ethics Statement).

This disease-specific biobank was conceptualised and is administered by researchers (the CureME team) at the
London School of Hygiene & Tropical Medicine (LSHTM); while the University College London-Royal Free Hospital (UCL-RFH Biobank), a partnering licensed infrastructure for biobanking, is responsible for processing, aliquoting and storing samples. Release of samples is coordinate between both teams.

The Biobank was concomitantly developed with our research proposal on immunological, virological and gene expression aspects of ME/CFS, which was later funded by the National Institutes of Health (NIH/NIAID – Grant Number: 1R01AI103629-01A1). The latter allowed the expansion of the initial cohort, which now includes 284 participants with ME/CFS, 60 with multiple sclerosis and 135 healthy. A subset was followed-up at 6–12 months.

Samples has been successfully released to the LSHTM laboratory teams and results will be published in 2017, upon follow-ups completion. The Biobank currently accepts external research applications.

**Classification (1)**
Human.

**Species**
N/A.

**Classification (2)**
Biological samples and associated data, clinical data.

**Context**

**Spatial coverage**

Description: All data and samples have been collected in the United Kingdom, with the majority from East Anglia and the Greater London region and a small minority from Yorkshire, Sussex, Devon, and Scotland.

**Temporal coverage**

Data and sample collection began on 03/03/2012 and is ongoing as of December 2016. The team plans to continue recruitment of new participants and longitudinal follow-up of selected participants until at least 2021, contingent on funding.

**Temporal coverage for accessibility**

There is no planned destruction of the data or samples and participant consent covers unrestricted future use of both data and samples.

(2) Methods

**Steps**

The UK ME/CFS Biobank requires a recruitment process that encompasses collection of data, clinical measures, and biosamples. All steps follow specific Standard Operating Procedures (SOPs). Most participants are recruited through the UK National Health Service (NHS) and ME/CFS diagnosis is ascertained for compliance with the Canadian Consensus or CDC-1994 criteria [4, 5]. Home visits were used for recruitment of severely ill patients, who have not been proportionately included in previous studies.

SOPs are followed for: participant identification and invitation; determination of eligibility; clinical assessments; blood collection kit preparation and phlebotomy; laboratory blood tests; data entry; sample transportation, receipt, preparation, processing, and storage; peer

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**Figure 1:** UK ME/CFS Biobank procedures from participant recruitment to storage of samples at the UCL/RFH BioBank.
review of applications to the Biobank; and data and sample release. Figure 1 summarises the Biobank recruitment procedures. Figure 2 details sample processing procedures and blood derivative outputs.

Researchers are encouraged to contact the CureME team at the London School of Hygiene & Tropical Medicine for detailed SOPs and protocols related to the above activities.

**Stabilization/preservation**

Whole blood, serum, plasma, granulocyte (and red blood cell) post density gradient, and peripheral blood mononuclear cell (PBMC) samples are stored in volumes of 200 µl to 2ml in 1.0 ml or 2 ml cryotubes. PAXgene tubes (2.5 ml) are stored as is, without processing. Each aliquot is labelled with a 2D bar code corresponding to a unique 15 digit ISBT 128 (global standard) identifier. De-identified information relating to the subject and samples is securely stored on the University College London/Royal Free Hospital (UCL/RFH) BioBank database.

**Type of long-term preservation**

The UK ME/CFS Biobank samples are stored at the UCL/RFH BioBank facility, where samples are received, logged, and processed (see Figure 2) before frozen for long-term storage. Located at the Royal Free London NHS Trust (London UK), access to this facility is limited to authorised users only. Staff are fully trained in all sample handling procedures.

The repository has a planned capacity of 1 million samples. The liquid nitrogen (LN$_2$) Cryo-Storage Room is 200 m$^2$ and the laboratory is 400 m$^2$.

The UCL/RFH BioBank has established full contingency plans, including 24-hour monitoring and alarm equipment. Back-up LN$_2$ storage tanks and freezers are kept at working temperature in case of equipment failure, and a dedicated supply of LN$_2$ covers two weeks’ usage. To protect samples during electricity loss, the UCL/RFH BioBank is connected to the Royal Free Hospital emergency power generator.

Both institutions – the LSHTM and the UCL/RFH BioBank, have be-spoke databases holding the data related to participants (the former) and to individual aliquots (the latter). Information systems and data are managed and daily backed up by their respective IT departments.

Both institutions operate in compliance with the Human Tissue Authority (HTA) guidance, which is the UK regulator for human tissue and organs.

**Storage temperature**

PAXgene tubes are kept at –80°C and all other samples are stored in vapour phase liquid nitrogen at –180°C.

**Shipping temperature from patient/source to preservation or research use**

Blood samples are collected in BD vacutainers and transported within Pathopak containers in compliance with UN 3373 regulations in 2” thick polystyrene boxes at room temperature (18–25°C), protected from direct sunlight and air conditioning.
Samples are delivered to laboratories for routine testing and/or biobanking within six hours of collection by the research nurse.

**Shipping temperature from storage to research use** –80°C (on dry ice).

**Quality assurance measures**
Quality control checks on stored samples have assessed PBMC viability and yield, cell phenotype and NK cell function, and RNA integrity and yield from PAXgene tubes (*Figures 3 and 4*), with reproducibly good results.

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**Figure 3: PBMC quality control.** PBMC are thawed and stained with either a leukocyte antibody cocktail or a T cell cocktail. To test NK cell function, PBMC are incubated with cytokines at 5 ng/ml for rhIL–12 (Peprotec) and 50 ng/ml for IL–18 (R&D Systems) or with MHC Class I-deficient K562 cells for 18 hours, in the presence of anti-CD107a-FITC (BD Biosciences), then stained with an NK cell function panel. Data are collected using an LSRII flow cytometer using FACS Diva software and analysed using FlowJo software. This figure shows data from samples from 10 participants recruited at different sites throughout 2012. Cell recovery yielded an average of $3.9 \times 10^6$ cells/tube. The viability of the recovered cells, assessed by trypan blue exclusion, was excellent in all samples, ranging from 89.2% to 100%. NK cells in the recovered PBMC were responsive in functional NK cell assays, responding to both high-dose cytokines (IL–12 and IL–18) and MHC Class I-deficient (K562) target cells by upregulating expression of the high affinity IL–2 receptor (CD25), increasing production of IFN-γ, and undergoing degranulation, measured by surface expression of CD107a.
Source of associated data

At enrolment, all participants complete a Symptoms Assessment form to confirm case definition compliance and study eligibility. Participants are asked to complete additional questionnaires within 48 hours of blood collection, including standard instruments allowing further characterisation of cases by clinical phenotype and disease severity [7]. Participants complete the questionnaires themselves except in very severe cases, when a family member or carer may help. Clinical variables have been chosen based on the team’s experience with ME/CFS research [1, 8, 9] and those routinely collected by the UK Biobank [10]. Clinical variables include:

- **Symptoms experienced**
  - urinalysis by dipstick (glucose, protein, blood, and specific gravity)
- **Sociodemographic variables**
  - pulse oximetry
- **Family and individual health histories**
  - blood pressure (seated and standing)
- **Potential risk factors (exposures)**
  - standing height
- **Medical Outcomes Survey Short Form (SF-36v2) [11]**
  - weight and bioimpedance
- **General Health Questionnaire (GHQ-28) [12, 13]**
  - waist circumference
- **Epworth sleepiness score [14, 15]**
  - hand grip strength test
- **Fatigue scales assessing severity [16–18] and disability [17, 19]**
  - spirometry
- **Pain and fatigue analogue scale [20, 21]**
  - rheumatoid factor*

* Available for baseline only.
assessment data have been collected using standard equipment by a research nurse trained in the study’s clinical assessment protocol. Data collected from the questionnaires, clinical assessments and blood tests are detailed in Table 1.

The subset of participants being followed-up, are assessed with the same procedures for data and sample collection, except for some blood tests that are required at baseline only (see Table 1).

A bespoke database securely stores data from questionnaires, clinical assessment, and blood tests results, and features algorithms to categorise participants according to distinct ME/CFS criteria.

(3) **Bioresource description**

**Object name**
Clinical data and blood samples from people with ME/CFS, people with MS, and healthy controls.

**Bioresource name**
The UK ME/CFS Biobank.

**Bioresource location**
The project is led and managed by the CureMe team, located within the International Centre for Evidence in Disability (ICED), Clinical Research Department, Faculty of Infectious and Tropical Diseases, K/490, London School of Hygiene & Tropical Medicine (LSHTM), Keppel St, London WC1E 7HT, UK. Participant data is securely stored at LSHTM in locked files and in a bespoke database.

De-identified biological samples are stored at the UCL/RFH BioBank facility, 1st Floor, UCL Medical School, Royal Free Campus, Rowland Hill St., London NW3 2PF, UK. Email: uclrfhibiobank@ucl.ac.uk; website: https://www.ucl.ac.uk/biobank/uclpphysicalbiobank.

**Bioresource contact**
mecfsbiobank@LSHTM.ac.uk.

**Bioresource URL**
http://cureme.LSHTM.ac.uk/the-uk-mecfs-biobank/.

The UK ME/CFS Biobank is one of the Cure-ME projects, therefore, the bioresource’s URL is linked to the Cure-ME webpage. Researchers are encouraged to to seek information through this site and/or contact the Cure-ME team.

**Bioresource type**
A Disease-specific biobank, the UK ME/CFS Biobank stores data and blood samples collected from consenting participants with ME/CFS, with MS, and healthy controls. The data includes socio-demographic variables, clinical measurements, routine blood test results. Blood samples are aliquoted for a wide range of research applications including in virology, immunology, genetic, and other types of research. Clinical data is stored at the LSHTM and all biological samples are stored at UCL/RFH BioBank facility. We plan to expand the collection to include oral fluid, urine, stool, and other samples, subject to funding.

**Type of sampling**
Participants with ME/CFS and MS are recruited based on their disease status, and all must have received a diagnosis from a medical professional prior to enrolment. Sampling of all participants is largely population-based, from primary and secondary health services within the study areas. Recruitment of healthy controls is augmented through personal referrals and via higher education institutions, and recruitment of severely-affected participants is supplemented through personal referrals and support groups to ensure proportional representation in our study population, since this segment of the ME/CFS population is under-represented in the NHS electronic databases.

Participating with MS and healthy controls serve as comparison cohorts and are frequency matched to ME/CFS cases by sex, area of residence, and age (within five years).

To enable longitudinal studies, a subset of participants has been followed up at 6–12 months (including a clinical assessment, blood collection, and completion of questionnaires), and additional follow-ups are planned, contingent on funding.

**Anatomical site**
Venous peripheral blood samples are taken from the arm.

**Disease status of patients/source**
Participants with “mild” to severe (bed-or housebound) ME/CFS; participants with multiple sclerosis; healthy controls who do not have any major morbidity.

**Clinical characteristics of patients/source**
The team initially assesses case or control eligibility using the project’s Symptoms Assessment screening questionnaire.

**Inclusion criteria**

All participants are aged between 18 and 60 years at the time of participation.

Participants with ME/CFS have been diagnosed by a clinician and are compliant with the Canadian Consensus [4] and/or CDC-1994 (“Fukuda”) [5] criteria, as determined using responses on the Symptoms Assessment form, which feeds a computerised algorithm that maps reported symptoms onto ME/CFS study case definitions.

MS comparators have been diagnosed by a UK NHS neurology consultant [22].

**Exclusion criteria**
Participants must not have:

- used drugs in the preceding three months known to alter immune function or taken anti-viral medications;
- had any vaccinations in the preceding three months;
- a history of acute or chronic infectious diseases (excluding herpes viruses);
- another severe illness such as cancer, coronary heart disease, or uncontrolled diabetes;
- a severe mood disorder;
- been pregnant or breastfeeding in the preceding 12 months; or
a BMI ≥ 40 (be morbidly obese).

**Vital state of patients/source**
Alive.

**Clinical diagnosis of patients/source**
As described in the *Clinical characteristics* section above, participants with ME/CFS have been diagnosed by a clinician and meet the Canadian Consensus [4] and/or CDC-1994 (“Fukuda”) [5] criteria. Some participants are recruited with a diagnosis of ME/CFS from a medical professional but do not fulfil the study’s aforementioned criteria after completion of baseline assessments; these participants are classified as having “non-ME/CFS chronic fatigue”. Compliance with four additional criteria – Oxford [23], London [24], Systemic Exertion Intolerance Disease [25], and International Consensus Criteria for ME [26] – is also assessed, but is not used to determine eligibility.

MS cases have been diagnosed by a UK NHS neurologist consultant [22].

**Pathology diagnosis**
N/A.

**Control samples**
Healthy controls must not have had any major morbidity and must have a BMI < 40. Further details are described in the *Clinical characteristics* section above.

**Biospecimen type**
Blood, peripheral blood mononuclear cells, DNA, RNA, serum, plasma (outlined in Table 2).

**Size of the bioresource**
As of October 2016, the UK ME/CFS Biobank included data and samples from 532 people (including 284 ME/CFS, 60 MS, 135 healthy control, and 53 non-ME/CFS chronic fatigue groups), 253 of whom had completed a 6–12 month follow-up (Figure 5). Current holdings include samples from both time-points; 29,863 aliquots are stored as of the end of October, 2016. We collect > 700 variables for the baseline time-point and > 300 for follow-ups. Figure 5 details the process for participant invitation and enrolment, including total number of participants recruited by category and recruitment rates.

The recruitment target for the end of 2016 is 300 people with ME/CFS, 75 people with MS, and 150 healthy controls.

There is no planned limit on the number of participants recruited or samples collected, although we intend that samples are released routinely for use in ethically-approved studies, and then replenished through additional recruitment. Recruitment is planned to continue at least through 2021, including additional time-point follow-ups of the current cohort, contingent to funding.

**Release date**
Samples and data are available upon successful application (see Figure 6).

**Access criteria**
The Cure-ME team had established the UK ME/CFS Biobank, with a governance structure that involves the collaboration between two academic institutions – the LSHTM (housing the Cure-ME) and the UCL (housing UCL-RFH BioBank facility). The UK ME/CFS Biobank Steering Committee oversees the above team-work, and is also involved in assessing research applications, ensuring community participation in the process.

Academic, non-commercial, and commercial researchers are all eligible to apply to use samples and/or anonymised data. Applications and review procedures are in place (see Bioresource URL). Researchers should present a sound scientific rationale for the proposed study, have a good research track record, and be supported by their institution. The following types of studies are eligible:

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Stored aliquot/ tube volume</th>
<th>Collection tube (BD)</th>
<th>Number of aliquots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>0.5 ml</td>
<td>EDTA</td>
<td>3,021</td>
</tr>
<tr>
<td>Serum</td>
<td>200 µl</td>
<td>Red serum</td>
<td>6,770</td>
</tr>
<tr>
<td>Plasma</td>
<td>1 ml</td>
<td>EDTA</td>
<td>2,029</td>
</tr>
<tr>
<td>Plasma</td>
<td>250 µl</td>
<td>Na Hep</td>
<td>5,382</td>
</tr>
<tr>
<td>Red Blood Cells (RBCs)</td>
<td>2 ml</td>
<td>Na Hep</td>
<td>773</td>
</tr>
<tr>
<td>Peripheral Blood Mononuclear Cells (PBMCs)</td>
<td>5x10⁶ cells in 1 ml</td>
<td>Na Hep</td>
<td>9,106</td>
</tr>
<tr>
<td>Peripheral Blood Mononuclear Cells (PBMCs)</td>
<td>5x10⁶ cells in 1 ml</td>
<td>EDTA</td>
<td>2,198</td>
</tr>
<tr>
<td>Blood for RNA</td>
<td>2.5 ml</td>
<td>PAXgene</td>
<td>584</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>29,863</strong></td>
</tr>
</tbody>
</table>

*Table 2: UK ME/CFS Biobank sample holdings at the UCL/RFH BioBank (through October 2016).*
will be prioritised: testing or generating new hypotheses on pathophysiology of ME/CFS; improving diagnosis and phenotyping; and/or, basic science, e.g. pharmacological in vitro studies, potentially leading to clinical trials on therapeutic approaches.

Applicants are asked to submit a short outline application, which will be reviewed by representatives of the UK ME/CFS Biobank Steering Committee (i.e. a patient, a clinical and a biomedical researcher). Upon approval of the outline application, applicants are invited to submit a full proposal to be peer-reviewed.

Both the LSHTM and UCL teams work in close contact for ensuring adherence of SOPs, from recruitment to sample and/or data release. Requests for usage of samples have to follow procedures in compliance with current regulations. These includes the local and the UCL-RFH BioBank Ethics Review Committee approvals. The latter, checks if the proposals are in compliance with the UK regulations (Figure 6). Release of data and/or samples will occur upon signing of a Materials Transfer Agreement and/or Data Transfer Agreement, and researchers must not attempt to identify any individuals.

The Biobank was planned to operate on a cost recovery basis to replace released samples, thereby helping to ensure the long-term sustainability of the resource. Further details are available upon request.

(4) Reuse potential

The standardisation and extensiveness of the clinical data collected allows classification and stratification of participants with ME/CFS according to clinical phenotype and six disease definitions. Similar data and identical sample collection procedures have been followed for all cohorts, maximizing the potential of identifying intergroup differences.

The team has developed protocols to ensure whole blood, plasma, serum, red blood cells, PBMCs, and PAXgene samples are suitable for a variety of applications, including for immunological, virological, genetic, and other types of studies. Quality analysis of a subset of stored samples has validated sample collection and storage protocols, ensuring that samples are fit for purpose.

Data and samples can be used for high quality, high-throughput studies, avoiding the limitations of previous studies, which have often been impacted by small sample sizes, significant selection bias due to
recruitment procedures, and uncertainty about case classification accuracy.

The Biobank’s open access to data and samples from our well-defined cohorts will enable high quality research with significant savings to users. We are confident that this resource will benefit the international community of researchers interested in conducting ethical and progressive research on ME/CFS, leading to the improved diagnosis and treatment of millions of people with ME/CFS worldwide.

**Ethics Statement**

The UK ME/CFS Biobank protocol was launched in 2011 following extensive consultation. Ethical approval granted by LSHTM Ethics Committee January 16th, 2012 (Ref.6123) and National Research Ethics Service (NRES) London-Bloomsbury Research Ethics Committee December 22nd, 2011 (REC ref.11/10/1760, IRAS ID: 77765). LSHTM and UCL-RFH Biobank hold Human Tissue Act licences – HTA-12066 and HTA-11016, respectively. Recruitment of participants started March 2012.

The Biobank Steering Committee comprises ME/CFS representatives, ME/CFS charity representatives, CureME and external researchers. This Committee has overseen the Biobank project from onset, and is part of its governance, monitoring progress and assessment of external applications.

All participants provide written consent for questionnaire, clinical measurement, and laboratory test data, and samples to be made available for ethically-approved research (including genetic analyses). Participants are posted the consent form and an extensive information sheet. The consent form includes an option to withdraw from the study at any time. Samples/data will be respectfully disposed of under exceptional circumstances, e.g. withdrawal of consent with a request of destruction.

No individual research results from the Biobank will return to participants or nominated doctors.

All data/samples are fully de-identified. The key linking the anonymised data with identifiers is kept securely by the LSHTM project lead.

**Constraints**

There are no constraints on the ethically-approved usage of data or samples, although applications for their use will be prioritised according to the UK ME/CFS Biobank’s mission, three priority areas (outlined in the Access criteria section), and available inventory.

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**Figure 6: Workflow for accessing samples and/or data.**

[Diagram of workflow for accessing samples and/or data]

Application & Review

- Submit outline research proposal for initial review to UK ME/CFS Biobank scientific subcommittee

- Submit full research proposal for full peer review to UK ME/CFS Biobank scientific subcommittee

- Submit application to UCL-RFH Biobank Ethical Review Committee (B-ERC)

- Signature by all parties of Material Transfer Agreement and/or Data Transfer Agreement

- Shipment of samples and/or transfer of data

Expected wait

- 2 weeks

- 4 weeks

- 2-3 weeks

- 4-8 weeks

- 1 week

Ethics Review

- Submit application to Local Ethics Committee

- Submit application to Research & Development (R&D) Office

- If proposal based in NHS institution

The workflow illustrates the process from application to ethical review and shipment of samples or transfer of data.
Acknowledgements

We would like to thank our Steering Committee members, including representatives of the initial funders, who have actively participated in the oversight of project development and implementation, shared insights, revised documents and protocols, and helped strengthen our invaluable connections with the ME/CFS community.

We thank the UCL/RFH BioBank staff (in particular Prof Mark Lowdell, Janet North and Pang Kwok) as the success of the UK ME/CFS Biobank would not have been possible without their contributions; and the UK Primary Care Network, who have been instrumental to our successful recruitment strategy.

Most of all, we would like to express our gratitude and appreciation to the hundreds of people who have so generously contributed to the UK ME/CFS Biobank by donating their time, resources, and often very precious energy to participate in the project.

Competing Interests

The authors have no competing interests to declare.

Author Roles

1. Lacerda, Eliana M.; Creator, Bioresource Manager, Scientific support
2. Bowman, Erinna W.; Creator, Collector, Scientific support
3. Cliff, Jacqueline M.; Lab scientific support
4. Kingdon, Caroline C.; Collector of Data or Samples
5. King, Elizabeth C.; Lab support
6. Lee, Ji-Sook; Lab support
7. Clark, Taane G.; Scientific support
8. Dockrell, Hazel M.; Scientific support
9. Riley, Eleanor M.; Scientific support
10. Curran, Hayley; Bioresource Manager
11. Nacul, Luís; Director, Creator

References


