Study 3.

Version 1.0 April 2016

PHE-MOHS Ebola Biobank Sample Access Form

Applicants for materials from the biobank must complete and submit this application form to the PHE Head of Research Governance. Decisions to grant access should (i) help ensure that any uses of the resource are consistent with the purpose of the biobank; (ii) ensure that research projects have relevant scientific and ethical approval, and (iii) make information publicly available about uses of the PHE-MOHS biobank collection. Review of the application will be undertaken by the PHE-MOHS Ebola Biobank Governance Group (EBGG), who will consider the following eligibility criteria.

1. The proposed research is scientifically sound
2. There is sufficient funding to enable completion of the research
3. The research is designed in such a way that ensures integrity, quality and transparency
4. The research team are suitably qualified by education, training and experience.
5. The research site has adequate capacity and capability to undertake the research
6. The research has all necessary ethical and regulatory permissions.
7. The materials will be used to ensure the greatest benefit to the public
8. The research has relevance to the people of Sierra Leone, and a likelihood that the people will be able to benefit from it.

The level of scrutiny used to assess applications will be proportionate to the nature and scale of the research project, taking into account (by way of example) whether a significant amount of material is required relative to the project objectives.

In reaching its decision on a particular research application, particularly with regard to items 1 – 6 inclusive, above, the EBGG will accept the outcomes of peer review that is undertaken by a research funder. Where that is not possible or where there is doubt that an application may not satisfy any of the eight criteria, applications may be scrutinised by an independent scientific reviewer selected by the EBGG in order to ensure independence, competence and rigour of peer review

Additionally the following will be considered by EBGG:

- Quantity of material requested
- Arrangements to maintain data security and confidentiality
- Biosafety and biosecurity
- Proposed dissemination of results

When the review is complete the application will either be:

1. Approved
2. Approved pending receipt of further information/approvals within a set period of time.
3. Approved subject to a project amendment
4. Declined.

Applicants awaiting the outcome of an application for funding will receive an 'approval in principle' letter. Access will be conditional on there being sufficient research funding to use the Biobank samples for the intended purpose.

When funding is in place the research study lead should forward a copy of the funding proposal and the letter of offer to the EBGG, along with any amendments to the original project that have been required by the funder.

1 **Title of Proposal** *Please give a succinct descriptive title for the proposal*
Ebola Virus: Modern Approaches for developing bedside Rapid Diagnostics EbolaMoDRAD
2 **Study team** *Please list all collaborators, lead investigator named first, with institute affiliations*
3 **Background** *Project rationale including a brief review of relevant literature with key references (max 400 words, references additional)*
The overall aim of is to develop and deliver rapid and bedside diagnostic tool(s) that will significantly increase our capacity to handle the current Ebola Virus Disease (EVD) in West Africa and also future outbreak in within a 36 month period. This will be done via a multidisciplinary research consortium drawn from key European and African research organisations thus consolidating previous tools and knowledge. will also put in place a strong capacity building programme in West Africa and hands-on training in non-endemic countries (neighbouring to endemic areas) in the fields of outbreak management, diagnostics and application tools/results. will disseminate widely all results as they become available notably to public health bodies, Non Govermental Organistions (NGOs), outbreak management teams and local hospitals in West Africa via a strong outreach programme. To achieve this overall aim an intensive work plan will be put in place with the following specific objectives: Objective 1: to develop a biosafe detection method (diagnostic tool) for use at points of care in EVD endemic countries (via WP2 and WP3) ; Objective 2: to validate the diagnostic tools first in BSL4 and reference laboratories and then in the field (via WP4); Objective 3: to implement a strong capacity building programme in West Africa with focus to rapid diagnostic, biosafety measure and outbreak management via WP4 and WP5. Objective 4: to disseminate widely the project and its results to public health bodies, NGOs, outbreak management teams and local hospitals in West Africa via WP5.
4 **Summary of the research with overview of methods** *Include numbers, sample types and volumes (see notes on page 3) required with statistical justification if appropriate. Include methods of analysis distinguishing those requiring CL4 containment and those that can take place at lower containment level.*

The samples requested will be used to improve and validate rapid point-of-care diagnostics for use in the field.

The main activities will be:

1. Validation of an Ebola blood inactivation tube for serology.
For this we require approximately 0.1 to 0.5ml of 5 to10 EBOV positive diagnostic samples with low Ct values (<25). The analysis will compare untreated vs treated inactivation experiments. The inactivation reagents will have been initially tested with blood spiked with live Ebola virus isolated from cell culture and the real patient samples from the Biobank will be used to validate the spiking experiments.

2. IgM (IgG) antigen microarray epitope mapping of patient sera
For this we require a selection of 10-20 IgM positive samples (with different IgM concentrations) and 20 IgM negative samples. If available or known, IgG positive samples could also be tested. 30-50 microliters of each sera are sufficient for a thorough analysis, running experiments in triplicates. The analysis/experiment could be performed in the Alternatively, microarray slides and SOP could be sent to. We will disseminate the results by presentation on international conferences and in peer reviewed journals as well as within the. These results are essential to improve specificity and sensitivity of the next LFD generation (see 3. Validation on Ebola antigen / IgM antibody lateral flow device)

3. Validation of an Ebola antigen / IgM antibody lateral flow device
For this we require 50 to 150 PCR positive samples at 100 - 300µl per sample. Ideally this would include 25 of each sample where the samples were tracked over the time course of the infection until the patient became PCR negative or the patient subsequently died. This will allow us to expand an initial trial of 20 antigen lateral flow devices and trial and improve a prototype IgM lateral flow device. We would also require 25 samples at 100 - 300µl per sample from the same population that tested negative for Ebola by PCR, but that were Malaria positive.

4. Validation of rapid point-of-care isothermal assay
For this we require 80-100 PCR positive samples (10-30 CT < 30, 30-40 CT > 30, 10-20 negatives 280µl each) with varying Ct values to validate isothermal Loop Mediated Isothermal Amplification (LAMP), Recombinase Polymerase Amplification (RPA) lateral flow assays, which are a further development of a recently published assay[1], and Rolling Circle Amplification (RCA). We also require up to 50 Ebola PCR negative samples (280µl each), ideally with 25 of those which are Malaria positive, and 10 of those which are haemolysed. We will compare the isothermal assays to the Trombley Ebola Zaire real-time PCR to test for concordance. Al of these assays either have already been tested with cell-culture derived Ebola genomic RNA or will be before clinical patient samples are used.

For assays being performed at we require 3 EBOV PCR positive samples taken during monitoring of treatment of those who subsequently died and 3 EBOV PCR positive samples taken during monitoring of treatment of those who subsequently survived. The samples will be inactivated by MagNA Pure lysis binding buffer. The EBOV RNA extraction and analysis will be performed at

For all of these activities we will disseminate the results by presenting at international conferences, publication in peer-reviewed journals and at EU-Africa workshops organised by

The benefits of developing these tests is that they will become available to the laboratories in Sierra Leone. The blood inactivation tubes will provide safe transport of blood to laboratories and will lead to reduced laboratory infrastructure e.g.

containment required to test samples as they will be inactivated and made safe at source. Scientists from Sierra Leone will be able to participate in workshops organised by Ebola
5 **Database variables** *Please list any metadata associated with the samples required for selection of samples or interpretation of results.*
We would need access to information about location, clinical outcomes and Ct values from initial tests performed in Sierra Leone to allow comparison to our diagnostic test development.
6 **Resource required and available** *Please give details of funding available (or being applied for) to carry out the proposed research. Include any resource available or required for the processing of samples at PHE's CL4 laboratory*
This project is being funded by and started in January 2015 and runs to January 2018. There is budget already in allocation available as part of this project to perform experiments in CL4 laboratory. Other partners have budgets available for CL4 (BSL4) work in their respective laboratories as part of this project.
7 **Biosafety and Biosecurity** *All applications must be accompanied by a biosafety and biosecurity assessment and risk management plan.*
All partners involved in this project are already involved with handling inactive viral material or human samples from convalescent patients. Each of the CL4 (BSL4) laboratories in this partnership are experienced in handling Ebola and other BSL4 pathogens. All are part of the network where biosafety and biosecurity assessments have been completed. If further information is required the partners will be able to provide this information. Samples used for rapid point-of-care isothermal assays will be inactivated using MagNA Pure lysis binding buffer. The inactivated samples can then be handled under standard BSL-2 conditions.

Notes

Categories of samples in Biobank

N.B. Samples are known to be EBOV positive or negative by PCR, some may be further categorised as below:

1. EBOV positive diagnostic samples (ie 1st positive sample per patient)
2. EBOV PCR positive samples taken during monitoring of treatment of those who subsequently died
3. EBOV PCR positive samples taken during monitoring of treatment of those who subsequently survived
4. EBOV PCR negative samples of those who subsequently tested positive
5. EBOV PCR negative samples of those previously testing positive
6. EBOV PCR negative patients or contacts

Database

Some further information is associated with some samples in the biobank. This includes laboratory of origin, laboratory ID number, name, age, gender, specimen type, original or follow up sample, facility from where the patient was referred, date of hospitalisation, symptom onset, date tested, clinical chemistry results, viral load and Ebola test result.