Study 4

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

Pathogen Identification in Children Attending Ebola Holding Units testing Negative for Ebola Virus Disease in Freetown, Sierra Leone

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 West African Ebola virus disease (EVD) outbreak claimed >11000 lives with nearly 30000 cases. However, the wider impact on health systems and mortality rates from non-EVD conditions is likely to be far greater[1-5]. To reduce nosocomial transmission, anyone with symptoms consistent with EVD was isolated and tested in an Ebola holding unit (EHU) prior to hospital admission[6, 7]. The suspect case definition for EVD in children was broader than for adults, meaning that many sick children were admitted to EHUs who subsequently tested negative for the disease[4, 8-10] This had implications for the management of their underlying diagnosis and their potential exposure to EVD in an EHU. The mean duration of admission in a Western Area EHU was 48 hours[11].

The majority of under 5s mortality in Sierra Leone is due to pneumonia, diarrhoeal diseases, malaria, tuberculosis and HIV[12, 13]. In EHUs it was challenging to manage these conditions successfully. Even in better equipped facilities, the lack of diagnostic tools available within many EHUs (e.g. malarial and HIV rapid diagnostic tests) hampered adequate caregiving[10]. Routine vaccination levels fell during the outbreak.[1] Understanding the prevalence of measles in EHUs will contribute to forward planning for both vaccination programmes and outbreak management, particularly important in the context of cross-infection risk (for both measles and EVD).

There may have been under-diagnosis of other viral infections such as Dengue and chikungunya in Sierra Leone[14, 15]. This project offers the potential to further understand the presentation and prevalence of these viral infections, contributing to national epidemiological planning.

Identifying the pathogens in children with febrile illnesses testing negative for EVD is crucial for both current and future service provision. Understanding the epidemiology of the commonest causative pathogens will enable therapy targeting common aetiological agents other than EVD in EHUs, and could inform a more specific case definition, allowing "ruling out" of EVD without necessitating admission to an EHU. Data about the distribution and frequency of these pathogens will also inform public health planning for the future.

Molecular testing using a specific polymerase chain reactions (PCR) allows accurate and sensitive identification of a range of pathogens, along with a broad range PCR for more unpredictable bacterial pathogens. This panel would enable a comprehensive description of pathogens underlying febrile illnesses in children attending EHUs who test negative for EVD.

Combining clinical data available from a prior cohort study with laboratory data generated by this study offers an unprecedented opportunity to investigate the epidemiology of infection in febrile children in Sierra Leone, as well as informing future outbreak planning.

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.

Study Aim

To identify the pathogens underlying febrile illnesses in children attending Ebola Holding Units testing negative for Ebola Virus Disease in order to identify opportunities for improving clinical outcomes.

Study Objectives

1. To identify pathogens present in the blood of children attending EHUs who subsequently test negative for EVD
2. To relate identified pathogen(s) present with clinical presentation
3. To describe the outcomes of children attending Holding Units testing negative for EVD with reference to the pathogen identified.

Study Design

To achieve the study objectives we propose to undertake a retrospective cohort study. The study population consists of 100 children (under 13 years old) with blood samples sent to the Kerry Town Public Health England laboratory in Sierra Leone and testing negative for EVD, for whom we already hold clinical, demographic and outcome data.

Methods

Stored plasma from 100 children (120 samples) attending Holding Units in the four areas served by PHE laboratories will be tested. These will be subjected to a panel of molecular assays. Bacterial assays will include *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Streptococcus agalactiae, Neisseria meningitidis,* Enterobacteriaceae (family), *Enterococcus* spp, *Ureaplasma* spp, *Candida* spp. Samples for which a pathogen is not detected using specific PCR assays will undergo broad range 16S rDNA PCR with subsequent next generation sequencing to identify further bacterial pathogens such as *Burkholderia pseudomallei* and *Yersinia pestis*. Samples have been screened for *Plasmodium falciparum* using a rapid diagnostic test since December 2014. Measles will be screened for, and in a proportion of samples other pathogens incorporated in PHE's imported fever panel will be included, alongside alphabunyaviridae, arenaviridae, flaviviridae and a pan-filovirus assay. Dengue, West Nile virus and Rift Valley Fever will also be incorporated in the panel, with the possibility of adding in Enterovirus and Parechovirus.

DNA will be extracted from frozen plasma as per standard operating procedures and then the PCR assays performed according to the standard operating procedures of and the Microbiology department at laboratories. **We are requesting access to 200uL pre-extracted DNA of Category 6 Samples (EBOV PCR negative samples) to be transported within the UK to laboratories at.**

Clinical Data and Participants

The samples requested (for which we have PHE Kerry Town laboratory numbers) have been chosen pragmatically as the children from whom they were collected form part of a retrospective cohort study[9] . This means we will be able to combine the results from this proposed study with the detailed clinical, demographic and outcome data we already have stored as part of the pre-existing study.

Data Analysis

Clinical and demographic features of children testing negative for EVD will be described using descriptive statistics incorporating the prevalence of alternative pathogens. The sample size of 120

assuming a sample positivity rate of 50% will allow give 95% confidence intervals of 40-60% positivity (modified Wald method). Logistic and linear regression will be used to identify features (including presence or absence of a specific pathogen) associated with mortality.

Ethical Considerations

The study has obtained approval from the Sierra Leone Ethics and Scientific Review Committee and from Ethics committee (where it was submitted as an amendment to the protocol of the research PhD study).

There will be no additional samples taken from children as part of this study, with stored remnant samples only being used. If epidemiologically significant pathogens are identified these results will be fed back to the heads of the various facilities during the course of the study. As remnant samples that have already been collected will be used with anonymisation of clinical data, specific consent will not be sought from patients as part of this study.

Data will be input directly into password-protected databases on the applicant's password secured computer. The raw database and password will only be shared with individuals directly involved in the study. All personal identifiers (name, address, telephone numbers) and potentially sensitive information will be removed from the final database at the completion of the project.

Dissemination of Results

It is anticipated that this study will result in at least one peer-reviewed paper in a high impact journal and will be presented in an academic forum such as the. The cohort study which will be allied to this study was presented as an oral session at this year's and as a plenary session at both the annual congress and the annual conference. The first paper from the cohort study has been accepted (pending response to reviewers) by the so the group has a strong track record for both presentation and publication of results.

The study results will be fed back to local stakeholders in Sierra Leone by one of the national collaborators in both academic and non-academic forums. Again, the group has a good track record for presenting results locally: presented the cohort results at in Freetown in March this year, and gave a presentation to local community stakeholders about the impact of the cohort study results in Freetown.

5 Database variables

Please list any metadata associated with the samples required for selection of samples or interpretation of results.

We have the Kerry Town laboratory numbers for the samples we are requesting. We already have necessary clinical and demographic and outcome data we need for the study.

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

We have been approved funding from the network to carry out the study pending access to the samples.

Resources Requested from PHE

We are requesting that carry out DNA extraction according to their standard protocol, if possible with an additional bead beating step (after sample inactivation) to maximise yield of bacterial DNA. It has been provisionally agreed that will also carry out their in-house imported fever panel PCR

(and this has been included in the budget) but if this is no longer feasible these tests could be carried out at instead.

Resources Available

The molecular diagnostic service provided by the Microbiology Department at has been established for over 15 years. has considerable expertise in both bacterial and virological PCR methods including developing a broad range 16S rDNA PCR as a clinical service[16-19]. The research PhD has involved the use of next generation sequencing in addition to both broad range and specific PCRs to identify bacterial DNA in the blood stream of children with HIV from Uganda and London, so we have both the competence and equipment to carry out the proposed assays on extracted DNA[20].

7 Biosafety and Biosecurity

All applications must be accompanied by a biosafety and biosecurity assessment and risk management plan.

Procedure

1. Transport of extracted DNA from PHE laboratories to Laboratories,
2. Exposure to biological samples containing DNA from any pathogens present in sample.
3. Toxic substances (enzymes and chemicals in general use in the laboratories)
4. Electrical equipment: PCR machine, Centrifuge, incubator, pH meter, water bath, magnetic stirrer, laminar flow hood, safety cabinets, phosphorimager, computers, printers, fridges, freezers, vortexers, scanner
5. Dangerous apparatus (centrifuge, ovens, autoclaves)

Hazard

1. Exposure to biological samples containing DNA from any pathogens present in sample including blood borne viruses (BBVs)
2. Exposure to biological samples containing DNA from any pathogens present in sample including blood borne viruses (BBVs)
3. Chemical burns, carcinogenesis, inhalation
4. Electrocution
5. Unbalanced centrifuge, heat burns

Risk Assessment

1. Transport of DNA extracted from samples potentially infected with EVD or other BBVs- Risk: Low
2. Exposure to DNA: Risk: Low
3. Toxic substances: Risk: Low
4. Electrical Equipment: Risk: Low/medium
5. Dangerous apparatus: Risk: Low/medium

Control Measures

1. **Transport of extracted DNA** in appropriately sealed containers (considered 'safe' by) (will be performed by specialised couriers according to standard operating procedures of PHE laboratories and Microbiology laboratory at and received on arrival: **Risk: Low**
2. **Exposure to DNA:**) The workers will wear laboratory gowns, aprons and gloves. Cut or

damaged skin surfaces will be covered before any work is carried out. Work will be carried out in accordance with the health and safety procedures in place in the Microbiology Department at and all waste disposed of according to the local standard operating procedures. All staff will be given, free access to Hepatitis B immunisation and will be required to ensure that they are immune to Hepatitis B virus before starting work. Human Resources will inform OH when new staff are appointed, and OH will subsequently manage appropriate immunisation procedures. Any Hep B non-responders will be given the opportunity to sign a disclaimer indicating that they are aware of the risks of working with biological specimens, including human tissue, without being immune to Hep B. **Risk: low.**

3. **Toxic substances:** risks are minimized by following established laboratory guidelines for sample processing and waster disposal and by observing the control measures in the relevant safety data sheets and COSHH assessments. All SOPPs and Health and safety documentation are held in the document control system in the Microbiology department at. **Risk: low**

4. **Electrical equipment**: (all) All will be advised on the safe use of electrical equipment by the laboratory manager as part of departmental safety induction. A visual check for obvious damage will be performed prior to each use. Electrical equipment in the unit is checked annually for electrical safety by in house works department. Equipment failing tests is removed from use until repaired or replaced. **Risk: low.**

5. **Dangerous apparatus:** all personnel working on the project have been or will be instructed in the safe use of the equipment by a designated person responsible for the equipment. A Safety Training Log is in place. Hazardous equipment will be kept in a good state of repair. Specific equipment will be maintained regularly by engineers from the supplier company or similar. **Risk: low**

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.