Study 2.

Version 1.5 Dated 12th July 2016

## 1 SAMPLES REQUESTED

| Cohort | Type of sample required | Sample volume |
|---|---|---|
| PHE Makeni EBOV + | 20x Sera | 20 x 0.5ml |
| PHE Makeni EBOV - | 20x Sera | 20 x 0.5ml |
| PHE Port Loko EBOV + | 20x Sera | 20 x 0.5ml |
| PHE Port Loko EBOV + | 20x Sera | 20 x 0.5ml |
| PHE Kerry Town EBOV + | 20x Sera | 20 x 0.5ml |
| PHE Kerry Town EBOV - | 20x Sera | 20 x 0.5ml |
| | | |
| | | |
| TOTAL | 120 x sera | 20 x 0.5 ml |

**2. Characterisation of the Human Virome in Febrile Patients admitted to the PHE Ebola treatment centres in Kerry Town, Port Loko and Makeni, Sierra Leone.**

## 3 STUDY TEAM

## 4 BACKGROUND

Viral haemorrhagic fever (VHF) is caused by a diverse group of viruses that may share commonalities in the process whereby they produce systemic and frequently fatal disease. In the two years since the beginning of the Ebola outbreak in West Africa, significant developments have been made in further understanding the biology of the Ebola virus (EBOV), one of the most known examples of a VHF. While this knowledge has advanced our thinking about VHF pathogens, there remain critical gaps in our knowledge of the effects of co-infection with non-VHF pathogens that co-circulate during a VHF outbreak. The common ability of many VHFs to disable the host immune response by attacking and manipulating the cells that initiate the antiviral response is well established. However, the role that non-VHF pathogen co-infection may play in affecting clinical outcome and survival of Ebola patients remains largely unknown. We hypothesize that the clinical outcome of patients infected with EBOV during the West African outbreak was detrimentally affected by co-infection with other circulating VHF and non-VHF pathogens. To establish what other pathogens were co-circulating, we will analyse the viromes (collection of viruses) of 120 patients admitted to the three geographically distinct PHE run DFID supported Ebola Treatment Centres in Sierra Leone in 2014-2015. We will focus on 60 patients (20 per ETC lab: Kerry Town, Port Loko, Makeni) that tested negative for EBOV to ascertain what non-VHF co-circulating pathogen may have contributed to their VHF-like clinical symptoms and 60 patients that tested positive for EBOV (20 per ETC lab: Kerry Town, Port Loko, Makeni). We will also fully characterise any viruses isolated from patient sera and investigate cytokine levels in patient sera and analyse the inflammatory responses they induce in healthy human peripheral blood mononuclear cells (PBMCs).

## 5 SUMMARY OF THE RESEARCH WITH OVERVIEW OF METHODS

**Sample type:** Sera

**Numbers of samples:** 120

60 Ebolavirus positive with Ct values < 25

60 Ebolavirus negative

**Location of samples:** 20 EBOV Pos & 20 EBOV Neg from KT, PL and MK labs respectively.

**Volume requested:** 0.5ml / sample:

- 2x 140ul (280ul) for RNA extraction
- 220ul for serological analysis and virus isolation

**Containment and methodology:**

All 120 samples will initially be handled at CL4 with duplicate aliquots of 140ul being inactivated in 560ul of Guanidine Thiocynate (Qiagen AVL chaotropic buffer) Inactivated samples will then be transferred in new o-ringed tubes from CL4 to a CL2 labwhere a second chemical inactivation (100% Ethanol) will take place within the Class III Microbiological Safety Cabinet (MSCIII) where RNA will be extracted and transferred from the CL3 to the CL2 laboratory for molecular analysis.

All 120 extracted RNA samples will be tested at CL2 by realtime RT-PCR (qPCR) for the following hazard group 4 pathogens: Ebola, Marburg, Lassa and Crimean Congo Haemorrhagic Fever virus.

280ul of the original sera samples testing negative will be transferred directly from CL4 to CL3). 220ul of those samples testing positive for any of the hazard group 4 (HG4) pathogens, remain at CL4 for any subsequent in vitro virus isolation attempts. All samples testing negative for HG4 pathogens will be transferred to CL3 for subsequent virus isolation and immunological analysies (PBMC stimulation, FACS, and ELISA).

Following this initial triage of samples from CL4 to CL3, subsequent qPCRs will be carried out on the extracted RNA for the following pathogens: Rift valley fever virus, Chikungunya virus, Dengue viruses 1-4, Zika virus, Yellow fever virus, West Nile virus, Influenza A and B, Plasmodium spp, Rickettsia spp and Leptospira spp. Conventional pan-species RT-PCRs will be carried out for alphaviruses, flaviviruses, Hantaviruses and bunyviruses.

HG4 negative sera will be analysed at CL3 for cytokine profiles and stimulation of non-infected health volunteer PBMCs,.

The viromes of 120 RNA samples will then be anyalysed by metagenomic sequencing.

Attempted virus isolation will take place within CL4 and CL3 labs at for identified HG4 and HG3 (&HG2) pathogens respectively. Challenge of health human PBMCs with EBOV negative sera and isolated HG3 and HG2 pathogens will take place at Containment Level 3 laboratory

Any viruses islolated from these 120 patient samples will be deposited in the PHE-MOHS Biobank, for other scientists to use upon request and agreement by PHE MOHS.

***Containment:*** *All EBOV positive samples will initially be aliquoted at Containment Level 4 (CL4.) Aliquots will be appropriately inactivated and transferred to a Containment Level 3 (CL3) laboratory where additional chemical inactivation within a Class III Microbiological Safety Cabinet (MSC) will be carried out as part of the nucleic acid extraction standard operating procedure (SOP EDL/003). EBOV negative samples will be handled at CL3 and chemically inactivated using the same SOP. The extracted RNA will then be transferred to a containment level 2 (CL2) laboratory for molecular diagnostic testing. This is a workflow process that has been tested and tried on a routine basis for molecular diagnosis of EBOV and other hazard group 4 pathogens*

## 5 DATABASE VARIABLES

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|---|
| Laboratory of origin |
| Laboratory ID number |
| Facility from where the patient was referred |
| Patient age |
| Gender |
| Original or follow up sample |
| Ebola test result |
| Date of hospitalisation |
| Symptom onset |
| Date tested |
| Clinical chemistry results |
| Viral load |
| Malaria test result |
| |
| **6 RESOURCE REQUIRED AND AVAILABLE** |
| |
| **7 BIOSAFETY AND BIOSECURITY** |
| *The principle investigator of this application is the author of the Risk assessment (RA03986) "Ebola Deployment – Laboratory work in Field Labs" and co-author of the "Laboratory Code of Practice for Ebola Diagnostic Laboratories, Sierra Leone" used by all three labs in Sierra Leone. The Novel and Dangerous Pathogens training group, within where the project lies, have over 15 years' experience of delivering training in the latest Biosafety and Biosecurity.*<br><br>*A full management plan will be provided upon successful outcome of this request for samples.*<br><br>*Additionally, the commencement of this project is subject to receiving successful outcome in our request for ethical approval to the relevant bodies in the UK and Sierra Leone. Funds have been allocated to ensure all fees will be covered.*<br><br>*Well established Biosafety and Biosecurity teams with decades of experience in working with HG4 clinical samples and schedule 5 and SAPO pathogens in accordance with home office and HSE guidelines.*<br><br>*A unique risk assessment for this work will be developed through reference to the existing risk assessments outlined in section 8.* |