ZIKA RAPID RESPONSE PROJECTS

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ZIKA Reference	PI Name	PI Research Organisation	Project Title	Mth	FINAL MRC AWARD
ZK/16-010	Heather Ferguson	University of Glasgow	Zika: The Ecology of Zika transmission in Colombia and Ecuador	18	£108,815
ZK/16-011	Yorgo Modis	University of Cambridge	Zika: Defining the antigenic epitopes in the Zika virus envelope protein	18	£155,917
ZK/16-012	David Bhella	CVR, University of Glasgow	Zika: Characterisation of Zika virus neutralisation and virion structure by cryogenic electron microscopy and 3D reconstruction	18	£133,141
ZK/16-017	Jimmy Whitworth	London School of Hygiene and Tropical Medicine	Zika: Transmission dynamics and impact of zika virus on population health in a large urban centre in northeastern Brazil	18	£148,000
ZK/16-018	Janet Daly	University of Nottingham	Zika: Harnessing plant power for rational design of immunogens for use in diagnostic assays	18	£93,638
ZK/16-019	Gerry Killeen	Liverpool School of Tropical Medicine	Zika: Affordable, scalable, low-technology transfluthrin emanators for protecting against transmission in low-income countries	18	£148,971
ZK/16-021	Steven Sinkins	Lancaster University	Zika: Vector competence and interactions with Wolbachia	12	£101,995
ZK/16-023	Laura Rodrigues	London School of Hygiene and Tropical Medicine	Zika: Cohort study of pregnant women with rash in Pernambuco State	18	£112,361
ZK/16-025	Luis E Cuevas	Liverpool School of Tropical Medicine	Zika: Novel point-of-care molecular diagnostics for the simultaneous diagnosis of Zika, chikungunya and dengue infections in Latin America	18	£150,000
ZK/16-041	Matthew Bayliss	University of Liverpool	Zika: susceptibility of South American and European vectors to ZIKV infection, and influence of temperature	18	£116,615

ZK/16-045	Neil M. Ferguson	Imperial College London	Zika: Zika in Colombia: Characterisation of exposure and epidemiology in a flavivirus endemic setting	18	£149,824
ZK/16-046	George Warimwe	University of Oxford	Zika: Estimating the transmission and case burden of Zika virus in Kenya	18	£149,996
ZK/16-047	Professor Richard Seton Tedder	University College London	Zika: Development of a type specific Zika virus antibody assay for use in Brazil	12	£121,935
ZK/16-050	Lisa Ng	University of Liverpool	Zika: Investigating the link between Zika virus infection and neurological disease in ex vivo and in vivo models	12	£104,560
ZK/16-061	Stephen Kennedy	University of Oxford	Zika: Development of an online data-sharing platform for images of fetal and newborn heads - An urgent need in the context of the Zika virus outbreak	18	£144,456
ZK/16-067	Tom Solomon	University of Liverpool	Zika: A prospective case-control study to examine the role of Zika virus in Guillain-Barré syndrome in Brazil	9	£117,830
ZK/16-068	Laith Yakob	London School of Hygiene & Tropical Medicine	Zika: surveillance, risk factors and vector management in Brazil	18	£100,000
ZK/16-075	Helen Dolk	Ulster University	Zika: Establishment of enhanced birth defect surveillance in South America	18	£50,000
ZK/16-076	Pontiano Kaleebu	MRC - Uganda Virus Research Institute	Zika: Is the Ugandan Population Vulnerable to a Zika Virus Epidemic?	18	£150,000
ZK/16-078	Nicholas James Loman	University of Birmingham	Zika: Open genomic surveillance of Zika virus in Brazil using a novel portable real-time sequencing device	12	£123,955
ZK/16-081	Michael John Griffiths	University of Liverpool	Zika: Improved diagnostics for Zika virus infection in South America through an established laboratory partnership between Brazil, Colombia and the UK.	12	£80,634
ZK/16-084	Zoltán Molnár	University of Oxford	Zika: Cellular mechanisms of microcephaly due to Zika virus infection in mice	18	£79,926

ZK/16-097	Taane G. Clark	London School of Hygiene and Tropical Medicine	Zika: Zika virus surveillance in human and mosquito populations in Cape Verde	16	£150,000
ZK/16-098	Tom Blanchard	University of Manchester	Zika: a safe recombinant vaccine with proof of efficacy in rodents	18	£177,713
ZK/16-100	Hugh Willison	Universtity of Glasgow	Zika: Assocation studies with Guillain-Barre syndrome and neurotropism.	18	£120,000
ZK/16-104	Daniel Altmann	Imperial College London	Zika: CD4 T cell immune correlates of Zika virus exposure	12	£100,000

PROJECT SUMMARIES

ZK/16-010
Zika: The Ecology of Zika transmission in Colombia and Ecuador

Heather Ferguson;	University of Glasgow
Alain Kohl;	MRC Glasgow Centre for Virus Research, UK
Felio Bello;	Universidad Antonio Narino, Colombia
Nidya Alexandra Segura;	Universidad Manuela Beltran, Colombia
Jonathan Kerr;	Universidad del Rosario, Colombia
Renato Leon;	Universidad San Francisco de Quito, Ecuador
Leonardo Ortega;	University of Glasgow, UK & Universidad San Francisco de Quito

The ability of South American countries to effectively respond to the unprecedented recent outbreak of Zika virus (ZIKV) is severely hampered by limited understanding of the ecology of transmission within rapid expanding foci. The current outbreak may be a product of changes in vector transmission potential as a consequence of i. climate change (in particular the current El Niño event), ii. the expansion of new vector species like Aedes albopictus, and/or iii. interactions with other arboviruses such as Dengue (DENV) and Chikungunya (CHIKV) which are highly prevalent within current outbreak areas. Additionally, the Zika virus itself may have underwent mutations that have increased its virulence and/or transmission efficiency in mosquito vectors. Identifying the contribution of these factors and other ecological drivers to the current outbreak is necessary both for planning effective vector control strategies and predicting the future course of the outbreak. Here we propose to conduct a comprehensive programme of mosquito vector surveillance and viral genotyping within four south american settings where ZIKV cases are recently emerging.
Studies will be conducted within two South American countries where ZIKV has been reported but at different frequency: Colombia where the current burden is high (31555 cases), and Ecuador where cases are present but much lower frequency (50). Proposed sites also differ in environmental characteristics, and diversity of potential vector species. By investigating the ecology of mosquito vectors and their infection rates with ZIKV, DENV and CHIKV, we will gather knowledge of critical importance for reducing human exposure and planning vector control.

ZK/16-011
Zika: Defining the antigenic epitopes in the Zika virus envelope protein

Yorgo Modis; University of Cambridge

Flaviviruses are enveloped viruses with a lipid membrane surrounded by an outer protein shell consisting of two structural, transmembrane glycoproteins, prM and E. In mature and infectious flaviviruses, the E protein forms a continuous icosahedral protein shell, which contains encompasses the cellular attachment interfaces, and all of the dominant neutralization antibody epiotpes. Moreover, the E protein catalyzes the fusion of the viral membrane to the host cell membrane, the critical step in cell entry that delivers the viral genome into the cytoplasm. We previously determined the crystal structures of the E proteins from various flaviviruses, including dengue virus, in the pre- and postfusion conformations. These structures provided detailed maps of the antigenic landscape of the virus, and revealed the molecular mechanism of membrane fusion. Here, we propose to harness our expertise and apply the experimental approaches we established in our previous work on flaviviruses to determine the atomic structure of the Zika virus E protein, how it assembles in virus particles, and how it catalyzes membrane fusion during cell entry. By identifying the structure and composition of the complete antigenic surface of Zika virus, this work will provide a powerful framework for the design and production of preventative and therapeutic treatments including therapeutic antibodies, subunit-based vaccines and virus cell-entry inhibitors.

ZK/16-012
Zika: Characterisation of Zika virus neutralisation and virion structure by cryogenic electron microscopy and 3D reconstruction.

Dr David Bhella; CVR, University of Glasgow

Dr Arvind Patel; MRC Glasgow Centre for Virus Research, UK

Cryogenic electron microscopy (CryoEM) has the potential to determine close-to atomic resolution structures for macromolecular assemblies and in particular icosahedral viruses. Such structures provide insights into mechanisms of virus assembly, maturation and entry. Mechanistic studies of antibody neutralisation are also informed by structure analysis of viruses decorated with Monoclonal antibody fragments (MAbs). Zika virus (ZIKV), being a member of the Flaviviridae is expected to exhibit an icosahedral virion structure similar to that of dengue virus (DENV), in which heterotetramers of E-M-M-E coat the outer surface of the virion (1). An atomic model of the mature virion exterior, determined by cryoEM at 2-4 angstroms resolution would be a considerable asset to researchers working towards the production of an effective vaccine. Recent studies of DENV2 virions decorated with a neutralising monoclonal antibody revealed that the bound antibody locks E dimers together, preventing the rearrangement necessary to expose the fusion peptide and allow entry (2). We propose to calculate an atomic resolution model of the ZIV particle alone and bound to both broadly-reactive anti-flavivirus MAbs and specific MAbs directed at ZIV. This study has the potential to inform both vaccine design and development of improved diagnostics."

ZK/16-017
Zika: Transmission dynamics and impact of zika virus on population health in a large urban centre in northeastern Brazil

Jimmy Whitworth; London School of Hygiene and Tropical Medicine
Maria da Gloria Teixeira; Public Health Institute /UFBA- Brazil
Mauricio Barreto; Public Health Institute /UFBA- Brazil
Maria da Conceiçao N. Costa; Public Health Institute /UFBA- Brazil
Enny da Paixao Cruz; London School of Hygiene and Tropical Medicine
Pedro Vasconcelos; Evandro Chagas Institute - Brazil
Laura Rodrigues; London School of Hygiene and Tropical Medicine

Zika virus (ZIKV) is suspected of causing congenital anomalies and neurological disorders and this virus is spreading rapidly throughout Brazil and over 20 countries in the Americas. Consequently this outbreak has been declared a Public Health Emergency of International Concern by WHO. There are many gaps in our knowledge of the natural history of ZIKV, the majority of the studies addressing zika are case reports and small serological surveys mainly conducted before this illness spread to the Americas. We aim to conduct epidemiological studies on ZIKV and potential complications linked with this infection. We will estimate the sero-prevalence and sero-incidence of ZIKV, the proportion of asymptomatic infections; investigate possible associated factors (biological indicators, socioeconomic and entomological); estimate the proportion of complications and atypical clinical presentations; verify the occurrence of re-infection. We will use an existing collaboration between UK and Brazilian researchers working at an established population surveillance site to carry out a prospective (individual and ecological) study in a city in Northeast Brazil. It is important for health workers to understand the clinical and epidemiological features of ZIKV in this complex context. The results of this study will produce information to enable health authorities and professionals to better conduct the process of decision making, treatment, prevention and control supported by solid scientific evidence.

ZK/16-018
Zika: Harnessing plant power for rational design of immunogens for use in diagnostic assays

Dr Janet Daly; University of Nottingham
Professor George P. Lomonossoff; John Innes Centre, UK
Professor Luis Ferreira; São Paulo University, Brazil

Laboratory confirmation of Zika virus infection is complicated by cross-reactivity of antibodies with other members of the same virus family. This includes Dengue virus, which is transmitted by the same species of mosquito as Zika and therefore also infects people in the same regions where Zika virus is currently emerging. Therefore, it is important to be able to distinguish between antibodies that have been raised against a previously encountered virus and antibodies that indicate a recent or current virus infection. A traditional approach to address this problem, which also occurs for Dengue and Japanese encephalitis

virus in Asia, is to test for antibodies generated early in the response to infection (IgM) to two viruses in parallel. The virus that gives the highest value in the MAC-ELISA is taken to be the most recently encountered virus. In the past decade, plant-expression of proteins, including virus like particles, has really come of age. This powerful technology will be harnessed in the current project to generate more specific diagnostic reagents to allow antibodies to Zika virus to be distinguished from antibodies generated to other flaviviruses such as Dengue."

ZK/16-019
Zika: Affordable, scalable, low-technology transfluthrin emanators for protecting against transmission in low-income countries

Gerry Killeen; Liverpool School of Tropical Medicine
Nicodem Govella; Ifakara Health Institute, Dar es Salaam, Tanzania
Sheila Ogoma; US Army Medical Research Unit, Kisumu, Kenya

While Zika transmission in Africa has been historically enzoonotic, the new pandemic virus lineage spreading across Asia and Latin America has adapted to transmission between humans [1,2]. Diverse Aedes, Culex, Mansonia and Anopheles mosquitoes may act as vectors, but pandemic urban transmission across Africa, Asia and Latin America appears predominantly mediated by Aedes aegypti and Ae. albopictus [3-7]. Existing repellent products for protecting against such day-biting mosquitoes, especially while active outdoors, only last hours, days or weeks per dose or application. Sustaining continuous protection is therefore impractical, and repeated replacement is unaffordable to low-income populations. We recently developed a low-technology emanator [8], which releases repellent transfluthrin vapour more slowly, providing at least 4 months of >90% protection against night-biting Culex and Anopheles mosquitoes in urban Dar es Salaam, Tanzania, despite the presence of considerable pyrethroid resistance [9]. This novel emanator consists only of a Hessian fabric strip, which can be safely treated and re-treated by any individual, community, program or local manufacturer [8,9]. Furthermore, equivalent efficacy and durability has since been achieved with a 10-fold lower transfluthrin dosage of only 1ml, costing only £0.09 per treatment. We have also recently developed a new electric grid trap for measuring attack rates of mosquitoes, which prevents exposure of human volunteers to potentially infectious bites [10]. We therefore propose to apply this novel trapping device to demonstrate that this new repellent technology provides ≥6 months of ≥80% protection against day-biting Aedes aegypti, probably the most important vector of pandemic Zika transmission globally.

ZK/16-021
Zika: Vector competence and interactions with Wolbachia

Steven Sinkins; Lancaster University

Wolbachia are maternally inherited endosymbiotic intracellular bacteria able to spread through insect populations using cytoplasmic incompatibility. The mosquito Aedes aegypti is naturally Wolbachia-free, but artificial transinfections have been generated in the lab, while Aedes albopictus naturally carries two Wolbachia low density co-infecting strains. Higher density Wolbachia transinfections in both species have been shown to block or strongly inhibit the transmission of dengue and chikungunya viruses and several other pathogens. In Ae. aegypti Wolbachia are becoming important biocontrol agents for the prevention of arbovirus transmission. Ae. aegypti is widely acknowledged as the primary vector of zika virus; Ae. albopictus has been shown to be a competent zika vector but controlled comparative assays to properly estimate its competence relative to aegypti have not been reported. Using a unique resource available in the applicant’s lab, a set of Ae. aegypti and Ae. albopictus lines transinfected with different strains of Wolbachia at varying densities (several not previously reported), we will test zika vector competence at different temperatures. We will also test the zika vector competence of Culex quinquefasciatus, an important vector of West Nile Virus (like zika a flavivirus); if it proves a fully competent vector we will also evaluate the transmission-blocking potential of a transinfection that reaches much higher density that its natural Wolbachia. The data obtained will enable us to assess the biocontrol potential of Wolbachia against zika, and to better evaluate the role of two potential secondary vectors in its transmission / epidemiology and as control targets"

ZK/16-023
Zika: Cohort study of pregnant women with rash in Pernambuco State

Prof Laura Rodrigues;	London School of Hygiene and Tropical Medicine,
Prof Ricardo A. de A. Ximenes;	(MERG) Universidade de Pernambuco and Universidade Federal de Pernambuco, Recife, Brazil
Prof Thalia V.B. de Araújo;	(MERG) Saúde Coletiva-ISC, Universidade Federal de Pernambuco, Recife, Brazil
Prof Celina Turchi Martelli;	(MERG; Instituto de Patologia Tropical e Saúde Pública da Universidade Federal de Goiás; Centro de Pesquisa Aggeu Magalhães - Fiocruz/Pernambuco, Recife, Brazil
Prof Demócrito de BM Filho;	(MERG) Universidade de Pernambuco, Recife, Brazil
Dr Rafael Dhalia;	Centro de Pesquisa Aggeu Magalhães - Fiocruz/Pernambuco, Recife, Brazil"
Dr Maria José Couto Oliveira	Director of the Diagnosis Section of the State Reference Laboratory (LACEN)
Dr Luciana Carolina A. Bezerra	Executive Secretary of Health, Surveillance, State of Pernambuco Department of Health"

We propose to identify and follow up pregnant women who develop a rash during pregnancy, establish the cause of the rash by clinical examination and laboratory confirmation (PCR for ZIKV and paired serology); follow the women until miscarriage or birth and estimate the risk of spontaneous abortions, foetal death and of microcephaly and other abnormalities by gestational week at viraemia. Our study site is in Pernambuco State, Brazil's region with highest numbers of suspected cases of microcephaly. The State Department of Health set up surveillance and control procedures for pregnant women with rash; 1440 pregnant women presented during the first two and a half months since implementation in December. We will work within this structure, recruiting the pregnant women presenting while with rash. Blood and urine samples will be tested for Zika (ZIKV), Dengue (DENV) and Chikungunya (CHIKV) by PCR and paired serology. After birth a multidisciplinary team will examine the neonates. Statistical analysis will provide the absolute risk of microcephaly and other abnormalities by gestational week of viraemia (for those with positive ZIKV PCR and for those with serologic evidence of acute infection by ZIKV). The study will also estimate the excess rate of microcephaly and of other abnormalities by gestational week of rash for those with negative exams for ZIKV, DENV and CHIKV but with rash and clinical symptoms suggestive of ZIKV infection (since not all women with ZIKA will be positive at PCR) and describe the whole range of abnormalities including those in neonates without microcephaly.

ZK/16-025
Zika: Novel point-of-care molecular diagnostics for the simultaneous diagnosis of Zika, chikungunya and dengue infections in Latin America.

Luis E Cuevas;	Liverpool School of Tropical Medicine
Prof. David Lalloo;	LSTM
Dr Emily Adams;	LSTM
Dr Thomas Edwards;	LSTM
Prof. Ricardo Q. Gurgel;	Federal University of Sergipe, Sergipe, Brazil
Dr Eliane Miyaji;	Butantan Institute, Sao Paulo, Brazil
Dr Alessandra S. Schanoski;	Butantan Institute, Sao Paulo, Brazil
Licda. Leticia Castillo Signor;	National Surveillance Laboratory, Guatemala
Licda. Danuza Duarte Costa;	LACEN, Sergipe, Brazil
Dr David Edge	BioGene Limited

The Americas are experiencing simultaneous arbovirus epidemics which are transmitted by the same vectors and have overlapping clinical presentation. The difficulties experienced in confirming the role of Zika as a potential teratogenic pathogen and understanding the risk of Guillain-Barre have demonstrated the deficiencies of current diagnostics for epidemiological investigations and clinical management [1]. Until recently, dengue infections were identified by confirming the first few clinical cases in reference laboratories; all subsequent cases were assumed to be due to the same virus. This approach no longer works as several arboviruses coexist in the same populations and assays suitable for the rapid confirmation of infection in patients at the primary health care level are needed. We propose to develop

sensitive and specific assays to identify Zika, chikungunya and dengue simultaneously in a platform suitable for use at the primary health care level. Assays will build on dengue and chikungunya assays developed at LSTM and public domain CDC primers and probes for Zika. These assays will be adapted to the BioGene QuRapID instrument, which is a platform initially designed for detection of bacteria and subsequently successfully adapted to an RT-QPCR approach for detection of Ebola. The system is able to process blood samples directly without DNA/RNA extraction and detects RNA targets without extraction steps within 35 minutes. On completion of the project we will have developed point-of-care molecular assays that are rapid, sensitive and specific, and suitable for the diagnosis and clinical management of populations at risk of arboviruses infections in Latin America.

ZK/16-041
Zika: susceptibility of South American and European vectors to ZIKV infection, and influence of temperature

Matthew Bayliss;	University of Liverpool
Dr. Marcus Blagrove;	University of Liverpool,UK
Prof. Tom Solomon;	University of Liverpool,UK
Dr Phil. McCall;	Liverpool School of Tropical Medicine
Dr. Gareth Lycett;	Liverpool School of Tropical Medicine

Zika virus (ZIKV) is transmitted by Aedes mosquitoes, primarily Ae. aegypti, a significant virus vector in tropical regions worldwide. The highly invasive species, Ae. albopictus, can be infected with ZIKV and may be an important vector too, especially as it occurs in temperate regions, including mainland Europe, and threatens the UK. Mosquito species native to UK and Europe, such as the human biting Aedes detritus and Ae. vexans, can support other flaviviruses and may present an as yet unknown risk from ZIKV. UoL/LSTM hold the UK's first infection system for high-containment viruses in live mosquitoes. Our main goal is to establish the first such system for ZIKV, and use it to answer key questions regarding the vector competences of Ae aegypti and Ae albopictus, the environmental temperature threshold required for transmission, and the risk presented by indigenous UK or European Aedes mosquitoes, including Ae. detritus and Ae vexans. Such data are essential to determine the risk of transmission of ZIKV in temperate zones of western Europe and elsewhere. The project has four complementary objectives: Establish the UK's first Zika virus – mosquito infection system; Compare vector competence of field-derived Ae. aegypti and Ae. albopictus. Quantify the effect of temperature on the extrinsic incubation period of ZIKV in Ae. aegypti and Ae. albopictus and determine temperature thresholds for transmission. Assess the vector competence of UK and European mosquitoes for ZIKV."

ZK/16-045
Zika: Zika in Colombia: Characterisation of exposure and epidemiology in a flavivirus endemic setting

Neil M. Ferguson;	Imperial College London
Dr Luis-Angel Villar;	Universidad Industrial de Santander (Colombia)
Dr María-Consuelo Miranda;	Universidad Industrial de Santander (Colombia)
Dr Isabel Rodríguez-Barraquer;	Johns Hopkins Bloomberg School of Public Health (USA)
Dr Jorge E. Osorio;	University of Wisconsin (USA)
Prof Jean-Claude Manuguerra;	Institut Pasteur (France)
Dr Jessica Vanhomwegen;	Institut Pasteur (France)
Dr Pierre Nouvellet;	Imperial College London (UK)
Prof Maria-Gloria Basáñez;	Imperial College London (UK)
Prof Christl A. Donnelly;	Imperial College London (UK)
Dr Thibaut Jombart;	Imperial College London (UK)

Following the large-scale epidemic in Brazil in 2015, Zika continues to spread across Latin America. However, our understanding of the transmission dynamics and epidemiology of this new threat remains poor. In particular we have limited information on the extent of population exposure: many infections remain asymptomatic or cause mild disease which can easily be misdiagnosed, while antibody cross-reactivity between flaviviruses make the results of serological testing difficult to interpret given much of the region is endemic for dengue. With 31.000 cases reported by Feb 2016 (4 months after the first case was diagnosed), Colombia is currently the second most affected country. Our proposal seeks to characterise

the epidemiology of Zika in Colombia and establish a Colombia-focussed research network of partners with complementary expertise. We will install state-of-the-art serological testing facilities in Colombia and conduct serological surveys in four cities with varying levels of dengue transmission intensity and reported Zika case incidence. We will develop statistical models to analyse age-stratified flavivirus seroprevalence data in a context where multiple flaviviruses co-circulate. These will allow us to characterise the force of infection, attack rate and transmissibility of Zika. We will then use these estimates to assess current levels of herd immunity to Zika thus predict possible future attack rates. Finally, we will estimate the proportion of infections which are asymptomatic and the per-infection risk of severe outcomes (e.g. microcephaly). The outputs from this project will be of immediate value in enabling improved situational awareness and in informing the development of evidence-based control strategies.

ZK/16-046
Zika: Estimating the transmission and case burden of Zika virus in Kenya

Dr George Warimwe;	University of Oxford
Prof Philip Bejon;	Kenya Medical Research Institute-Wellcome Trust Research Programme, Kenya, (KEMRI-WTRP)
Dr Rosemary Sang;	Kenya Medical Research Institute, Kenya
Prof James Nokes;	KEMRI-WTRP

Zika virus (ZIKV) was discovered in Uganda, and given the presence of vectors throughout East Africa it is likely to be endemic in the region. High ZIKV antibody sero-prevalence, ~50%, was reported in coastal Kenya in 1970, where coincident transmission of other arboviruses also occurs. However, this may be due to cross-reactivity with other flaviviruses and the epidemiology of ZIKV in East Africa remains unknown. To address these knowledge gaps we will utilise a unique biobank of samples at the Kenya Medical Research Institute-Wellcome Trust Research Programme (KWTRP) collected over a 25-year period from coastal Kenya residents. The biobank is a sample archive of >100k acute hospital admissions, paired maternal and cord blood from >10k deliveries, longitudinal cohort samples from >2k individuals, and a large archive of local mosquito collections. Hospital case records and other metadata are available for all these samples. Using qRT-PCR and plaque reduction neutralisation assays (PRNT) we will determine the case burden of ZIKV in matched case-control studies of: 1) cord blood samples for microcephalic vs. normocephalic births, 2) plasma samples for acute undifferentiated fevers vs. community controls, and 3) perform qRT-PCR assessments of Aedes mosquito collections for ZIKV. In addition to genome sequencing of any qRT-PCR positive samples, PRNT on co-circulating flaviviruses will be performed to ensure diagnostic accuracy. Together, this will provide the first comprehensive analysis of ZIKV transmission and case burden in East Africa.

ZK/16-047
Zika: Development of a type specific Zika virus antibody assay for use in Brazil

Professor Richard Tedder;	University College London
Dr Ana Maria Bispo;	Fundação Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil
Professor David Brown;	Fundação Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil and Virus Reference Department, PHE Colindale, UK"
Dr Alfredo Mendrone;	Fundação Pro Sangue Hemocentro São Paulo, Brazil
Dr Eduardo José Levi;	Fundação Pro Sangue Hemocentro São Paulo, Brazil
Dr Ernesto T. A. Marques;	Fundação Oswaldo Cruz – FIOCRUZ Recife, Brazil and University of Pittsburgh, School of Public Health, USA
Dr Rafael Dhalia;	Fundação Oswaldo Cruz - FIOCRUZ, Centro de Pesquisa Aggeu Magalhães - CPqAM, Recife, Brazil"
Dr Ines Ushiro-Lumb;	NHS Blood and Transplant, UK and Virus Reference Department, PHE Colindale, UK"
Professor Maria Zambon;	Virus Reference Department, PHE Colindale, UK
Dr Samreen Ijaz;	Virus Reference Department, PHE Colindale, UK
Dr Marcus Eder;	Frimley Park NHS Trust, UK
Dr Emma Aarons;	Rare and Imported Pathogens Lab, PHE Porton Down, UK

The emergence and rapid spread of Zika virus (ZIKV) in Latin America, with possible links to Guillain Barré Syndrome (GBS) and clusters of microcephaly has led WHO to announce a Public Health Emergency of International Concern on 1st February 2016 (1, 3, 7.). Currently, diagnosis of acute disease is largely based on virus genome detection. There are no reliable serological assays which can be used to assess exposure, and therefore contribute to risk assessment for individual pregnant women or population susceptibility, highlighting the urgent clinical and public health need to develop a highly specific serological assay for ZIKV. Affordable and reliable serological tools that can be produced locally, at scale and used outside reference laboratories, must be part of a rapid emergency response to a newly emerging infection. Flavivirus serology is notoriously challenging because of antigenic cross reactivity between different flaviviruses including Dengue 1 to 4 which have circulated widely in South America in the last 20 years. We intend primarily to develop an enzyme immunoassay (EIA) for the detection of anti-ZIKV IgG in a competitive format using native viral antigens, a methodology optimal for distinguishing serological responses to very closelyrelated viruses. Our direct links, exchange programmes and longstanding relationships with public health and transfusion centres in Brazil enable us to directly implement local field assessments and embed a local production capability in country, working through existing collaborations at the heart of the Brazilian public health system.

ZK/16-050
Zika: Investigating the link between Zika virus infection and neurological disease in ex vivo and in vivo models

Lisa Ng;	University of Liverpool
Prof. Julian A. Hiscox;	University of Liverpool, UK
Prof. Tom Solomon;	University of Liverpool, UK

The link between Zika virus (ZIKV) infection and neurological disease is not established, and this proposal will seek to investigate this link using both in vivo models and ex vivo human tissue. Reports have demonstrated the presence of ZIKV in the brain and placenta of babies born to women who were infected during pregnancy (Mlakar et al. 2016), but these case studies remain limited. The complete viral genome has also been recovered from the foetal brain. Importantly, there seems to be different outcomes of infection in Brazil compared to cases from the Pacific Islands, South-East Asia, and African. Although the focus has been on the potential link with microcephaly, ZIKV has also been implicated in other neurological complications such as Guillain-Barre syndrome (Oehler et al. 2014), with evidence of virus in the spinal fluid (Brasil et al, under review, Lancet). Development of microcephaly and ZIKV associated neurological disease has been associated with the host response through inflammation and autophagy. Classically Koch's postulates have not been fully established for virus infection of humans. Over the past year we have optimised the virus production set up for ZIKV (see accompanying figure), and also developed a novel mouse model that has been infected with different isolates. In this proposal, we will use the mouse model to investigate whether different ZIKV isolates cause neurological disease. This will be correlated with infection of primary human tissues representing the proposed route of infection: skin, blood and neuronal tissues in the foetus and adult.

ZK/16-061
Zika:Development of an online data-sharing platform for images of fetal and newborn heads - An urgent need in the context of the Zika virus outbreak

Stephen Kennedy;	University of Oxford
Peter Hammond;	Nuffield Department of Obstetrics & Gynaecology (NDOG), University of Oxford, UK"
Chris Nellaker;	NDOG and Institute of Biomedical Engineering (IBME), University of Oxford, UK
Alison Noble;	IBME, University of Oxford, UK
José Villar;	Oxford Maternal & Perinatal Health Institute (OMPHI), University of Oxford, UK
Aris Papageorghiou;	OMPHI, University of Oxford, UK
Andrew Wilkie;	Weatherall Institute of Molecular Medicine, University of Oxford,
Deirdre Cilliers;	Oxford Craniofacial Unit, UK
Usha Kini;	Oxford Brain Abnormalities Group, UK

Laura Merson;	Infectious Diseases Data Observatory (IDDO), Centre for Tropical Medicine/Global Health, University of Oxford, UK
Philippe Guerin;	IDDO, Centre for Tropical Medicine/Global Health, University of Oxford, UK
Gail Carson;	International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC) Coordinating Centre, University of Oxford,
Laura C Rodrigues;	London School of Hygiene and Tropical Medicine, UK
Lavinia Schuler-Faccini;	Universidade Federal do Rio Grande do Sul, Brasil
Celina Turchi;	Centro de Pesquisas Aggeu Magalhães, Fundação Oswaldo Cruz, Recife, Brasil
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International organisations in the global health community, including WHO, have recently stressed the critical need to share data about emerging infections amongst researchers around the world to extract the maximum scientific knowledge from the available datasets. This applies particularly during the current Zika virus outbreak because a causal association between maternal exposure and congenital microcephaly has not been definitively established. Some Brasilian groups are already acquiring ultrasound, CT and MR images of affected infants. However, no facility exists to enable those images to be shared with other scientists so as to improve the phenotypic characterisation of microcephaly and facilitate research into the causal association with Zika virus exposure. We have, therefore, assembled a multi-disciplinary team of clinician scientists, epidemiologists, geneticists, engineers, computational biologists and radiologists from Brasil, the USA and UK, to produce a secure, online, digital information platform for images of fetal and newborn heads (plus associated clinical and laboratory records) as a global data-sharing resource. The platform will be developed (in a basic form within 3 months) in partnership with a consortium of three software companies with considerable experience of designing databases that can store millions of images in a high-security environment for rapid searching and interrogation with a range of analytical and visualisation tools. The growing image datasets in Brasil will be further expanded by acquiring 2D and 3D photographs of affected newborns (and controls) to help screen for microcephaly by automating the measurement of head size and shape, and exploring facial characteristics as potential diagnostic markers.

ZK/16-067
Zika: A prospective case-control study to examine the role of Zika virus in Guillain-Barré syndrome in Brazil

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Guillain-Barré Syndrome (GBS) is an acute immune-mediated disease of the peripheral nervous system which can lead to death or debilitating neurological disability. The number of GBS cases in Zika-affected areas is rising sharply, but the WHO has urged caution in attributing them to the virus. Through the Zika Neurology Network, established in 2015, we will conduct a prospective case-control study of GBS in Rio de Janeiro, focusing on Zika as a risk factor. The project builds on Liverpool expertise in flavivirus

neurological disease, Public Health England's diagnostic capabilities, flavivirus research at the Oswaldo Cruz Foundation (Fiocruz), Rio di Janeiro, and the International GBS Outcome Study's experience. We will recruit 60 adults with GBS referred to major hospitals in the Network, with scope to expand if needed. GBS will be defined through recognised clinical and electrophysiological criteria, and Zika infection diagnosed with investigations already established at Fiocruz, including PCR of blood and urine, and IgM and IgG ELISA of blood. Outcome will be assessed at 2 months. Four community controls will be recruited for each case. Underpinned by the NIHR Health Protection Research Unit in Emerging and Zoonotic Infections, which already has team members in Rio supporting protocol, case record form, and diagnostic developments, the project provides excellent value for money, and is ready to deliver in a timely manner. Within nine months our study will define the role of Zika as a risk factor for GBS, to help individual patient management, and importantly to support public health and policy decisions.

ZK/16-068
Zika: surveillance, risk factors and vector management in Brazil

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Rapid developments in Zika virus surveillance, risk factor analysis and optimal control strategy are immediately required to curtail the American outbreak. Since 2012, our Brazilian partner has been leading an arbovirus serosurveillance cohort study of 117 pregnant women and 278 infants in a favela of Manguinhos, Rio de Janeiro; and we will be piggybacking CI Brasil's study for the currently proposed project. Half the requested funds will be transferred to Brazil to support the PCR-confirmation of 181 suspected Zika cases among the cohort (in addition to any suspected cases that arise over the next 18 months). PCR analysis will also be conducted on mosquito samples collected from the households of febrile cases and their neighbours. All collected mosquitoes of all species have been retained over the past 2 years and stored appropriately for this follow-up analysis. This will allow for the incrimination of the vector species that is/are primarily responsible for Zika transmission – a critical current knowledge gap. Taking advantage of the years of collected data on the cohort (socioeconomics, geo-referenced locations, febrile history, mosquito numbers/species collected, etc) coupled with the new diagnostic results, risk factor analysis will be conducted using cutting-edge Model Based Geostatistical methods in order to produce the first Zika risk maps for (primarily) Rio and (secondarily) Brazil. These data and risk factors will then inform the construction of mathematical models developed to analyse optimal mosquito control strategy employing both traditional tools (insecticides, breeding site elimination) and novel approaches (sterile insect technique, genetic control and Wolbachia releases)"

ZK/16-075
Zika: Establishment of enhanced birth defect surveillance in South America

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Although one of the main reasons that WHO has pronounced Zika Virus (ZIKV) a Public Health Emergency of International Concern, is its putative link with microcephaly, the scientific evidence remains unclear and uncertain. To increase the knowledge needed to guide public health actions, this project will use an established network dedicated to the congenital defect research in the last 49 years in South America, the Latin American Collaborative Study of Congenital Malformations (ECLAMC), currently operating in 21 hospitals in 7 countries. First we propose an epidemiological analysis of microcephaly data from ECLAMC in the 10 years pre-Zika, and a comparison to the period of Zika outbreak for each country.

ZK/16-076
Zika: Is the Ugandan Population Vulnerable to a Zika Virus Epidemic?

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This pilot study will address the question, whether Uganda is vulnerable to a Zika virus (ZIKV) epidemic. Since its discovery in 1947, no evidence of endemic/epidemic ZIKV infection has been found in Uganda. However, a virulent, pathogenic ZIKV lineage has emerged in Asia, quickly spreading to other continents. The emergence of this lineage raises the question, whether ZIKV strains from Uganda are non-pathogenic or if Ugandans have built immunity over time or through cross-reactivity to related viral species. Our aims are 1) to search for ZIKV among mosquitoes and key human cohorts and 2) to molecularly and phylogenetically characterize Ugandan strains. To this end, a large mosquito collection representing a broad geographical distribution with seasonal variation will be screened for ZIKV RNA by RT-PCR and full ZIKV genomes molecularly characterized using metagenomics next generation sequencing on the MiSeq platform. ZIKV sequences derived from mosquitoes will be phylogenetically compared to estimate evolutionary radiation and divergence from the 1947 strain. Similarly, stored samples from key human populations (Measles/Rubella Surveillance and Acute Febrile Illness cohorts, and AIDS Indicator Surveys) will be screened for ZIKV RNA. Plasma ZIKV sequences will be phylogenetically compared to local mosquitoes' sequences and African/Asian lineages (GenBank). Clinical specimens will also be screened serologically for evidence of past exposure to ZIKV, already in use at UVRI. The data generated through this study will form the foundation for further studies to evaluate Ugandan ZIKV as a prototype target for the development of ZIKV-specific diagnostic assays and the development of a vaccine using a non-pathogenic live/attenuated strain of the virus.

ZK/16-078
Zika: Open genomic surveillance of Zika virus in Brazil using a novel portable real-time sequencing device

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A major epidemic of Zika virus (ZIKV) is ongoing in Latin America. Critically, there is evidence for an association between infection with ZIKV and both microcephaly in newborns and Guillain-Barré syndrome. Currently there is a paucity of complete genome sequences for this virus, in part due to difficulties in transporting material outside of Brazil for sequencing, hindering attempts to determine virus origins, epidemiology and any genomic basis to microcephaly. We have recently established real-time portable genome sequencing using the Oxford Nanopore MinION device, and successfully used this to characterize Ebola virus genetic diversity in Guinea during the 2014-2015 outbreak [1]. We propose to extend this ground-breaking achievement to ZIKV by establishing two portable genome sequencing laboratories in Brazil. Through collaboration with the Oswaldo Cruz Foundation (FIOCRUZ) and the Instituto Evandro Chagas public health laboratory in Brazil we will sequence 750 complete genomes of ZIKV, covering a broad geographical region including historical samples, and from patients with a range of clinical presentations. These novel genomic data will provide key information on how and when ZIKV was introduced to Brazil, the pattern and determinants of spread through the country and to neighbouring localities, the extent of genetic diversity (of importance to vaccine and diagnostic design), and whether there are any associations between changes in the virus genome and the likelihood of ZIKV complications such as microcephaly. Crucially these data will provide a surveillance framework for tracking further spread into other geographic regions. In common with our previous efforts, this effort will serve as a beacon for open science during a public health emergency [1-4]. Data will be subject to open release as it is generated.

ZK/16-081
Zika: Improved diagnostics for Zika virus infection in South America through an established laboratory partnership between Brazil, Colombia and the UK.

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Accurate diagnostics for Zika virus (ZIKV) underpins all public health responses and epidemiology studies of the outbreak. Currently available diagnostics: nucleic acid amplification tests (NAAT) and antibody ELISA, have critical problems [1]. NAAT are potentially highly sensitive. However, ZIKV is cleared rapidly during recovery (often within 1 week), limiting the time post-infection NAAT can usefully be used. ELISAs, IgM and IgG, detect antibody reponses to ZIKV over a longer time-frame. However, they exhibit reduced specificity through cross-reactivity against other flaviviruses, e.g. dengue, circulating in South America. Guidance from WHO, CDC and ECDC caution interpretation of these tests [2]. We aim to refine and validate more specific serological tests (Plaque Reduction Neutralisation Test [PRNT] and Non-structural protein 1 [NS1] ELISA) and assess the time-frame postinfection that portable NAAT provide accurate diagnosis. This will be achieved through an established partnership between the Health Protection Research Unit in Emerging and Zoonotic Infections (HPRU-EZI), Public Health England, Fiocruz (Rio,Brazil) and Laboratorio de Virología (Colombia). OBJECTIVES Establish and confirm

diagnostic accuracy of new serological tests; ZIKV PRNT (most specific serological test for ZIKV). ZIKV NS1 ELISA (reduced cross-reactivity with other flaviviral antibodies). Field validation of new rapid ZIKV point-of-care NAAT. OUTPUTS Tests, protocols and training will be available to local laboratory networks for patient use and to support further diagnostic studies. OUTCOMES Fiocruz and Laboratorio de Virología provide central diagnostic services for their national public health teams. Tests and training will strengthen diagnostic services, and improve management of suspected ZIKV patients.

ZK/16-084
Zika: Cellular mechanisms of microcephaly due to Zika virus infection in mice

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Since October 2015, the occurrence microcephaly has dramatically increased in South America, a phenomenon that has been tied to the widespread epidemic of the Zika virus (ZIKV). However, as correlations does not imply causation, we aim to investigate this relationship by studying the anatomical and cellular brain alterations caused by the ZIKV at different gestational stages. Microcephaly is a severe brain malformation defined as a head circumference more than two standard deviations below the mean that can be associated with decreased neuronal production due to proliferation defects and death of cortical progenitors. It is called primary microcephaly when diagnosed before the 36th gestation week. The known causes vary from genetic mutations, such as MCPH1-MCPH11, to extrinsic insults such as the so-called TORCH factors: Toxoplasmosis, Rubella, Cytomegalovirus (CMV) and Herpes. We aim to model microcephaly using in utero injection of ZIKV in pregnant mice during the peak of foetal neurogenesis. We will be able to determine the capacity for ZIKV infection to produce microcephaly and compare the cellular mechanism of injury with other well-known causes such as CMV and downregulation of MCPH6. This project will shed light on the etiological nature of ZIKV infection for microcephaly, using an animal model simulating the human condition. In addition, it will define the critical developmental period for ZIKV infection, characterize the morphological abnormalities in brain anatomy with high-resolution magnetic resonance, and unfold the developmental processes impaired by ZIKV infection.

ZK/16-097
Zika: Zika virus surveillance in human and mosquito populations in Cape Verde

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The Zika virus, originally identified in Uganda, has spread rapidly across regions occupied by Aedes mosquitos in Africa, Asia, and recently to the Americas. Although originally only causing mild illness, the recent outbreak in Brazil has been linked to a sudden spike of neurological disorders and congenital microcephaly cases. Since the current outbreak in the Americas, Cape Verde has become the first African country declaring an epidemic of Zika, reporting ~7000 cases since October 2015. The strong historical and economic links with Brazil suggest that the Cape Verde epidemic is caused by the same viral strain. Due to Cape Verde's international travel links there is a fear of the potentially severe viral strain spreading to mainland Africa and Europe.
To determine the origin and spread of infection, and support the implementation of a rapid public health response, we will perform a surveillance study of the Zika virus in Cape Verde.
We propose to provide accurate clinical and epidemiological data on Zika infection, by
(1) Identifying transmission hotspots, and survey Aedes mosquito populations therein;

(2) Using serological techniques to estimate Zika virus community exposure;
(3) Sequence viral samples sourced from the collected mosquitos and confirmed patient cases;
(4) Compare the genetic diversity of viral samples to sequenced strains from elsewhere (Brazil, other African), thereby determining the origin of Zika infection in Cape Verde.
This project has a multidisciplinary approach- viro-entomo-epidemio-genomics working together to support the investigation of the Zika virus infection epidemic. The surveillance and research components of this proposal will support the development of timely effective actions to help contain Zika infections, and insights could assist other global control efforts, especially in the Americas.

ZK/16-098
Zika: a safe recombinant vaccine with proof of efficacy in rodents

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We will construct a recombinant modified vaccinia Ankara (MVA) Zika virus vaccine in Manchester, and obtain proof of protection in a rodent model of Zika infection under development at Porton Down. Recombinant modified vaccinia Ankara has been widely used as a human vaccine candidate. MVA has an excellent safety record, does not replicate in mammals, has a desirable cytokine receptor profile, and is particularly immunogenic when employed as a boosting agent(1-3). Such a vaccine would be suitable for large-scale use across whole populations giving simultaneous protection whilst interrupting the transmission of Zika. Recombinant MVA has already been employed to generate vaccine candidates for related flaviviruses such as Japanese B encephalitis virus (JEV) and dengue(4-6). These employ prM and E sequences, sometimes truncated. We will employ capsid sequences as well, in order to generate more immunogenic virus-like particles. Recombinant vaccines such as these may be difficult to generate because of syncytia formation in which case strategies such as T7 expression system regulated by coinfection will be employed. Licensed vaccines for the closely related dengue virus and for JEV do exist. In the case of the Sanofi-Pasteur dengue vaccine this is based on recombinant variants of the 17D yellow fever vaccine: there would be concern that an equivalent Zika vaccine might be embryotoxic. The JEV vaccine is an inactivated derivative of JEV: manufacture of such a vaccine would require large scale production of Zika (not possible at present) and always carries the risk of environmental release or failure of inactivation.

ZK/16-100
Zika: Association studies with Guillain-Barre syndrome and neurotropism.

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The recent global outbreak of Zika virus infection has been linked to severe neurological phenotypes affecting the peripheral and central nervous systems. While these relationships are currently undergoing epidemiological monitoring, it seems prudent to assume that these associations will be confirmed (Cao-Lormeau VM et al., Lancet 2016, in press). In anticipation, this study will investigate the underlying causation of neurological deficits, focusing on Guillan-Barré syndrome (GBS); generally considered an autoantibody-mediated disorder. What is not clear in the current epidemic is whether Zika-GBS is due to direct infection of neural cells or a post-infectious autoimmune syndrome. We are poised to begin work to examine the effects immediately of (i) direct viral infection, and shortly of (ii) humoral factors in serum from Zika-associated GBS and control cases (currently being obtained with consent from Recife, Brazil

by Willison/Kohl), on neural cell viability and function, using physiologically intact murine living peripheral nerve preparations and cultured neurons and Schwann cells. Comparative cultures of murine CNS cells will be examined in parallel to gain insights into the CNS/PNS distinctions in neurological complications. Further, human sera will be screened to identify autoantibodies to antigens associated with conventional GBS, to facilitate diagnosis. Once Category 3 permissions are granted, we will substantiate relevant findings in vivo. These studies will lead to a deeper understanding of the nature of Zika-associated GBS and allow treatment and diagnostic strategies to be developed.

ZK/16-104
Zika: CD4 T cell immune correlates of Zika virus exposure

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Current knowledge of T cell immunity to Zika virus is minimal. For a disease of such diverse outcomes, from asymptomatic exposure to Guilain-Barre syndrome or neonatal microcpephaly, there is a pressing need to characterise immunity to identify the difference between protective and pathogenic T cell responses. In West Nile virus (WNV) infection, for example, neuropathogenic complications are themselves attributed to effects of T cells. We aim to supply the Zika research community with the first detailed dataset of CD4 T cell immunity to Zika virus, establishing the immune correlates of different disease outcomes after exposure. Our expertise and track-record in this regard is based in >12 years funding to the Altmann/Boyton and Kwok/James teams within the consortia of the NIH Epitope Discovery Program (IEDB). This has been a programme of high-throughput analysis to achieve rapid characterisation of immunity in response to emerging pathogens. As such, the teams are ready to go with precisely the toolkit required for the proposed study. Kwok/James have led the way in characterisation of protective/pathogenic flavivirus immune correlates, generating tetramers for multiple HLA alleles. The London and Seattle labs will here team with Silva's lab in Sao Paulo. Patients will be characterised, covering phenotypes from mild to severe, and CD4 T cell immunity analysed against the protein antigens ENV, NS5, NS3, and NS1. This will include qualitative and quantitative aspects, by ELIspot and HLA/Zika tetramer flow cytometry, allowing immune correlates of disease outcome to be annotated. The reagent set will subsequently be invaluable for monitoring of Zika vaccine trials."