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Centre de Recherche sur les Filarioses & Autres Maladies Tropicales

Cross-reactive antigenemia and treatment-related adverse events in loiasis

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Version (date)	Key changes
1.4	Addition of a 1-month follow-up visit to test for Mf counts and circulating antigen (by FTS)

1. Ethical clearance request

Prof Joseph Kamgno (MD, PhD),
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June 28, 2019

To the President of the Cameroon Regional Ethics Committee for Research in Human Health for the Centre Region

Subject: Request for Ethical Clearance

Dear Mrs President,
I am writing on behalf of the investigators (including myself) of the collaborative research project entitled **'' Cross-reactive antigenemia and treatment-related adverse events in loiasis''** to request for ethical clearance.

Lymphatic Filariasis (LF) is a debilitating parasitic infection endemic in tropical countries (including Cameroon) and targeted for elimination by 2020. The strategy to eliminate LF relies on yearly Mass Drug Administration (MDA) of albendazole and ivermectin (IVM) or diethylcarbamazine (DEC). Despite the success of this strategy (the transmission interruption have been achieved in many countries), none of the 11 loiasis-endemic African nations have interrupted LF transmission, because of (i) cross reactivity of LF-RDT with *Loa loa*, and (ii) severe adverse events (SAEs) potentially fatal that have been reported among persons with very high *L. loa* microfilaria counts treated with IVM or DEC against LF or onchocerciasis. The objective of this study is to determine what are the cross-reactive *L. loa* antigens, and investigate how and when are they released, as well as whether their release contribute to treatment-related SAEs observed in persons with

heavy loiasis infections. This study will provide insights in the progress towards elimination of LF.

I remain at your disposal to bring any additional information if needed.
Yours sincerely,

Prof Joseph Kamgno.

Prof Joseph Kamgno.

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3. List of abbreviations

AEs: Adverse Events

CFA: Circulating Filarial Antigen

DALYs: Disability-Adjusted Life Years

DEC: Diethylcarbamazine

FTS: Filariasis Test Strip

GPELF: Global Program to Eliminate Lymphatic Filariasis

ICT: Immuno-Chromatographic Test

IVM: Ivermectin

LF: Lymphatic filariasis

MDA: Mass Drug Administration

Mf: Microfilaria

MMDP: Morbidity Management and Disability Prevention

NTD: Neglected Tropical Disease

RDT: Rapid Diagnostic Test

SAEs: Severe Adverse Events

TaNT: Test and Not Treat

WHO: World Health Organization

4. Summary of the proposal

Lymphatic filariasis (LF) is a disfiguring and disabling, mosquito-borne, parasitic neglected tropical disease. The majority of LF worldwide, and all LF in Africa, is caused by the filarial worm, *Wuchereria bancrofti.* The Global Program to Eliminate Lymphatic Filariasis (GPELF) is the world's largest disease elimination program relying on population-based mass drug administration (MDA). Since its inception in 2000, more than 7.1 billion doses of LF elimination medications have been administered to more than 890 million people. However, none of the 11 loiasis-endemic African nations have interrupted LF transmission, partly due to two major obstacles in loiasis-endemic populations. (1) Severe adverse events (AEs), including coma and death, which sometimes occur when LF MDA medications are given to persons with heavy loiasis infections (>20,000 microfilariae per mL of blood); (2) Up to 6% of persons in loiasis-endemic populations test positive by LF rapid diagnostic tests (RDTs) in the absence of *W. bancrofti* infection. Because a filarial antigenemia prevalence of 1% is the GPELF threshold for implementing MDA for LF, cross-reactivity with loiasis makes it impossible for national programs to determine with certainty where LF MDA is required and when it can be stopped. Therefore, improved RDTs that can reliably distinguish *W. bancrofti* infection from cross-reactive loiasis are urgently needed to prevent unnecessary and potentially harmful mass drug administration in areas where LF is not endemic.

This study aims to determine what the cross-reactive *L. loa* antigens are, how and when they are released, and whether their release contributes to treatment-related SAEs observed in persons with heavy loiasis infections.

5. Résumé du protocole

La filariose lymphatique (FL) est une maladie tropicale négligée parasitaire à transmission vectorielle (moustique) débilitante et invalidante. La plupart des cas d'infection associés à FL dans le monde, et tous ceux retrouvés en Afrique, est due à la filaire *Wuchereria bancrofti.* Le Programme Global pour l'Elimination de la Filariose Lymphatique (GPELF) s'appuie sur la distribution de masse des médicaments aux populations à risque. Depuis sa création en 2000, plus de 7,1 milliards de doses de médicaments (Albendazole + ivermectine/diethylcarbamazine) contre la FL ont été distribués à près de 890 millions de personnes à travers le monde. Cependant, aucun des 11 pays Africains où la loase est endémique n'a réussi à interrompre la transmission de la filariose lymphatique pour deux principales raisons. (1) les effets secondaires graves (ESG) tels que le coma et la mort, qui surviennent souvent lorsque l'ivermectine ou la diethylcarbamazine sont administrés, dans la cadre de la lutte contre la FL, aux personnes ayant une forte charge de loase (> 20 000 microfilaires par ml de sang); (2) près de 6% de personnes dans les zones d'endémie à la loase sont réagissent (testées positives) zux tests de diagnostic rapide (TDR) de la FL, même en l'absence du parasite responsable de la FL (*W. bancrofti*). Compte tenu du fait qu'un taux d'antigènes filariens de 1% est recommandé par le GPELF pour l'implémentation de la distribution de masse des médicaments contre cette maladie, la réactivité croisée des antigènes de la loase rend impossible l'identification avec certitude des zones où la distribution de masse est nécessaire et là où elle doit être stoppée. Ainsi, une amélioration de l'efficacité des TDRs à distinguer l'infection à *W. bancrofti* de la loase est urgemment nécessaire pour éviter une administration de masse de médicaments inutile et potentiellement dangereuse dans les zones où la FL n'est pas endémique.

Cette étude a pour objectifs de déterminer les antigènes de la loase ayant une réactivité croisée avec les TDR dédiés à la FL, quand et comment ces antigènes sont produits et si leur libération contribue aux effets secondaires graves associés au traitement des personnes ayant une forte charge de loase.

6. Introduction

Lymphatic filariasis (LF) is a vector-borne neglected tropical disease (NTD) targeted for elimination as a public health problem. The infection transmitted by different species of mosquitoes (*Culex, Anopheles* and *Aedes*) is caused by thread-like filarial worms (nematodes) (CDC, 2019). In majority of the cases (90%), the infection is caused by *Wuchereria Bancrofti* and remainder by *Brugia* Species (*Brugia malayi* & *Brugia timori*). It is estimated that 120 million people in tropical and subtropical areas of the world are infected with lymphatic filariasis; among which, almost 25 million have genital disease (most commonly hydrocele in men) and almost 15 million (both men and women) have lymphoedema of the leg. The majority (57%) of population requiring preventative chemotherapy lives in the South-East Asia Region (9 countries) and remaining (37%) live in the WHO Africa Region (35 countries). WHO estimates indicate that around 36 million people are living with these chronic disease manifestations globally. LF accounts for at least 2.8 million Disability-Adjusted Life Years (DALYs); this does not include significant co-morbidity of mental illness commonly experienced by patients and their caregivers (WHO, 2018). Fortunately, LF elimination is possible by interrupting the transmission cycle. Providing treatment on a large-scale to entire communities where the infection is present can stop the spread of infection. A key intervention strategy is MDA in all endemic areas with a microfilaria (Mf) or antigen prevalence of at least 1% (GPELF, 2017).

The Global Programme to Eliminate LF (GPELF), the world's largest disease elimination program relying on population-based mass drug administration, has worked towards elimination for nearly two decades. Since its inception in 2000, more than 7.1 billion doses of LF elimination medications have been administered to more than 890 million people (WHO, 2018). However, the disease is still persisting in 11 countries in Central and West Africa where Loiasis is also endemic. Loiasis, better known as the African eye worm, is a parasitic disease caused by the filarial nematode *Loa loa* and transmitted to humans by tabanids belonging to the genus *Chrysops*. It afflicts approximately 10 million people and clinical symptoms include localized skin swelling and irritation due to migration of the adult worms in subcutaneous tissue, as well as occasional passage of a worm across the eye under the bulbar conjunctiva (Boussinesq, 2006). Although loiasis has traditionally been considered benign and not classified on the WHO' official list of NTD (Metzger WG 2014), it poses two major obstacles for LF elimination. First, Severe adverse events (AEs)

including coma and death sometimes occur when LF MDA medications are given to persons with heavy loiasis infections (>20,000 microfilariae per mL of blood) (Gardon *et al.,* 1997). Secondly, up to 6% of persons in loiasis-endemic populations test positive by LF-RDTs in the absence of *W. bancrofti* infection (Pion *et al.,* 2016 ; Wanji *et al.,* 2015). Therefore, there is a need for a rapid test that can reliably distinguish *W. bancrofti* infection from cross-reactive loiasis.

Recently many *L. loa* antigens that cause RDT cross-reactivity in two samples from persons with loiasis were identified. These included both secreted and somatic antigens, suggesting indiscriminate release of *L. loa* antigen from dying adult worms and/or microfilariae (Hertz *et al.,* 2018). A similar pattern of antigen release has been observed following treatment of microfilaremic LF patients with medications known to kill microfilariae (Anderson and Hertz, unpublished data), this suggests that filarial antigens might be released from dying worms. In addition, it was found that cross-reactivity with LF-RDTs is not constant in many loiasis patients despite persistence of high microfilarial loads. All these data above suggest intermittent release and/or clearance of cross-reactive antigen in persons with loiasis. Understanding how, why, and when cross-reactive Loiasis antigen are released and cleared will be essential to defining strategies to distinguish true LF-RDT positives from cross-reactive loiasis.

7. Research questions

This study addresses the following questions:

- What are the cross-reactive *L. loa* antigens, and how and when are they released?
- Does their release and their quantity correlate with the occurrence and the severity of treatment-related AEs and cytokine profiles?

8. Research hypothesis

- Death of *L. loa* microfilariae releases antigens responsible for RDT cross-reactivity, and an imbalance in release and clearance of these antigens accounts for their intermittent presence in serum.

- The antigen profile of those with spontaneous antigenemia will not differ significantly from ivermectin-induced antigenemia.
- The nature and duration of treatment-related adverse events will correlate with the presence and levels of cross-reactive antigen and cytokine profiles.

9. Objectives

9.1. General objective

To define the cross-reactive antigen profile of persons with spontaneous loiasis antigenemia and in those treated with Ivermectin, and to determine whether specific *L. loa* antigens are related to treatment-related adverse events.

9.2. Specific objectives

- To define the cross-reactive antigen profile of persons with spontaneous loiasis antigenemia;
- To identify which cross-reactive Loa antigens are released in response to ivermectin (IVM) treatment of loiasis, and define the kinetics of antigen clearance;
- To determine whether the release of specific *Loa loa* antigens or the amount of released antigen correlate with the occurrence and severity of treatment-related AEs and cytokine profiles.

10. Litterature review

LF is a vector-borne neglected tropical disease targeted for elimination as a public health problem. The infection transmitted by different species of mosquitoes (*Culex*, *Anopheles* and Aedes) is caused by a thread like filarial nematodes roundworms (CDC, 2019). Three species of lymphatic dwelling filarial worms (*Wuchereria bancrofti*, *Brugia malayi* and *B. timori*) cause lymphatic filariasis in humans. The majority of the cases worldwide (90%), and all cases of LF in Africa are caused by *Wuchereria Bancrofti* (Michael *et al.,* 1996). Adult worms reside in the lymphatic vessels interrupting the normal function of the lymphatic system. The worms have life span of about 6–8 years and produce millions of Mf (immature larvae) that circulate in the blood. Mosquitoes are infected with Mf by consuming blood when biting an infected person. Mf then mature into the infective L3 larval stage within the mosquito. When mosquitoes harbouring L3 larvae bite people, the larvae are deposited on the skin from where they can enter the body. The L3 larvae then migrate to the lymphatic vessels where they develop into adult worms, thus continuing a cycle of transmission (CDC, 2019). LF infection can cause a variety of clinical manifestations, including genital disease (hydrocele and/or chylocele) and lymphoedema of the limbs, which can be complicated by recurrent bacterial superinfections called acute attacks, which are extremely painful and are accompanied by fever. The majority of infected people are asymptomatic, but virtually all of them have subclinical lymphatic damage. It takes years to manifest chronic and disfiguring conditions. These conditions lead to mental, social and financial losses contributing to social stigma and poverty (WHO, 2018).

It is estimated that 120 million people in tropical and subtropical areas of the world are infected with lymphatic filariasis; among them, almost 25 million men have genital disease (most commonly hydrocele) and almost 15 million men and women, have lymphoedema of the leg. The majority (57%) of population requiring preventative chemotherapy live in the South-East Asia Region (9 countries) and remaining (37%) live in the African Region (35 countries). An estimation of WHO indicated that around 36 million people are living with these chronic disease manifestations globally. LF accounts for at least 2.8 million DALYs; this does not include significant co-morbidity of mental illness commonly experienced by patients and their caregivers (WHO, 2018).

Elimination of LF as a public health problem means reducing infection prevalence in an area to below target thresholds and providing the recommended basic package of care in all areas with lymphoedema or hydrocele patients (WHO, 2018). GPELF has worked towards elimination for nearly two decades; first by interrupting transmission by MDA using different regimen combinations of albendazole, IVM and diethylcarbamazine (DEC), and second by alleviating suffering through morbidity management and disability prevention (MMDP) (WHO, 2017). Since its inception in 2000, more than 7.1 billion doses of LF elimination medications have been administered to more than 890 million people (WHO, 2018). Overall significant progress has been made towards elimination, but greater efforts are required in the WHO Africa Region, where many countries remain behind the elimination targets (WHO, 2017). In 2016, the WHO Africa region was estimated to have 371.2 million people requiring MDA across 32 endemic countries, with a reported coverage of 56.9% (WHO, 2017). While this marks an increase in coverage from previous years there are still 17 African countries yet to start or scale-up MDA to full nationwide geographical coverage (Molyneux *et al.,* 2014). The reasons for limited coverage are complex and related to several factors including but not limited to political will, conflict, financial commitment, and technical support, difficult to access populations, stakeholder interest, poverty, poor infrastructure, limited human capacity, competing priority diseases, and co-endemicity with *L. loa* (Hope *et al.,* 2018).

Loiasis, also known as African eye-worm disease, is a vector-borne parasitic infection caused by *L. loa*, a filarial worm endemic to Central and West Africa (Pion et *al.,* 2017). It is restricted to the equatorial rain forest regions of Central and West Africa. Epidemiological data collected from 11 African countries indicates that at least 10 million people are infected (Zoure *et al.,* 2011). *L. loa* is transmitted by daytime biting flies of the genus *Chrysops* during a blood meal. The fly injects into the human host infective larvae (L3 development stage) that develop over time into adult worms that reside and migrate in the subcutaneous tissues. These then mate to produce Mf that circulate in peripheral blood (Pion et *al.,* 2017). The major clinical manifestations of loiasis are Calabar swellings (evanescent episodic angioedema) and occasional subconjunctival migration of the adult worm (eye-worm). Less specific manifestations include urticaria, pruritus, myalgias, and arthralgia (Herrick *et al.,* 2015). Moreover, *L. loa* infection can cause renal, cardiac, pulmonary and neurological diseases, and a recent study found *L. loa* infection to be

associated with a decreased life expectancy (Chesnais *et al.,* 2017). The primary health risk from loiasis is the risk for severe or fatal AEs that can occur when individuals with *L. loa* Mf loads >30,000/mL are treated in MDA programs for LF and onchocerciasis (river blindness) (Gardon *et al.,* 1997).

Loiasis has recently emerged as a disease of public health importance, not because of its own clinical manifestations but because of its negative impact on the control of onchocerciasis and LF in areas of co-endemicity (Zouré *et al.,* 2011). In fact, loiasis poses two problems for LF elimination :

- First, severe AEs including coma and death sometimes occur when LF MDA medications are given to persons with heavy loiasis infections (>20,000 microfilariae per mL of blood) (Gardon *et al.,* 1997). In fact, systemic AEs are common following treatment of filarial infections, including LF, onchocerciasis and loiasis, and are thought to be due to inflammation caused by dying filarial worms (Budge et al., 2018). These can be severe or fatal in persons with loiasis and high microfilarial loads. It has been shown that circulating Mf can reach 500,000 Mf/mL in loiasis and the risk of AEs is positively associated with this Mf density, with Mf counts >50,000 Mf/mL conferring high risk for serious/fatal AEs (Gardon *et al.,* 1997). The "Test and Not Treat" (TaNT) studies have recently demonstrate that there was no risk for serious AEs following ivermectin treatment of persons with loiasis Mf counts <20,000 Mf/mL (Kamgno *et al.,* 2018; Kamgno *et al.,* 2017). Among persons with microfilaremic LF or low-level loiasis, treatment-related AEs remain common but are mild and self-limited, usually resolving within 2-3 days following treatment. Typical reactions include fever, headache, myalgias, and malaise, which are similar to those seen following treatment of LF (Budge *et al.,* 2018). Treatment-related AEs in LF are thought to be related to the immune response to filarial or *Wolbachia* antigens released by dying Mf (Kumaraswami *et al.*, 1988; Taylor *et al.,* 2001). However, since *L. loa* does not harbour endosymbiont *Wolbachia* (McGarry *et al.,* 2003), the pathogenesis of AEs following loiasis treatment may differ. In LF and loiasis, eosinophil counts and serum concentrations of type 2 cytokines increase following treatment with antifilarial drugs (Herrick *et al.,* 2017; Andersen *et al.,* 2018). The relationship between specific cytokine activation and AEs in people

with loiasis has not been elucidated. In LF, there is a post treatment increase in circulating filarial antigen and filarial DNA in patients who went on to develop moderate AEs compared to those who had mild or no AEs (Andersen *et al.,* 2018).

- Second, *L. loa* infection complicates the mapping of LF distribution since standard RDTs for LF [e.g. BinaxNOW Filariasis immunochromatographic test (ICT) and/or the new Alere Filariasis Test Strip (FTS Alere, Scarborough, ME, USA)] (Weil *et al.,* 2013), cross-react with *L. loa,* resulting in false positives for *W. bancrofti*, with strong associations between ICT positivity and *L. loa* Mf counts (around 30% and 50% of ICTs reacting to *L. loa* when densities were > 15,00 Mf/ml and > 30,000 Mf/ml respectively) (Pion *et al.,* 2016). Other studies also shown that up to 6% of persons in loiasis-endemic populations test positive by LF-RDTs in the absence of *W. bancrofti* infection (Wanji *et al.,* 2015; Pion *et al.,* 2016). This makes it impossible for national programs to determine with certainty where LF MDA is required and when it can be stopped, given that a filarial antigenemia prevalence of 1% is the GPELF threshold for implementing MDA for LF.

Recently, many *L. loa* antigens that cause LF-RDT cross-reactivity in two samples from persons with loiasis were identified. Surprisingly, these included both secreted and somatic antigens, suggesting indiscriminate release of *L. loa* antigen from dying adult worms and/or Mf (Hertz *et al.,* 2018). A similar pattern of antigen release has been observed following treatment of patients with microfilaremic LF with medications known to kill Mf (Anderson *et al,* 2019). In addition, it was found that cross-reactivity with LF RDTs is not constant in many loiasis patients despite persistence of high Mf loads. These data suggest intermittent release and/or clearance of cross-reactive antigen in persons with loiasis.

11. Methods

11.1. Study design

This study is a prospective cohort study that will include two cohorts of persons with loiasis.

- ***Cohort 1 (spontaneous antigenemia).*** This cohort will prospectively enroll up to 100 adults with *L. loa* Mf counts >20,000 Mf/mL including up to 75 participants with cross-reactive antigenemia and up to 15 without detectable antigenemia. Participants will be followed for one year and tested every three months for persistence of antigenemia. This cohort will not receive any filariasis treatment**.** Cohort 1 will allow us to define the cross-reactive antigen profile of persons with spontaneous loiasis antigenemia, and determine whether this varies with time.
- ***Cohort 2 (induced antigenemia)***. This cohort will include 50 adults with low to moderate *L. loa* microfilaremia (5,000 – 18,000 Mf/mL) who do not have cross-reactive antigenemia at the time of enrollment. All will receive ivermectin (150 µg/kg, once) and will be monitored daily for one week post-treatment to document AEs and collect venous blood for measurement of cytokines and cross-reactive *L. loa* antigens. We will also visit each participant at one month post-treatment to test for circulating antigen and Mf levels. Cohort 2 will allow us to identify cross-reactive *L. loa* antigens released by ivermectin treatment and define the kinetics of antigen clearance, and to determine whether the release of specific *L. loa* antigens or the amount of released antigen correlate with the occurrence and severity of treatment-related AEs and cytokine profiles.

11.2. Study sites

Study site for cohort 1 (spontaneous antigenemia)

The study site for cohort 1will be the Okola health district (HD). The Okola HD is a degraded forest area located at 20 km from Yaoundé that is highly endemic for loiasis and hypomesoendemic for onchocerciasis (Zoure *et al.,* 2011). MDA of ivermectin for onchocerciasis control has yet been implemented in this HD. Participants in this cohort will be recruited among those excluded from the ongoing TaNT study due to Mf counts >20,000/mL.

Study site for cohort 2 (induced antigenemia)

This cohort will be recruit and monitored in the Awae HD (Mefou and Afamba Division, Centre Region), which is located about 50 km south of Yaoundé, the political capital city of Cameroon. Because loiasis is highly endemic and onchocerciasis is hypoendemic in the area, no mass ivermectin distribution has ever been implemented in this area.

11.3. Period of the study

This study will be carried out over three years as shown in the chronogram. Data and sample collection in the field will begin in 2019 after ethical clearance have been granted.

11.4. Study population

Individuals eligible for this study will be of both sexes, aged from 18 years old and over, as follow:

- Participants of both sexes aged from 18 years and over in the ongoing TaNT study in Okola that were excluded from treatment due to Mf counts >20,000 Mf/mL will be included in the cohort 1 (spontaneous antigenemia).
- Participants of both sexes aged from 18 years and over in the Awae HD where no MDA of ivermectin is ongoing will be included in cohort 2 (induced antigenemia)

11.5. Selection criteria

11.5.1. Inclusion criteria

Inclusion criteria will be defined according to the cohort:

- Participants who will be included in cohort 1 will meet the following criteria:
  - Age ≥ 18 years
  - Ability to give informed consent
  - Loiasis Mf count > 20,000 Mf/mL
  - Resident of study area
  - No evidence of severe or systemic comorbidities
  - Consent to storage of blood samples for future study

- Participants who will be included in cohort 2 will meet the following criteria:

- Age ≥ 18 years
- Ability to give informed consent
- Loiasis Mf count between 5,000 Mf/mL and 18,000 Mf/mL
- Resident of study area
- No evidence of severe or systemic comorbidities
- Consent to storage of blood samples for future study

11.5.2. Exclusion criteria

Exclusion criteria will differ from one cohort to the other one. Participants who plans to move from study area during subsequent 12 months will be excluded from cohort 1, while participants will be excluded from cohort 2 if they meet the following criteria:

- Pregnancy or breastfeeding
- Alcohol or drug abuse
- History of severe adverse reaction to ivermectin
- Onchocerciasis (positive Ov16 test or positive skin snip)
- *M. perstans* infection (positive day blood smear)

11.6. Sample size

The sample size has been calculated according to the objectives and the corresponding cohort:

- The objective Cohort 1 is to identify one or more antigens that are sufficiently prevalent among persons with cross-reactive loiasis, that they can be used as biomarker(s) to distinguish cross-reactive loiasis from LF. We define a potential biomarker as an antigen that is detectable in at least **90%** of the population of interest. Assuming that one or more antigens is present in at least **90%** of the population of interest (those with cross-reactive loiasis), a sample size of **50** patients will give us **95%** confidence in excluding antigens that are present in less than **80%** of samples (1-sided Wilson score lower than the **95%** confidence interval). If more that 50 potential participants are identified during screening, we may enroll up to 75 participants for this minimal risk portion of the study.
- The objectives of cohort 2 are to define the cross-reactive antigen profile released by ivermectin treatment and the kinetics of antigen clearance, and to determine whether the release of specific *L. loa* antigens or the amount of released antigen

correlate with the occurrence and severity of treatment-related AEs and cytokine profiles. The sample size of **50** was chosen for cohort 2 to provide direct comparison to the objective of cohort 1 and will provide a similar level of confidence about the prevalence of antigens for this population.

11.7. Data and sample collection

Data and sample collection will be organized according to the objectives of the different cohorts.

11.7.1. Cohort 1 (recruitment and monitoring)

This cohort will include participants of the ongoing TaNT in Okola, who were excluded from IVM treatment due to Mf count ≥20,000 Mf/mL. Those participants will be visited in their home or other designated location. After providing consent for screening, they will be test for cross-reactivity using FTS. In addition, a LoaScope Mf count will be done for those who have not done this test during the last three months. Those with positive FTS test results who consent to participate to the study will be enrolled in cohort 1. For every 5 FTS-positive persons enrolled, we will enroll FTS-negative persons with high Mf loads. If, after screening all available participants excluded from TaNT due to high Mf counts, fewer than 50 FTS positive subjects are found, we will enroll some of the FTS negative subjects with highest MF loads.

The day of enrolment, each participant will provide 10 mL venous blood for subsequent mass spectrometry (detection of cross-reactive *L. loa* antigens) and quantitative ELISA (determination of the level of cross-reactive *L. loa* antigens). Additionally, daytime calibrated thick blood smear will be performed for *L. loa* and *Mansonella* species detection and quantification. Furthermore, nocturnal calibrated thick blood smears and dried blood spot (DBS) will be done for detection and quantification of *W. bancrofti* by microscopy and PCR.

For this cohort, the follow up will be done 3, 6, 9 and 12 months after enrolment. During each visit, a FTS test will be done for each participant and subjects will provide 10 mL venous blood for subsequent mass spectrometry (detection of cross-reactive L. loa antigens) and quantitative ELISA (determination of the level of cross-reactive L. loa

antigens). Due to COVID-19 restrictions the 3 month follow-up visit was cancelled and a 15-month visit is scheduled to retain our ability to examine seasonal variability without changing the overall number of study visits.

11.7.2. Cohort 2 (recruitment and monitoring)

Screening for Cohort 2 will involve community-based testing of potential participants. Initial screening tests will examine a small amount (~200 µL) of capillary blood (obtained by fingerprick); *L. loa* Mf will be measured using the LoaScope, cross-reactive antigen detected using the FTS tests. Consenting Individuals with negative FTS tests and meeting inclusion criteria will undergo further testing to rule out co-infection with other filarial species (*O. volvulus, M. perstans*) that may contribute to treatment-related AEs. Cohort 2 participants will then be admitted to the local health center for observation. All participants will receive a single dose of ivermectin (150 µg/kg) the day after admission. This dose is the same as that given for onchocerciasis MDA annually. Physical examination will be performed daily starting on day –1 (pre-treatment, the day of admission). Vital signs will be recorded on admission and at least daily through day 7. Venous blood will be drawn before treatment on day 0, and on days 1, 2, 3, and 7 post-treatment. We will also collect fingerprick and venous blood at one month post-treatment Procedure for data and samples collection for this cohort are resumed Ain the table below.

Visit	**Cohort 2 activities and assessments**
Screening 1 (0-4 weeks pre-treatment)	• Consent for screening • LoaScope Mf count • FTS • Daytime thick blood smear (*Loa, Mansonella* Mf count) • Skin snips (rule out Onchocerciasis) • Urine pregnancy test (if female)

Enrollment (Day 0)	• FTS (*exclude if positive*) • LoaScope Mf count • Thick blood smear • Admit to health center • Pre-treatment physical examination • Pre-treatment AE questionnaire (*defer if febrile or other systemic illness*) • Venous blood (pre-treatment) for cytokines, antigen ELISA • Ivermectin treatment • Vital signs and AE monitoring q8hrs
Day 1	• FTS • LoaScope Mf count • Venous blood (antigen ELISA, cytokines, +/- mass spec) • Physical exam • Vital signs and AE monitoring q8hrs
Day 2	• FTS (if not positive day 1) • LoaScope Mf count • Venous blood (antigen ELISA, cytokines, +/- mass spec) • Physical exam • Vital signs and AE monitoring q8hrs
Day 3	• LoaScope Mf count • Thick blood smear • Venous blood (antigen ELISA, cytokines, +/- mass spec) • Physical exam • Vital signs and AE monitoring q8hrs
Day 4 – 6	• LoaScope Mf count (if not zero on day 3) • Venous blood (antigen ELISA, cytokines) • Physical exam • Vital signs and AE monitoring daily
Day 7	• FTS • LoaScope Mf count • Thick blood smear • Venous blood (antigen ELISA, cytokines) • Physical exam • Vital signs and AE monitoring daily
Follow-up	• Weekly FTS until negative (if not negative on day 7)
1 Month post-treatmetn	• FTS • Loascope Mf count

	• Thick blood smear

11.7.3. Laboratory evaluation

- **FTS test**

The Alere/Abbott Diagnostics FTS test cassettes will be used, according to manufacturer specifications. Briefly, 75 µL of finger prick blood will be added to a sample application pad that contains dried anti-filarial polyclonal antibody that has been labeled with colloidal gold. The labeled antibody binds to circulating filarial antigen (CFA) if it is present. Blood cells are retained in the sample pad, and the labeled antigen–antibody complexes flow down a nitrocellulose strip with the plasma. The immune complexes are immobilized when they bind to a monoclonal antibody to CFA that has been striped across the nitrocellulose membrane, and this results in a positive test with a visible "T-line." The procedural control "C-line" develops when excess labeled polyclonal antibody crosses a line that contains a secondary antibody to the immunoglobulin that was in the sample pad. Thus, samples that contain CFA produce visible T- and C-lines in the FTS, while negative samples only produce the C-line (Chesnais *et al.,* 2016).

- **Calibrated thick blood smears**

Nocturnal as well as daytime calibrated thick blood smear will be performed according to established protocol on 70 µL of fingerprick blood. These will be stained and read by experienced microscopists at CRFilMT.

- **Skin Snips**

For cohort 2 participants will have skin snips (one punch over each iliac crest) to rule out onchocerciasis. The skin will be disinfected before a snip is taken with a sterile scleral punch. Each snip will be weighed on an analytical balance and incubated for at least 8 hours in isotonic saline in a well of a flat-bottomed micro-titre plate at ambient temperature. Microfilariae will be counted by microscopy. Mf number and skin snip weight will be recorded; skin microfilarial density will be calculated and recorded as mf/mg.

- **LoaScope Mf counts**

LoaScope Mf count will be done on fingerstick blood as described in (Kamgno *et al.,* 2017).

- **Mass spectrometry and ELISA**

Venous blood samples will be separated by centrifugation. Plasma will be frozen and shipped to Washington University in St. Louis for antigen testing (Weil lab ELISA protocol), and for specific antigens by mass spectrometry as previously described (Hertz *et al.,* 2018).

11.8. Statistical analysis

Data analysis will be specific to the cohorts:

- Cohort 1

The prevalence of antigens on Day 0 and at follow-up will be described using frequency (percentages) and their 95% confidence intervals will be reported. Potential biomarkers and the antigen profile of Cohort 1 will be defined as described according to demographic data (age, sex, and village), *L. loa* Mf counts, and presence of *M. perstans*. The presence of detectable cross-reactive antigen at each follow-up study will be analysed as a binary outcome and differences in the proportion of samples positive for cross-reactive antigen compared using Fischer's exact test.

- Cohort 2

Potential biomarkers and the antigen profile for Cohort 2 will be defined as described for Cohort 1. For the purpose of comparing spontaneous antigenemia (Cohort 1) to ivermectin induced antigenemia (Cohort 2), the cross-reactive antigen profile of Cohort 1 will be defined as the collection of antigens found in >90% of baseline samples. Comparison between the antigen profiles of Cohorts 1 and 2 will be assessed as the proportion of samples in Cohort 2 containing detectable amounts of the potential biomarkers identified in Cohort 1. The association of quantitative antigen levels and AE occurrence and severity will be assessed comparing total circulating filarial antigen levels (measured by Weil Lab ELISA) among participants with no AEs to those with mild AEs or moderate AEs and examining differences using t-tests or Mann-Whitney U tests. Data management for Cohort 2 will be the same as for Cohort 1.

12. Ethical considerations

Before the beginning of this study, an ethical clearance will be requested to National Ethics Committee for Research in Human Health of Cameroon and to the Institutional Review Board of the Washington University in St. Louis.

Participation in this study will be voluntary and the refusal to participate will have no consequences for the persons who refuse to take part in the study. The objectives and schedule of the study will be explained to the potential participants and those who agree to participate will sign an informed consent form and keep a copy. The sampling will be performed with non-invasive or sterile and disposable equipment. All participants in Cohort 2 will benefit from general medical consultations with free study-related medication. The laboratory filarial test results will be communicated individually to each participant in strict compliance with the confidentiality rules. The results of this study will be made available to the Ministry of Public Health and the the targeted districts.

12.1. Risks/Discomfort of the study

Drawing blood may be painful; as such, we shall assure the participants that the prick and venous puncture will last only a few seconds. Blood collection will be performed by trained professionals. Blood drawing will be carried out with sterile and single use materials. The risk is then very limited for the participants.

12.2. Benefits of the Study

The main benefit for participants will be to know their status about the diseases tested. Another benefit will be the satisfaction of contributing to the advancement of science.

12.3. Confidentiality

Participant identities, test results, and medical information will be maintained in a confidential manner by the study team and will not be released to anyone outside the staff conducting the project. Confidentiality of individual data will be protected insofar as legally as possible. The report of this study will be transmitted to the Regional Ethic Committee for Research in Human Health for the Centre Region as well as to the Ministry of Public Health. Participants will be informed about their personal test results will be disclosed only to the participant in a private setting.

13. Information notes

This study will have two separate information notes, according to the objectives and the cohort.

13.1. Information note, Cohort 1

I am Professor Joseph KAMGNO, Medical Doctor, and Director of the Centre for Research on Filariasis and other Tropical Diseases (CRFilMT). Our team works with the Ministry of Public Health and other international organizations to research strategies to control filariasis.

We invite you to participate to this research project entitled **'' Cross-reactive antigenemia and treatment-related adverse events in loiasis''** whose objective is to determine why some people with loiasis test positive for lymphatic filariasis even though they do not have lymphatic filariasis, and whether this varies with time. **Participation in the study is entirely voluntary and you may choose not to participate or to withdraw at any moment.**

Now we will describe the research study and explain to you how it will take place.

1- Study title: Cross-reactive antigenemia and treatment-related adverse events in loiasis.

2- Principal investigator: Joseph KAMGNO

3- Authorization number of the Rational Ethic Committee for Research in Human Health:

4- Background Information

Lymphatic Filariasis (elephantiasis) and loiasis (African eye worms) are important public health problem in our country. LF manifests itself mainly by lymphedema (swollen limbs), genital diseases (swollen scrotum) and recurrent acute attacks which are extremely painful and accompanied by fever. On the other hand, symptoms of loiasis include specific manifestations swellings of the hands again called Calabar swelling, or migration of the worm that causes loiasis across the surface of the eye.

To eliminate LF, Mass Drugs Administration (MDA) of Ivermectin, and Albendazole are organized each year by the Ministry of Public Health. However, MDA for LF is very difficult in areas where loiasis is common because some people with loiasis test positive for LF even though they do not have the disease. This makes it very difficult to tell whether MDA for LF is actually needed.

5- Purpose of the study

The objective of this study is to determine why some people with loiasis test positive for lymphatic filariasis even when they do not have the diseases, and to test whether this varies with time.

6- Research procedures

6.1- Study Population

This study will include people who are at least 18 years old who have loiasis and a positive LF test. People with very high levels of loiasis are more likely to have a positive LF test, so we will be testing people living in the Okala Health District who previously tested (during the TaNT study) and were found to have loiasis levels of at least 20,000 Mf/mL of blood.

6.2- Study Design

If you participate in this study, the study team will visit you in your home or village every three months for one year. At each visit, a study nurse or technician will prick your finger to obtain a few drops of blood that he/she will used to test how heavy your loiasis infection is using a handheld microscope called a LoaScope. This is the same procedure as you have had done previously in the Test and Not Treat Study. At the first study visit, we will also use a few drops of fingerprick blood to test for loiasis and related infections. Additionally, the study team will draw 10 mL of venous blood to test for loiasis antigens to help identify why many people with loiasis test falsely positive for LF. On the first study day the study team will also visit you at night for a second fingerprick to test for *W. bancrofti*, the parasite that causes LF.

At each subsequent study visit (3 months, 6 months, 9 months, and 12 months after the first visit), the study team will visit you in your home of village and will again collect daytime blood by fingerprick and by vein. All these visits will be during the day; the only time you will be asked to give blood at night is at the first study visit.

7- Risks/Discomfort of the study procedures.

Drawing blood may be painful; as such, we shall assure the participants that the prick and venous puncture will last only a few seconds. Blood drawing will be carried out by trained professionals using sterile and single use materials.

8- Benefits of the Study

The main benefit for you will be to know your status about these diseases. Another benefit will be the satisfaction of contributing to the advancement of science. Furthermore, at any time of the

visit if you have a health concern, a consultation will be done by a medical Doctor or a nurse, and you will receive generic drug or prescription if the drug is not available.

9- Compensation/Indemnification/Motivation

Participants in this cohort 1 will receive 5000 CFA francs (~$8.65) per study visit to compensate for their travel and lost work time.

10- Confidentiality and Reporting of Findings

Your name, test results, and medical information will be maintained in a confidential manner by the study team and will not be released to anyone outside the staff conducting the project. Confidentiality of individual data will be protected insofar as legally as possible. The report of this study will be transmitted to the Regional Ethic Committee for Research in Human Health for centre region as well as to the Ministry of Public Health. However, your personal results will be given to you and you only.

11- Voluntary feature of the participation

Participation in the study is entirely voluntary. You may withdraw from the study at any time. This will not affect at all your health care within the community.

12- New information

Storage of collected samples: your samples will be stored and transferred to the Washington University for further analysis. We will NOT test them for HIV nor perform any human genetic exam on your samples. Even in our research team, we will not share your name with any person using those samples. Samples collection supplies will be disposed according to security norms.

13- People to contact for further information

If you have any questions or concerns, or you feel you might have been hurt by the study, please contact Professor Joseph KAMGNO (Tel.: 222 202 442, PO Box 5797, Yaoundé). He is the Principal Investigator of this study.

14. Future Contact

We will keep your contact information. In the future, you may be asked to participate in follow up studies that would help to eliminate LF or Loiasis. At any time, you may choose not to participate in future studies.

13.2. Information note, Cohort 2

I am Professor Joseph KAMGNO, Medical Doctor and Director of the Centre for Research on Filariasis and other Tropical Diseases (CRFilMT). Our team works with the Ministry of Public Health and other international organizations to research strategies to control filariasis.

We invite you to participate in a research project entitled '' **Cross-reactive antigenemia and treatment-related adverse events in loiasis''**. The objective of this study is to learn more about what happens in the body when people infected with the parasite, *Loa loa,* are treated with the medication ivermectin. **Participation in the study is entirely voluntary and you may choose not to participate or to withdraw at any moment.**

Now we will describe the research study and explain to you how it will take place.

1- Study title: Cross-reactive antigenemia and treatment-related adverse events in loiasis.

2- Principal investigator: Joseph KAMGNO

3- Authorization number of the Rational Ethic Committee for Research in Human Health:

4- Background Information

Lymphatic Filariasis (elephantiasis) and loiasis (African eye worms) are important public health problem in our country. LF manifests itself mainly by lymphedema (swollen limbs), genital diseases (swollen scrotum) and recurrent acute attacks which are extremely painful and accompanied by fever. Also, symptoms of loiasis include specific manifestations swellings of the hands called Calabar swelling, or migration of the worm across the surface of the eye.

To eliminate LF, Mass Drugs Administration (MDA) of Ivermectin, and Albendazole are organized each year by the Ministry of Public Health. However, MDA for LF is very difficult in areas where loiasis is common because some people with loiasis test positive for LF even though they do not have the disease. This makes it very difficult to tell whether MDA for LF is actually needed. When ivermectin is used to treat loiasis, it kills the microscopic parasite larvae that live in the bloodstream. When these parasite die, the release antigens that cause inflammation in the body. When parasite levels are low, this inflammatory response to the dying parasites often causes headaches, muscle aches, fatigue, weakness, itching, and other symptoms that last 1-2 days and then resolve. However, when parasite levels are high (>20,000 Mf/mL), the inflammation caused by the dying parasites can be much more severe. For people with extremely high parasite levels (>50,000 Mf/mL), this reaction to dying parasites can even be life-threatening. For this reason, ivermectin should not be given to anyone with loiasis levels greater than 20,000 Mf/mL of blood.

5- Purpose of the study

The objectives of this part of the study are (i) to identify which parasite antigens are released by ivermectin treatment of loiasis, and (ii) how these parasite antigens cause the inflammation that leads to treatment-related side effects.

6- Research procedures

6.1- Study Population

This study will include adults at least 18 years old, who have low to moderate level loiasis (5,000 – 18,000 Mf/mL), who have not been previously treated with ivermectin.

6.2- Study Design

People who wish to be enroll in the study will first be tested to determine if they have loiasis, and how heavy their infection is. This will be done by testing a few drops of blood obtained by fingerprick and examining them under a microscope to count the number of parasites in the blood. A few drops of fingerprick blood will also be tested for the presence of parasite antigens. People who will have between 5,000 and 18,000 loaisis parasites per mL of blood and a negative antigen test will be invited to participate in the study.

Those wishing to participate will asked to come to the local health center for treatment, where they will stay for seven days. Each participant will receive a single dose of ivermectin (150 ug/kg) and will be monitored closely so the study team can observe and treat any treatment-related adverse events. Each participant will have a physical examination and monitoring of vital signs at least once a day. A small amount of venous blood (10 mL) will be collected from each participant on the day prior to treatment, the day of treatment, and on days 1, 2, 3, and 7 post-treatment.

7- Risks/Discomfort of the study procedures

Drawing blood may be painful; but the prick and venous puncture will last only a few seconds. Blood collection will be performed by trained professionals. Blood drawing will be carried out with sterile and single use materials to minimize the risk of bleeding or infection.

As mentioned above, treatment with ivermectin is expected to cause side effects in people with loiasis. These may include fever, headache, muscle pain, joint pain, cough, chest pain, rash, itching, fatigue, high or low blood pressure, nausea, abdominal pain, or diarrhea. These symptoms are usually mild, and self-resolve within one week in people with Mf counts less than 20,000 Mf/Ml.

8- Benefits of the Study

The main benefit for you will be to know your status about these diseases. Another benefit will be the satisfaction of contributing to the advancement of science. Furthermore, at any time of the

visit if you have a health concern, a consultation will be done by a medical Doctor or a nurse, and you will receive generic drug or prescription if the drug is not available.

9- Compensation/Indemnification/Motivation

Participants who will undergo both phases of screening will benefited of a medical consultation and delivery of generic druds and those who enroll will receive 15,000 (~$25) on completion of the study.

10- Confidentiality and Reporting of Findings

Your name, test results, and medical information will be maintained in a confidential manner by the study team and will not be released to anyone outside the staff conducting the project. Confidentiality of individual data will be protected insofar as legally as possible. The report of this study will be transmitted to the Regional Ethic Committee for Research in Human Health for Centre Region as well as to the Ministry of Public Health. However, your personal results will be given to you and you only.

11- Voluntary feature of the participation

Participation in the study is entirely voluntary. You may withdraw from the study at any time. This will not affect at all your health care within the community.

12- New information

Storage of collected samples: your samples will be stored and transferred to the Washington University (USA) for further analysis. However, we will not test them for HIV nor perform any human genetic exam on your samples. Even in our research team, we will not share your name with any person using those samples. Samples collection supplies will be disposed according to security norms.

13- People to contact for further information

If you have any questions or concerns, or you feel you might have been hurt by the study, please contact Prof Joseph KAMGNO (Tel.: 222 202 442, PO Box 5797, Yaoundé). He is the Principal Investigator of this study.

14. Future Contact

We will keep your contact information. In the future, you may be asked to participate in follow up studies that would help to eliminate LF or Loiasis. At any time, you may choose not to participate in future studies.

14. Informed consents

14.1. Assent form (Cohort 1: Participants aged from 18 to 20 years old)

I, the undersigned, M / Ms / Miss ....., attest to having been invited to participate in the research work entitled **''Cross-reactive antigenemia and treatment related adverse events in loiasis''** whose principal investigator is Professor Joseph KAMGNO (Director of the Center for Research on Filariasis and other Tropical Diseases.

- I understood the information note that was provided to me; moreover, this information leaflet has been read and explained by the investigators;
- I understood the purpose and objectives of this study;
- I received all the answers to the questions I asked;
- The risks and benefits of this study were presented to me and explained;
- I understand that I am free to accept or refuse to participate in this study;
- My consent does not discharge the investigators of the research of their responsibilities; I therefore retain all my rights guaranteed by law.

I freely agree to participate in this study under the conditions specified in the information leaflet by (1) answering questions from the investigators, (2) communicating my medical information, (3) submitting myself to the capillary as well as venous blood samples for the examinations of filariasis, detection of cross-reactive *L. loa* antigens and determination of the level of cross-reactive *L. loa* antigens, and (4) to be present at each follow up visit.

I agree that my samples taken for this study may be used in subsequent studies.

☐ Yes ☐ No

(*Please check one box only)*

Done at …… on ……. /……. / 2019

_____ _____

Signature of the Principal Investigator Fingerprint of the participant

_____

Name and Signature witness

14.2. Assent form (Cohort 2: Participants aged from 18 to 20 years old)

I, the undersigned, M / Ms / Miss ....., attest to having been invited to participate in the research work entitled **''Cross-reactive antigenemia and treatment related adverse events in loiasis''** whose principal investigator is Professor Joseph KAMGNO (Director of the Center for Research on Filariasis and other Tropical Diseases.

- I understood the information note that was provided to me; moreover, this information leaflet has been read and explained by the investigators;
- I understood the purpose and objectives of this study;
- I received all the answers to the questions I asked;
- The risks and benefits of this study were presented to me and explained;
- I understand that I am free to accept or refuse to participate in this study;
- My consent does not discharge the investigators of the research of their responsibilities; I therefore retain all my rights guaranteed by law.

I freely agree to participate in this study under the conditions specified in the information leaflet by (1) answering questions from the investigators, (2) communicating my medical information, (3) submitting myself to the capillary as well as venous blood samples for the examinations of filariasis, detection of cross-reactive L. loa antigens and determination of the level of cross-reactive *L. loa* antigens, and (4) to be admitted to a health center for treatment with single dose ivermectin and actively monitored for adverse events during seven days.
I agree that my samples taken for this study may be used in subsequent studies.

☐ Yes ☐ No

(*Please check one box only)*

Done at …… on ……. /……. / 2019

_____ _____

Signature of the Principal Investigator Fingerprint of the participant

_____

Name and Signature witness

14.3. Informed consent form (Cohort 1: Participants aged ≥21 years old)

I, the undersigned, M / Ms / Miss ....., attest to having been invited to participate in the research work entitled **"Cross-reactive antigenemia and treatment related adverse events in loiasis"** whose principal investigator is Professor Joseph KAMGNO (Director of the Center for Research on Filariasis and other Tropical Diseases.

- I understood the information note that was provided to me; moreover, this information leaflet has been read and explained by the investigators;
- I understood the purpose and objectives of this study;
- I received all the answers to the questions I asked;
- The risks and benefits of this study were presented to me and explained;
- I understand that I am free to accept or refuse to participate in this study;
- My consent does not discharge the investigators of the research of their responsibilities; I therefore retain all my rights guaranteed by law.

I freely agree to participate in this study under the conditions specified in the information leaflet by (1) answering questions from the investigators, (2) communicating my medical information, (3) submitting myself to the capillary as well as venous blood samples for the examinations of filariasis, detection of cross-reactive *L. loa* antigens and determination of the level of cross-reactive *L. loa* antigens, and (4) to be present at each follow up visit.

I agree that my samples taken for this study may be used in subsequent studies.

☐ Yes ☐ No

(*Please check one box only)*

Done at …… on ……. /……. / 2019

_____ _____

Signature of the Principal Investigator Signature of the participant

14.4. Informed consent form (Cohort 2: Participants aged ≥21 years old)

I, the undersigned, M / Ms / Miss ....., attest to having been invited to participate in the research work entitled **"Cross-reactive antigenemia and treatment related adverse events in loiasis"** whose principal investigator is Professor Joseph KAMGNO (Director of the Center for Research on Filariasis and other Tropical.

- I understood the information note that was provided to me; moreover, this information leaflet has been read and explained by the investigators;
- I understood the purpose and objectives of this study;
- I received all the answers to the questions I asked;
- The risks and benefits of this study were presented to me and explained;
- I understand that I am free to accept or refuse to participate in this study;
- My consent does not discharge the investigators of the research of their responsibilities; I therefore retain all my rights guaranteed by law.

I freely agree to participate in this study under the conditions specified in the information leaflet by (1) answering questions from the investigators, (2) communicating my medical information, (3) submitting myself to the capillary as well as venous blood samples for the examinations of filariasis, detection of cross-reactive *L. loa* antigens and determination of the level of cross-reactive *L. loa* antigens, and (4) to be admitted to a health center for treatment with single dose ivermectin and actively monitored for adverse events during seven days.

I agree that my samples taken for this study may be used in subsequent studies.

☐ Yes ☐ No

(*Please check one box only)*

Done at …… on ……. /……. / 2019

_____ _____

Signature of the Principal Investigator Signature of the participant

15. Chronogram

Years	2019						2020												2021											
Activities	**July**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
Ethical clearance / administrative authorization																														
Cohort 1																														
Screening and enrolment																														
Follow-up visits																														
Laboratory analysis (LCMS, CFA ELISA)																														
Cohort 2																														
Screening and enrolment																														
Ivermectin treatment and monitoring																														
Laboratory analysis (LCMS, CFA & cytokines ELISA)																														
Valorization																														
Preparation and submit publication																														
Meeting attendance																														
Prepare and submit R01																														
Final Report (Thesis defense)																														

16. Detailed budget and Source of funding

16.1. Detailed budget

Budget Line Items	Number	Units	Unit Price	Total Cost	Total Cost in US Dollar
Ethical clearance	1	1	200000	200000	347,43
Survey materials				**0**	**0,00**
Microscope (available at CRFilMT)	1	2	0	0	0,00
Centrifuge (available at CRFilMT)	1	1	0	0	0,00
LoaScope (available at CRFilMT)	1	4	0	0	0,00
Lancets (Only for blood smears) (200/pack)	100	1	2500	250000	434,28
Capillary tubes (Only for blood smears) (100/box)	10	1	5000	50000	86,86
Capillary tubes (Only for LoaScope) (100/box)	15			0	0,00
Slides (50/pack) / both for thick blood smear and Skin Snip	40	1	3500	140000	243,20
Holth type punches for skin snip (available at CRFilMT)	20	1	0	0	0,00
96 wells Microtiter plates (round bottom)	7	1	500	3500	6,08
Parafilm	1	2	35000	70000	121,60
Eau ou serum physiologique (Sachet de 500 ml)	4	1	2000	8000	13,90
Papier essui tout	2	10	1000	20000	34,74
Label (1000/tape)	1	5	10000	50000	86,86
Bin bags (50/pack)	1	6	10000	60000	104,23
Cotton ball (500g)	2	4	3500	28000	48,64
95° Alcohol (Should be diluted at 70°)	2	4	1500	12000	20,85
Methanol	1	1	15000	15000	26,06
Giemsa	1	1	30000	30000	52,11
EDTA tubes (100/pack)	10	1	10000	100000	173,71
Filter paper (12mm) (100/pack)	2	1	4500	9000	15,63
Vacutainer needles (100/pack)	10	1	5000	50000	86,86
1.5 mL Eppendorf tubes (1000/pack)	2	1	29000	58000	100,75
1000 µL tips (1000/pack)	1	1	12000	12000	20,85
300 µL tips (1000/pack)	2	1	12000	24000	41,69
200 µL tips (1000/pack)	1	1	15000	15000	26,06
Ivermectin for loiasis treatment				0	0,00
FTS test	1200	1	900	1080000	1876,11
Prenancy test	300	1	500	150000	260,57
Isopropanol				0	0,00
Gloves (100/pack)	20	1	5000	100000	173,71
Reprography (Survey forms, information sheet and inform consent)	1800	6	20	216000	375,22
Drugs for general medicine	1	6	100000	600000	1042,28
Insurance for field surveyors	4	3	25000	300000	521,14
Hospitalization expense	7	50	2000	700000	1216,00
compensation cohort 1	4	50	5000	1000000	1737,14
compensation cohort 2 (Screening)	1	50	5000	250000	434,28
compensation cohort 2 (study)	1	50	15000	750000	1302,85
Antigens identification by Mass Spectrometry					
				0	0,00
Antigens quantification by ELISA					
				0	0,00
Cytokines measurement by ELISA					
				0	0,00
Honorarium and per diems (20 days of field work)					

Honorarium of the project coordinator	1	20	50000	1000000	1737,14
Perdiem for field activities (01 researcher, 02 lab technicians, 01 drivers)	2	20	120000	4800000	8338,26
Per diem for field supervision (Regional Delegations)	1	2	120000	240000	416,91
Per diem for field supervision (Health Districts)	1	6	50000	300000	521,14
Per diem for field supervision (Health Area)	10	6	10000	600000	1042,28
Per diem for sensitization and assistance (community distributors)	20	6	5000	600000	1042,28
Transportation and communication (20 days of field work)					
Vehicules maintenance	20	3	12500	750000	1302,85
Fuel	20	3	12000	720000	1250,74
Airtime credit for surveyor communication	4	3	20000	240000	416,91
Total				**15600500**	**27100,20**
Administrative fees (15% of the total budget)				2340075	4065,03
Grand Total				**17940575**	**31165,23**

16.2. Source of funding

This study will be funded by the Doris Duke Charitable Foundation (650 5th Ave, New York, NY 10019, USA).

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WU Ref. No. : A2020-0039

Material/data sharing agreement

Preamble

This document defines the arrangements for material transferring and data sharing between the **Centre for Research on Filariasis and other Tropical Diseases (CRFilMT)** and The **Washington University (WUSTL)** under the project **"Cross-reactive antigenemia and treatment-related adverse events in loiasis"** that binds them.

I. PARTIES TO THE AGREEMENT

Institution providing the material and/or data: Centre for Research on Filariasis and other Tropical Diseases (CRFilMT), Yaounde, Cameroon ("Provider")

Contact person: Joseph KAMGNO (MD, MPH, PhD)

Title: Director of the CRFilMT and Vice-Dean in charge of Research and Cooperation at the Faculty of Medicine and Biomedical Sciences of the University of Yaounde I.

Address: Street 1308, Quartier Fouda | PO Box 5797 Yaounde, Cameroon

Phone number: 00237 677 789 736 | 00237 222 202 442

Email: kamgno@crfilmt.org

Fax number: 00237 222 202 443

Institution receiving the material and/or data: The Washington University ("Recipient")

Contact person: Philip Budge, MD, PhD

Title: Assistant Professor of Medicine and Infectious Diseases

Address: 4444 Forest Park Ave room 4186c, St. Louis, MO, 63108. USA

Phone number: +1-314-747-5532 or +1-314-747-5198

Email: pbudge@dom.wustl.edu

Fax number: +1-314-454-5293

II. PURPOSE AND TERM OF AGREEMENT

A. Purpose of material and/or data sharing

To investigate what are the cross-reactive *L. loa* antigens, how and when are they released, and if their releases contribute to treatment-related adverse events observed in individuals with heavy loiasis infection. This study is of great importance for mapping, transmission assessment survey, surveillance and validation of the elimination of lymphatic filariasis in areas where loiasis is co-endemic. Indeed, it was demonstrated that people harboring high *L. loa* microfilarial densities can exhibit false-positive rapid diagnostic tests result while targeting LF.

B. Legality and credibility of parties

1. The Centre for Research on filariasis and other Tropical Diseases (CRFilMT) is a non-profit organization working in the medical field whose mission is to contribute to the improvement of the living conditions of the populations through an appropriate and applied scientific and medical research. The CRFilMT had signed a collaboration agreement with the Ministry of health of Cameroon as well as with many other national and international institutions and was positioned in 2009 as reference in Central Africa for surveillance of different filariasis. In addition, the CRFilMT also received in 2009 an official testimony of satisfaction from the Ministry of health of Cameroon.
2. The Washington University, a corporation established by special act of the Missouri General Assembly approved February 22, 1853 and acts amendatory thereto, having its principal office at One Brookings Drive, St. Louis, MO 63130, USA (WUSTL) is among the top-ranked medical research institutions in the United States.

C. Period of performance

This Agreement shall be effective when signed by both parties and shall continue until terminated pursuant to the termination clause contained herein.

III. MATERIAL AND DATA MANAGEMENT PLAN

Sample collection will be done after approval by WUSTL Institutional Review Board (IRB) and Cameroon Regional Ethic Committee for Research in Human Health for *Centre* region, only on those individuals who will agree to participate in the study by signing a consent form. Thick blood smears will be prepared and stored at the CRFilMT in a locked cabinet designated for sample storage. Venous blood samples will be used for the preparation of dried blood spots and isolation of plasma samples. These plasma samples will be transferred in anonymously recoded 4.8 ml cryovial tubes containing and stored at -20°C at the CRFilMT. These anonymous samples ("Samples") will then be shipped to WUSTL where they will be processed, blinded from the personal information of the subjects ("Results"). Hard copies with personal information of subjects will be stored at CRFilMT in a locked cabinet designated for data storage. Samples collection materials or used Samples will be destroyed according to the validated security norms.

IV. ACCESS TO MATERIAL AND/OR DATA

A. Method of access and transfer

Thick blood smears slides and hard copies of data collection forms, stored in locked cabinet, will only be accessible at the CRFilMT after specific authorizations. DBS and plasma Samples shipped at WUSTL will be processed to determine and quantify cross-reactive loiasis antigens and cytokines. Secured electronic databases generated by CRFilMT and WUSTL will be shared between both parties.

B. Persons having access to data

Only principal investigators (and/or authorized designee) will have access to data and/or stored material. Prior to the transfer of any data, team members and researchers who will have access to the data should request specific authorization to the principal investigators (both at CRFilMT and WUSTL).

C. Frequency of data exchange

Data will be exchanged as needed to meet reporting requirements as well as on an ongoing basis between CRFilMT and WUSTL teams for the entire length of the project.

V. SECURITY OF DATA AND CONFIDENTIALITY OF SUBJECT RECORDS

Neither CRFilMT team, nor WUSTL team will attempt to identify individuals' records by any method. Datasets containing protected personal information shall be encrypted. All reasonable precautions shall be taken to secure the data from individuals who do not specifically have authorized access. Data shall be kept on a password-protected file server located in a secure environment, both at CRFilMT and at WUSTL. Project data will be kept in a separate directory on the server which is also password-protected and will be accessible only by principal investigators or designee with specifically authorized access.

The report of this study will be transmitted to the Cameroon Regional Ethic Committee for Research in Human Health for *Centre* region as well as to the Ministry of Public Health. However, subject personal results will be given to them in strict compliance with privacy rules.

VI. WARRANTY AND LIMITATION OF LIABILITY

A. Warranty

Any Samples or Results delivered pursuant to this Agreement is understood to be experimental in nature and may have hazardous properties. THE PARTIES MAKE NO REPRESENTATIONS AND EXTEND NO WARRANTIES OF ANY KIND, EITHER EXPRESSED OR IMPLIED. THERE ARE NO EXPRESS OR IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, OR THAT THE USE OF THE SAMPLES OR THE RESULTS WILL NOT INFRINGE ANY PATENT, COPYRIGHT, TRADEMARK, OR OTHER PROPRIETARY RIGHTS

B. Limitation of Liability

Except to the extent prohibited by law, the Recipient assumes all liability for damages which may arise from its use, storage or disposal of the Samples. The Provider will not be liable to the Recipient for any loss, claim or demand made by the Recipient, or made against the Recipient by any other party, due to or

arising from the use of the Samples by the Recipient, except to the extent permitted by law when caused by the gross negligence or willful misconduct of the Provider.

Except to the extent prohibited by law, the Provider assumes all liability for damages which may arise from its use of the Results. The Recipient will not be liable to the Provider for any loss, claim or demand made by the Provider or made against the Provider by any other party, due to or arising from the use of the Material by the Provider, except to the extent permitted by law when caused by the gross negligence or willful misconduct of the Recipient.

This agreement must be formally approved and signed by both parties before any material transfer or data sharing takes place. Both parties will ensure that the material transfer and the data sharing agreements are known and understood by all staff involved in the process.

Centre for Research on Filariasis and other Tropical Diseases (CRFilMT)

Pr KATIGNO JOSEPH MD, PhD — Director

Name — Position



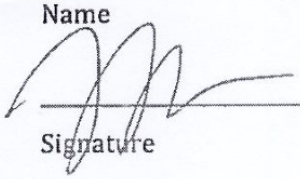
7/31/19

Signature — Date

The Washington University (WUSTL)

Nichole R. Mercier, PhD — Asst Vice Chancellor & Managing Director, OTM

Name — Position



7/24/19

Signature — Date