INVESTIGATION INTO THE RISK FACTORS FOR MALARIA TRANSMISSION IN THE OMUSATI REGION

A THESIS SUBMITTED IN FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTERS OF SCIENCE

OF

THE UNIVERSITY OF NAMIBIA

BY

ERASTUS H. HANGANENI HAINDONGO

200808907

JULY 2016

MAIN SUPERVISOR: DR DAVIS MUMBENGEGWI

Co-SUPERVISOR : DR RONNIE BÖCK

ABSTRACT

Namibia has achieved great success in the reduction of malaria case numbers from over 500 per 1000 population in 2000 to between 1 -2 per 1000 population in 2013 (MoHSS-NVDCP, 2009). The gains in the reduction of malaria case numbers have seen the country transitioning from the control phase of malaria epidemiology to the pre-elimination phase, and has subsequently adopted the goal of malaria elimination by 2020 (MoHSS-NVDCP, 2010; Pindolia *et al*., 2012). Since the year 2008, there were no significant reductions in cases per annum, despite continued interventions (such as Indoor Residual spraying, Insecticide treated nets and larviciding) geared towards vector and parasite control. These interventions are aimed at the realization of malaria elimination. These uninformed and untargeted interventions leave certain groups or individuals at an increased risk of infection. Furthermore in a low transmission setting, parasite density decreases and a considerable number of people harboring *Plasmodium* parasites are asymptomatic. As a consequence, untargeted at risk populations continue to harbor parasites, which continues to fuel transmission and thus poses as a challenge to elimination. This highlights the need for establishing the unknown risk factors and detection of low parasite density asymptomatic infections with more sensitive molecular diagnostics since the current Point of Care (P.O.C) diagnostics, the Rapid Diagnostic Tests (RDTs) do not detect some of these low density infections. These will ultimately allow for evidence-based targeting of interventions, for the final drive to eliminate malaria.

A household level cluster case-control study was carried out between January – May 2014 in the Oshikuku and Outapi health district of the Omusati Region, north central Namibia. Case households were identified by Re-Active Case Detection (RACD), a surveillance tool which involves the screening of individual residing in proximity of a case detected passively at a health

facility. Control households were randomly selected from National Census Enumeration Areas (EA). A semi- structured questionnaire was administered to all eligible and consenting members. Questions pertaining to the demographics (age and gender), net ownership and usage, presence of breeding site, travel in the past 6 weeks, outdoor nocturnal behavior, household spraying, and treatment seeking behavior following the self-reported history of fever were elicited from all eligible study participants. RDT and Dried Blood Spots were collected and stored for analysis of the presence of *Plasmodium* parasites using Loop-mediated isothermal Amplification (LAMP) and Cytochrome B nested Polymerase Chain Reaction (nPCR).

RACD identified 59 index case households for investigation and an additional randomly selected 77 households were investigated as controls. The distribution of males and females was comparable in both case and control households. The following factors were found to be associated with the increased risk of being a malaria case: Household with a low Socio-Economic status(SES); Long lasting insecticide treated bed net ownership (O.R=3.89, p-value: 0.05), net usage (O.R=1.4, p-value:0.01); Age group: 35 – 45 (O.R=15.06, p-value: <0.001); Presence of breeding site (O.R=2.21, p-value= 0.01) ; distance of household to a Health facility (O.R=6.26, p-value:0.01) and poor treatment seeking behaviour. RDTs had a sensitivity and specificity of 76.47(95%CI:50.10-93.19) and 95.88 (95%CI:92.45-96.51), with its calculated Positive Predictive Value (PPV) being 35.14 (95%CI:18.57-49.13). Hence, when compared to LAMP and nPCR, the sensitivity and specificity of RDTs was the lowest. When using the reference method of nPCR, the sensitivity (100%) and specificity (97.89%) of LAMP was comparable to that of nPCR although LAMP detected 48% more positives than nPCR. However, LAMP had a PPV of 52% since most of its positives were considered false. This study indicates that RACD can be used as a tool to establish the risk factors associated with malaria at household

level and can thus inform elimination programmes on the appropriate intervention tools that should be employed. LAMP had a higher sensitivity, specificity, PPV and NPV than RDT and may potentially perform better than RDTs in detecting low density infections. However, further research is required as LAMP results could not always be confirmed using PCR.

Keywords: Reactive Case Detection, Pre-elimination, elimination, risk factors, interventions, RDT, LAMP, nested PCR, malaria, sensitivity, specificity, PPV, case-control, Socio-Economic Status, Demographics, Behaviour, sleeping structure, net ownership, net usage, breeding site, Indoor Residual Spraying, Travel

Table of Contents

ABSTRACT i

LIST OF FIGURES vii

LIST OF TABLES x

LIST OF CONFERENCE AND POSTERS xi

ACKNOWLEDGEMENTS xii

DECLARATION xiv

DEDICATION xv

DEFINITION OF KEY TERMINOLOGY xvi

LIST OF ABBREVIATIONS xvii

CHAPTER 1: INTRODUCTION 1

1.1. Orientation of Study 1

1.1.1 WHO Malaria Continuum: 'From Control to Eradication' 5

1.2. Statement of the Problem 8

1.3. Research objectives 8

1.4. Significance of the study 8

1.5. Limitation of the study 9

CHAPTER 2: LITERATURE REVIEW 10

2.1 Malaria aetiology 10

2.2 Malaria life cycle 12

2.3 Distribution, transmission intensity (seasonality) and Endemicity of malaria 14

2.4 Burden of Malaria: Morbidity and Mortality 17

2.5 Vector Control Interventions: 18

2.5.1 Indoor Residual Spraying (IRS) 18

2.5.2 Insecticide Treated Bed Nets 20

2.5.3 Larviciding 21

2.6 Parasite Control Interventions: Treatment 22

2.7 Malaria surveillance as an Intervention tool 22

2.8 Malaria Risk factors 23

2.9 Detection and Diagnosis of Malaria parasitaemia 27
2.9.1 Microscopy 28
2.9.2 Serological: Immunochromatographic test (ICT) 29
2.9.3 Molecular detection and identification 33
CHAPTER 3: MATERIALS AND METHODS 38
3.1. Research design 38
3.2. Study Area and Population 39
3.3. Sample and sample size 42
3.4 Ethical Clearance 43
3.5. Field Procedures 44
3.5.1. Eligibility and Consent 45
3.5.2 Questionnaire: Determination of Risk factors of transmission 45
3.5.3 Blood sample collection and storage 45
3.6. Laboratory Procedures 46
3.6.1 Extraction of DNA by Chelex-100 method 47
3.6.2 Loop-mediated Isothermal Amplification (LAMP) 48
3.6.3 Nested Polymerase Chain Reaction (nPCR) 49
3.6.4 Cytochrome B digestion 51
3.6.5 Gel electrophoresis and Visualization 51
3.7. Data Analysis 51
CHAPTER 4: RESULTS 53
4.1 Demographics of study participants 54
4.2 Risk factors for malaria transmission 55
4.2.1 Risk factor by age group 55
4.2.2 Total number of nets owned and usage 56
4.2.3 Source, condition and treatment of Nets 59
4.2.4 Presence of Mosquito breeding site 60
4.2.5 History of fever, treatment seeking-behaviour, nocturnal behaviour and travel 61
4.2.6 Indoor Residual Spraying: Vector Control 64
4.2.7 Socio-Economic Indicators 65
4.2.8 Sleeping structure characteristics 69

4.3 Detection of *Plasmodium* infections by RDT, LAMP and nested PCR 69

4.3.1 Samples.. 69

4.3.2 Comparison of the detection of *Plasmodium* infections by LAMP, nested PCR and RDT in case and control households. 70

4.3.2 Comparison of symptomatic cases (index cases) and asymptomatic (secondary cases) 73

CHAPTER 5: DISCUSSION 77

CHAPTER 6: CONCLUSION AND RECOMMENDATION 91

REFERENCES 93

8. APPENDICES 111

Appendix 1: English Consent form 111

Appendix 2: Oshiwambo Consent form... 113

Appendix 3: Sample Questionnaire.. 116

Appendix 4: Carestart RDT Manufactures guideline.. 119

Appendix 5: Eiken PAN/Pf LAMP guidelines 120

Appendix 6: MoHSS Case Investigation Form.. 123

Appendix 7: Proportions and Odds ratio calculations for the type of material used in the floor, wall, window, construct of sleeping structures in case and control households 125

LIST OF FIGURES

Figure 1: Trend of confirmed malaria cases per 1000 in Namibia from 2001-2014. Source:WHO, (2015) 1

Figure 2: Health facility reported malaria cases from 2001-2011. Source: Smith Gueye et al.,(2014) 2

Figure 3: showing the 3 components of Global Malaria Action Plan (GMAP) elimination strategy 6

Figure 4: Diagram showing the WHO malaria continuum, from malaria control to eradication: WHO perspective (Mendis et al., 2009). 7

Figure 5: Global categorization of the predominant *Plasmodium* species causing human malaria. Adopted from Feachem et al., (2010) 11

Figure 6: Digenetic *Plasmodium* life cycle, adopted from Vale, Aguiar, & Gomes, (2014) 12

Figure 7: Drawings of the morphology of *Plasmodium* species at different stages of the life cycles. Retrieved from http://www.tulane.edu/ 14

Figure 8: Map of Namibia, showing Malaria incidence by regional stratification. Source: Ministry of Health and Social Services (MoHSS) 17

Figure 9: Different types of ELISA and the enzyme-substrate reaction to produce a visible colour change. Source: http://www.lifetechnologies.com Accessed: August, 24, 2015........ 29

Figure 10: A-C: Mechanism of Action of Immunochromatographic test (ICT) or RDT in detecting *Plasmodium* spp. Schematic adopted from Malaria Rapid Diagnostic Tests (2015).... 32

Figure 11: Schematic illustration of *Plasmodium* mitochondrial genome adopted from Sundararaman et al.,(2013) 35

Figure 12: Process flow chart of the research design. 38

Figure 13: Map of Namibia showing Omusati Region and the study health districts and health facilities (red crosses). Erastus Haindongo (2015) 40

Figure 14: Typical Oshiwambo homestead. Source: www.serasphere.net................................. 41

Figure 15: Diagram showing a process flow chart of the field procedure.................................. 44

Figure 16: *Plasmodium* Genus specific PCR reaction conditions curve 50

Figure 17: Location of case (red dots) and control (blue dots) of the visited households. Map created by Erastus H. Haindongo (2015)... 53

Figure 18: Pie charts showing the distribution of males and females in Case (A) and Control (B) households.. 54

Figure 19: Population pyramids in Case (below) and control (above) household by age and gender.. 55

Figure 20: Distribution of the number of nets per household in Case (A) and control (B) households.. 58

Figure 21: Images showing larvae in an uncovered container of water at a house in the Oshikuku district. Erastus Haindongo (2014) 61

Figure 22: Reasons for not seeking care for fevers in study household, case households (A) and control households (B)... 62

Figure 23: Proportions of Indoor Residual Spraying in case (B) and control (A) households..... 64

Figure 24: Response to why sleeping structures were not sprayed 65

Figure 25: Proportions of Assets owned in case and control household 67

Figure 26: Image of RDT with mixed infection (3 lines) (R) and Negative (only control line) (L). Erastus Haindongo (2015) 70

Figure 27: A nurse performing DBS in Katima Mulilo. Erastus Haindongo (2015)................... 70

Figure 28: Total *Plasmodium* positivity by RDT, LAMP and nested-PCR 72

Figure 29: Representative image of LAMP tubes under UV light- tube no 2 is positive (green fluorescence) and the rest are negative (no fluorescence). Source: Erastus Haindongo (2015)... 73

Figure 30: A representative gel image of a nested PCR product. Bands in lane 1,2,4,5,7,9,12,17,19,20,21,22 indicative of positives. 73

Figure 31: Results from diagnostic tests (RDT, LAMP and nested PCR) for case and control households.. 75

LIST OF TABLES

Table 1: Profile by programme type. Source: World Health Organization (2009) 7

Table 2: Shows the distribution, severity of disease, recurrent state for the different parasite species.Reproduced:Walker et al.,(2014). 11

Table 3: Endemicity by *Plasmodium* falciparum Parasite Rate (PfPR) with information from Smith, Guerra, Snow, & Hay,(2007) 16

Table 4: Univariate analysis for risk of association by age 56

Table 5: Indicating the total net count, distribution and general use of nets amongst children and adults 57

Table 6: Univariate analysis of association and frequency distribution of nets in case and control households 57

Table 7: Indicating the proportions and univariate association of net condition and status of net in sleeping structure. 60

Table 8: Univariate analysis for Individual risk factors 63

Table 9: Chi square and p-values for types of assets found in households 66

Table 10: Univariate measure of association for type of toilet facilities in a household 68

Table 11: Univariate measure of association for main source of drinking water 68

Table 12: Univariate measure of association for main source of fuel 68

Table 13: Sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) using PCR as the reference standard 76

LIST OF CONFERENCE AND POSTERS

1. Davis Mumbengegwi, Joyce Auala, Munyaradzi Tambo, **Erastus Haindongo** (2014). Molecular techniques and findings from malaria elimination research in Ohangwena and Omusati. Windhoek, Namibia.

2. **Haindongo, E. H.**, Mumbengegwi D.R., Bock R., Sturrock H., Smith J., Gosling R., (2015). An investigation into the risk factors of malaria in a pre-elimination rural and peri-urban setting of Northern Namibia. Poster presented at the ASTMH meeting 2015, Philadelphia, USA.

3. **Haindongo, E. H.**, Mumbengegwi D.R., Bock R., Sturrock H., Smith J., Gosling R., (2015). A comparison of malaria risk factors between case and control households in the low transmission, pre-elimination Omusati Region. Paper presented at the NCRST Conference 2015, Windhoek, Namibia.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank the almighty God with whom everything begins and ends under his watchful eye and protective hand. I would also like to thank the Bill and Melinda Gates foundation for generously funding the research through a grant by the University of California-San Francisco. Thank you Auntie Elise Shilongo, for forwarding the email that have seen me applying for this scholarship.

I would like to extend my heartfelt gratitude to my main supervisor Dr. Davis R. Mumbengegwi for investing his time and energy in my professional development. Dr. Davis, you have lived up to the quote by David Stoddard, which states that "*Getting the most out of life isn't about how much you keep for yourself, but how much you pour into others*". I will forever be indebted to you for what you have poured into me. I would also like to thank my co-supervisor, Dr Ronnie Bock, for his encouragement, motivation and for always availing himself to guide and direct me. In a nutshell, without the contribution of my enthusiastic academic supervisors this work would have been a daunting task.

I would also like to thank Dr. Jennifer Smith for giving me a "crash-introduction" to the R software. All in all, thank you for equipping me with this indispensable tool, with which data cleaning and analysis would not have been easy. In addition, I would also like to thank Prof. Lawrence Kazembe for his guidance and direction during this analysis. Prof, thank you for the prompt response, to my calls for help whenever I got stuck!

I would also like to thank the following Multi-disciplinary Research Centre/Biomedical Research laboratory colleagues for extending a helping hand in the field and in the laboratory in one way or another: Joyce Auala, Munyaradzi Tambo and Iwanette Du Preez.

I would also like to thank all the study participants for making time to respond to the study questions and providing samples that have made all subsequent analysis possible. Thank you to the Chief/Environmental Health Practioners (C/EHP), enrolled and registered nurses, Primary Health Care supervisors, Chief Medical Officers (CMO) from the Outapi and Oshikuku Health districts for the support they have rendered.

Lastly, I would like to thank everyone that gave me words of support and encouragement. You have made me believe in myself more. I thank God for the supportive family and friends that I have. Thank you for understanding my absence without requiring me to furnish a reason, especially during the course of this academic undertaking. I needed this more than ever!

"The LORD bless you and keep you;
The LORD make His face shine upon you,
And be gracious to you;

The LORD lift up His countenance upon you,
And give you peace." **Numbers 6:24-26**

DECLARATION

I, Erastus H. Hanganeni Haindongo, hereby declare that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

No part of this report may be reproduced, stored in any retrievable system, or transmitted in any form, or by means (e.g. electronic, mechanical, photocopying, recording or otherwise) without the prior permission of the author, or the University of Namibia in that behalf.

I, Erastus H. Hanganeni Haindongo, grant the University of Namibia the right to reproduce this thesis in whole or in part, in any manner of format, which the University of Namibia may deem fit for any person or institution requiring it for study and research: providing that the University of Namibia shall waive this right in the whole thesis has been or is being published in a manner satisfactory to the University.

.... Date..

Erastus H. Hanganeni Haindongo

DEDICATION

I dedicate this work to my late father:

Lieutenant Colonel Policarpus Gaddafi Haindongo.

The principles you have installed in me during my formative years have had a positive and indispensable impact on my life.

I apply your teachings daily, as I try to keep my head above the waters.

Ina kafa okwena, iha kashiva ukukutu wedu!

DEFINITION OF KEY TERMINOLOGY

Active Case Detection (ACD): the operation carried out by surveillance agents who visit every locality in a defined area at regular intervals (usually monthly during the transmission season), in order to enquire for fever cases through individual house visits, and to test for malaria (and treat if positive) each suspected person so discovered[1].

Case Investigation: the process of identifying the origin of infection[1].

Passive Case Detection (PCD): the finding of malaria cases through the notification by medical personnel to whom fever cases and other suspected cases are reported[2].

Pro-Acitve Case Detection: is the screening of a focal population without the trigger of a passively identified case[3].

Re-Active Case Detection (RACD): is triggered whenever a case is identified by passive case detection. Involve visits to the households of locally acquired cases, screening family members, and neighbors within a defined radius[3].

Sub-patent infections: parasitemia below the microscopic threshold or having >0 and <100 parasites/µl[4].

Surveillance: is the ongoing systematic collection, analysis, and interpretation of outcome-specific data of an infection for use in the planning, implementation, and evaluation of infection control (or elimination) initiatives[3].

---

[1] Kelly Sanders et.al (2012). Active Case Detection for Malaria Elimination: A confusion of acronyms and definitions.
[2] R. M Montanari (2001). Policy and Practice. Bulletion of WHO
[3] Fabrizio Molteni. Malaria Surveillance: The way forward towards malaria elimination.
[4] Jacklin F. Mosha et.al (2013). Epidemiology of subpatent infection: implication for detection of hotspots using imperfect diagnostics.

LIST OF ABBREVIATIONS

95% CI – 95% Confidence Interval

ACT- Artemisinin-based Combination Therapy

Bst-Bacillus staerothermophilus

CDC- Centre for Disease Control and Prevention

Cyt B- Cytochrome B

DBS- Dried Blood Spots

DNA- Deoxyribonucleic Acid

EA- Enumeration Area

EIR- Entomological Inoculation Rate

ELISA- Enzyme Linked Immunosorbent Assay

GPS- Geographic Positioning System

HRP-2- Histidine Rich Protein 2

ICT- Immunochromatographic test

IRS- Indoor Residual Spraying

ITN – Insecticide Treated bed Net

LAMP- Loop-mediated isothermal Amplification

LLIN-Long Lasting Insecticide Treated bed Net

MoHSS- Ministry of Health and Social Services

MRC- Multi-disciplinary Research Centre

MRDT- Malaria Rapid Diagnostic Test

mtDNA- mitochondrial DNA

nPCR- nested Polymerase Chain Reaction

NVDCP-National Vector Disease Control Programme

O.R – Odds Ratio

P.f- Plasmodium falciparum

P.v-Plasmodium vivax

PfPR- *Plasmodium* falciparum Parasite Rate

pLDH- *Plasmodium* Lactose DeHydrogenase

POC- Point of Care

RACD- Re-Active Case Detection

RDT- Rapid Diagnostic Test

SES- Socio-Economic Status

ssDNA- single stranded Deoxyribonucleic acid

T3- Test, Treat and Track

Taq- Thermophillus aquaticus

UNAM- University of Namibia

WHO – World Health Organisation

CHAPTER 1: INTRODUCTION

1.1. Orientation of Study

An estimated 3.4 billion people worldwide are estimated to be at the risk of malaria. In 2012, the World Health Organisation (WHO) reported 207 million cases and 627,000 deaths globally. About 80% of these cases and 90% of the deaths occurred on continental Africa. Malaria is confined to the northern regions of Namibia(Kamwi, 2005). Malaria continues to be a major health problem in Namibia, despite the downward trend of malaria cases and deaths since the 2001 outbreak. Namibia's malaria control program has had a successful track record, evident from the reduction of malaria cases from 537 115 in 2001 (which was the highest record of malaria cases since 1993) down to 1,546 in August of 2013. According to figure 1, there was an increase in 2014 to above 6 cases per 1000. This considerable reduction in the number of malaria cases down to an average of 1700 cases annually is an indication of Namibia entering the pre-elimination phase of the malaria control/elimination continuum (National Malaria M&E Plan, 2010, Roll Back Malaria Monitoring and Evaluation:Namibia, 2005, Ministry of Health and Social Services, 2009)

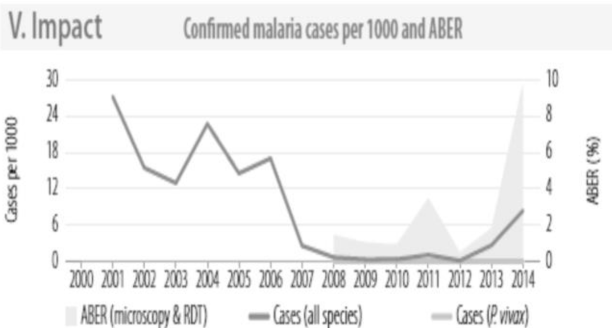


Figure 1: Trend of confirmed malaria cases per 1000 in Namibia from 2001-2014. Source:WHO, (2015)

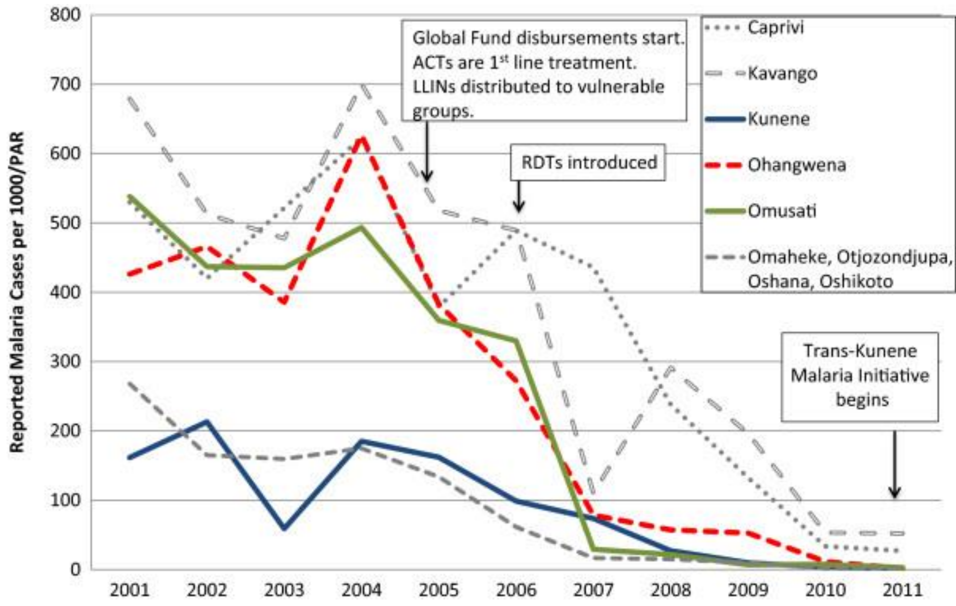


Figure 2: Health facility reported malaria cases from 2001-2011. Source: Smith Gueye et al.,(2014)

Figure 2 shows the trend of cases in Namibia as well as the introduction of several interventions, which brought about the successful reduction of case numbers. Between 2005-2011, a total of 625,000 Long Lasting Insecticide Treated Bed Nets (LLINs) were distributed. The nets were targeted for at risk groups, most particularly, children under 5 years and pregnant women. National Indoor Residual Spraying (IRS) covered 15.6% of the Populations At Risk (PAR). RDTs were first introduced in 2005 and by 2011 RDTs that are more sensitive and specific to the predominant *Plasmodium falciparum* parasite were procured (Smith Gueye *et al*., 2014).

By 2010 countries have made malaria program reviews and have undergone program reviews, essentially adopting new elimination strategies (WHO, 2011). Countries such as Cape Verde (WHO, 2012b), South Africa, Botswana, Namibia, Tanzania (Zanzibar) have made considerable progress in reducing disease burden and have thus transitioned from the control to the pre-elimination phase(Southern Africa Roll Back Malaria Network, 2012).

Malaria programmes have had significant gains in reducing case numbers, sustaining the downward trend in the gains made by these programmes remains important for attaining elimination goals (Berhane *et al.*, 2015; Karunaweera, Galappaththy, & Wirth, 2014). The maintenance of an effective surveillance and response system is important for elimination. The allocation of resources to malaria surveillance and response becomes challenging in the face of diseases such as dengue which has a higher disease burden (Karunaweera, Galappaththy, & Wirth, 2014). Hence, there is a need for the development of a surveillance research agenda for active and prompt detection of infection (Slutsker, 2012).

Across all transmission settings, malaria infections display clustering. The clusters are termed hotspots, and can be comprised of a group of household/a household experiencing high exposure to infectious mosquitoes. This hotspots can act as a source of infection to households outside the hotspot (Sturrock, Bousema, Mosha, & Gosling, 2012). Exposure to malaria is heterogeneous in different settings. One notable variation is that of the majority of infections in a local population being carried asymptomatically, whilst the symptomatic carriers account for about 20% of the population. Hotspots maintain transmission in low transmission seasons and fuel transmission in the high seasons (Bousema *et al.*, 2012). The majority of *P.falciparum* infections detected in community surveys are usually characterized by low-density parasitaemia and the absence of clinical symptoms (Bousema, Okell, Felger, & Drakeley, 2014). The detection of low-density infections is limited by the sensitivity of the diagnostic tool. Henceforth, transmission in Namibia is low, the Carestart RDT being the Point Of Care (P.O.C) diagnostic has reduced sensitivity in low transmission settings. High quality laboratory diagnostic services are therefore also crucial to the detection of both symptomatic and asymptomatic infections, which will allow for the provision of prompt treatment and effective treatment (Karunaweera *et al.*, 2014).

Molecular methods have shown that asymptomatic infections are more widespread in low transmission settings (Bousema *et al*., 2014). Nested-PCR and Loop mediated isothermal Amplification (LAMP) have been shown in several studies to have a detection limit of between 1-5 parasites/µl, whereas RDTs have a detection limit of 50-100 parasites/µl (Paris *et al*., 2007). Highlighting the need for molecular tools in low transmission settings where most of the infections are potentially low-density and asymptomatic.

With the changing disease epidemiology, there is a shift in the populations most at risk of malaria. These populations normally have a shared social behaviour, occupation, gender or geographic characteristic (Cotter *et al*., 2013). Low malaria transmission in Namibia is suggestive that elimination is possible, with an increased risk of importation from neighbouring Angola (Smith Gueye *et al*., 2014). As Sri Lanka was entering elimination, it experienced a large burden of imported malaria from illegal migrants *via* routes from Western Africa in 2012 (Wickramage, Premaratne, Peiris, & Mosca, 2013). Importation of malaria in Zanzibar and Swaziland sustains local transmission, with strategies such as rapid response following a reported case-known as Re-Active Case Detection (RACD) and border screening as interventions (Sturrock, Roberts, Wegbreit, Ohrt, & Gosling, 2015). Management of the risk of importation becomes a high priority for successful interventions especially in a country like Namibia which shares borders with southern Angola. The southern Angolan provinces are characterized by high seasonal transmission and are prone to epidemics (Presidents Malaria Iniative: Angola, 2012). There is an increase in cross border traffic between Angola and Namibia (Crush, 2005), which is a possible risk factor for importation and transmission of malaria.

Human activities and social-behaviour may provide an additional risk as a result of increased exposure. The high coverage and intensive use of Long Lasting Insecticide Treated Nets (LLIN)

and Indoor Residual Spraying (IRS) decreases exposure to infectious mosquito bites and thus the risk of infection (Mboera, Mazigo, Rumisha, & Kramer, 2013). However, with the main vector control interventions, the efficacy of LLINs are sometimes short-lived whilst with IRS, there is resistance to pyrethroid insecticides (Karunaweera *et al*., 2014).

As a consequence, traditional control interventions of Indoor residual spraying and mass net distribution are likely to be less effective as they are not appropriately targeted to at risk populations. Knowledge of the changing epidemiological trends of malaria will ensure improved targeting of interventions, as parasites are cleared and vector development is impeded (Cotter *et al*., 2013).

1.1.1 WHO Malaria Continuum: 'From Control to Eradication'

Roll Back Malaria (RBM) has established the Global Malaria Action Plan (GMAP). GMAP is aimed at supporting countries for a sustained reduction in global malaria and ultimately, for the eradication of global malaria. Figure 3 classifies the 3 part global strategy for the progression from control to elimination. Firstly, GMAP aims to **control** malaria by reducing the current burden as well as to sustain control for as long as necessary. Secondly, GMAP aims to eliminate malaria country by country, over the years. Furthermore, GMAP also sets out to research new tools and approaches to support global control and elimination (Roll Back Malaria Partnership (WHO), 2008).

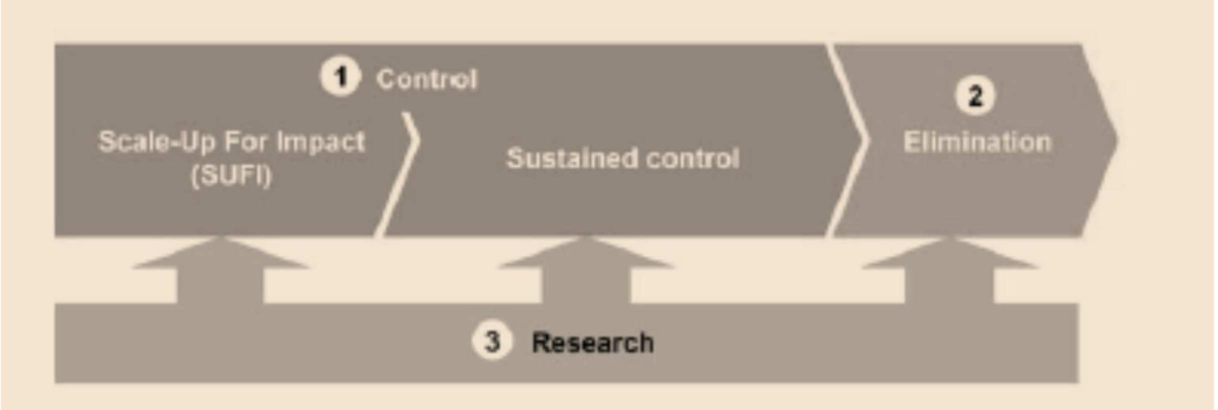


Figure 3: showing the 3 components of Global Malaria Action Plan (GMAP) elimination strategy

Figure 4 shows the WHO malaria continuum, in which malaria control is defined as reducing the malaria disease burden to a level at which it is no longer a public health problem, whilst elimination is defined as the interruption of local mosquito-borne malaria transmission. Thus, countries transition from one phase to another, every phase requires a programme re-orientation (World Health Organization, 2009). Table 1 below summarizes the main programme goal, epidemiological objective, transmission objective and unit of interaction from the control phase to the elimination phase.

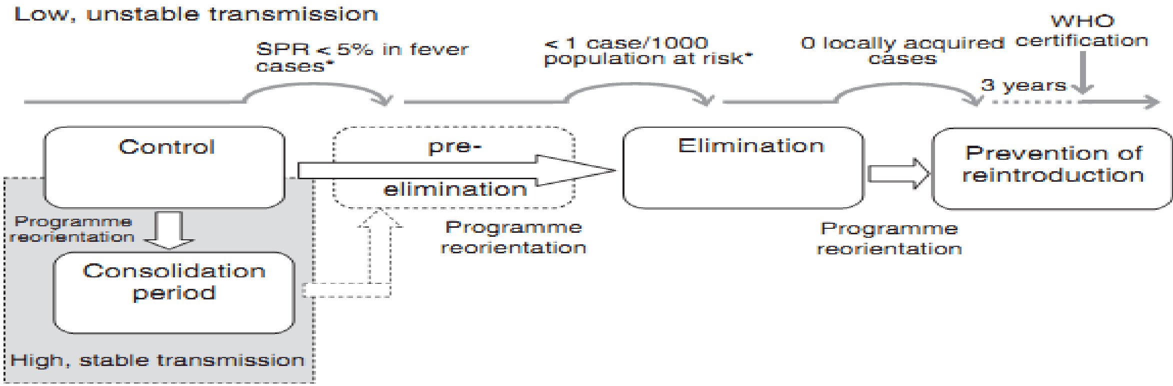


Figure 4: Diagram showing the WHO malaria continuum, from malaria control to eradication: WHO perspective (Mendis et al., 2009).

Table 1: Profile by programme type. Source: World Health Organization (2009)

	CONTROL PROGRAMME	Pre-elimination programme	Elimination programme	Prevention of reintroduction programmes
Main Programme goal	Reduce morbidity and mortality	Halt local transmission nationwide	Halt local transmission nationwide	Prevent re-establishment of local transmission
Epidemiological objective	Reduce burden of malaria	Reduce number of active foci to zero Reduce number of locally acquired case	Reduces number of active to zero Reduce number of locally acquired cases to zero	Prevent introduced cases and indigenous cases secondary to introduced cases
Transmission objective	Reduce transmission intensity	Reduce onward transmission from existing cases	Reduces onward transmission from existing cases	Reduce onward transmission from imported cases
Unit of Interaction	Country or area wide	Transmission foci	Transmission foci, individual cases (locally acquired and imported)	Recent transmission foci (receptive areas), individual cases (imported cases only)
Indicative milestones for transition to next programme type	SPR<5% in suspected malaria cases	<1 case per 1000 population at risk per year	Zero locally acquired cases	
Data source for measuring progress towards reaching milestones	Proxy data: health facility data Confirmatory data: population based	Proxy data: health facility data Confirmatory data: population-based surveys	Notification reports, individual case investigations, genotyping	

1.2. Statement of the Problem

Although malaria cases in Namibia have been on the decline, a plateau has been reached since 2008 (World Health Organisation, 2013) with actual increases in 2014. The plateau provides direct evidence that the current malaria interventions used in the control phase cannot be used to achieve the elimination phase. There is a gap in knowledge on the appropriate intervention tools required to reduce malaria cases in order to achieve the elimination phase.

1.3. Research objectives

In order to provide evidence based decisions on the appropriate interventions to achieve malaria elimination. The objectives of this study are to:

- Conduct RACD to determine malaria transmission patterns in Outapi and Oshikuku health districts.
- Determine the risk factors of infection in malaria cases and controls in Outapi and Oshikuku health districts using a household level clustered case-control study approach.
- Evaluate a new molecular diagnostic tool, Loop-mediated Isothermal Amplification (LAMP) as compared to nested Polymerase Chain Reaction (n-PCR) and RDTs for use with RACD

1.4.Significance of the study

Namibia has set a goal of achieving malaria elimination by the year 2020 (MoHSS-NVDCP, 2010). In Namibia, the epidemiology of the disease has also changed in recent years. These changes involved a shift in social risk factors and density of parasites. Such challenges have

compromised elimination efforts as interventions were not targeted to at risk groups. This study therefore fills the knowledge gap in recognizing the applicability of case-control studies, in determining risk factors. Thus the risk factors determined in this study will allow for targeted and evidence-based interventions, which will reduce resource wastage and maximize the gains and progress of the National Vector Disease Control Programme of the Ministry of Health and Social Services in attaining elimination. Re-Active Case Detection (RACD) can also be used to identify infection clusters that are epitomized by low density infections which can only be detected by molecular tools such as LAMP, which has proven to be more robust than currently used RDTs. Thus LAMP can be used as a surveillance tool during RACD to detect and to treat individuals that may act as reservoirs of infection.

1.5.Limitation of the study

Since the study traced cases reported from health facilities, there was no control on cases that did not visit health facilities and this data may be lost. The Oshikuku and Outapi health districts share borders with Angola and the findings of this study may not necessarily reflect the malaria situation in other regions that do not share borders with Angola.

CHAPTER 2: LITERATURE REVIEW

2.1 Malaria aetiology

Malaria is a vector-borne infectious disease (Ding *et al*., 2014), caused by Apicomplexan (Sub-phylum) parasites which belongs to the *Plasmodium* genus(Mandal, 2014). Five different species from the *Plasmodium* genus are known to infect humans, namely, *P. falciparum, P. vivax, P. ovale, P. malariae* and *P.knowlesi* (Gunn & Pitt, 2012; Vale, Aguiar, & Gomes, 2014). The latter species, being of non-human primates, but it occasionally infects humans. *P.falciparum* is the most virulent species and thus accounts for the greater portion or extent of malaria-related deaths in Africa(Walker, Nadjm, & Whitty, 2014). *Plasmodium* species are confined to different parts of the world, the severity of the disease and possibility of relapses depends on the type of species. Table 2 and figure 5 shows that *P. falciparum* and *P. vivax* are the most widespread species, with *P. falciparum* accounting for 90% of the infections on continental Africa whilst *P. vivax* is responsible for most of the infections in South America. Furthermore *P. falciparum* is the most virulent and is responsible for most of the fatalities. *P. ovale* and *P. malariae* are confined to West Africa and Africa respectively. In addition, *P. knowlesi* is found in Thailand, Mynmar and Malaysia.

Table 2: Shows the distribution, severity of disease, recurrent state for the different parasite species.Reproduced:Walker et al.,(2014).

Species of *Plasmodium* causing disease				
Species	**Distribution**	**Severe disease**	**Recurrent disease (relapsed)**	**Features**
P. falciparum	Widespread	+++	-	Most fatalities Severe disease
P. vivax	Widespread	+	+	Hypnozoites present: long incubation and relapse possible
P. ovale	West Africa	(+)	+	
P. malariae	Africa	(+)	-	Long incubation possible Cause of nephrotic syndrome
P. knowlesi	Malaysia, Thailand, Mynmar	+	-	Zoonosis
Key: (+) Very rare, + rare, +++ common				Microscopically similar to *P. malariae* Severe disease/fatalities

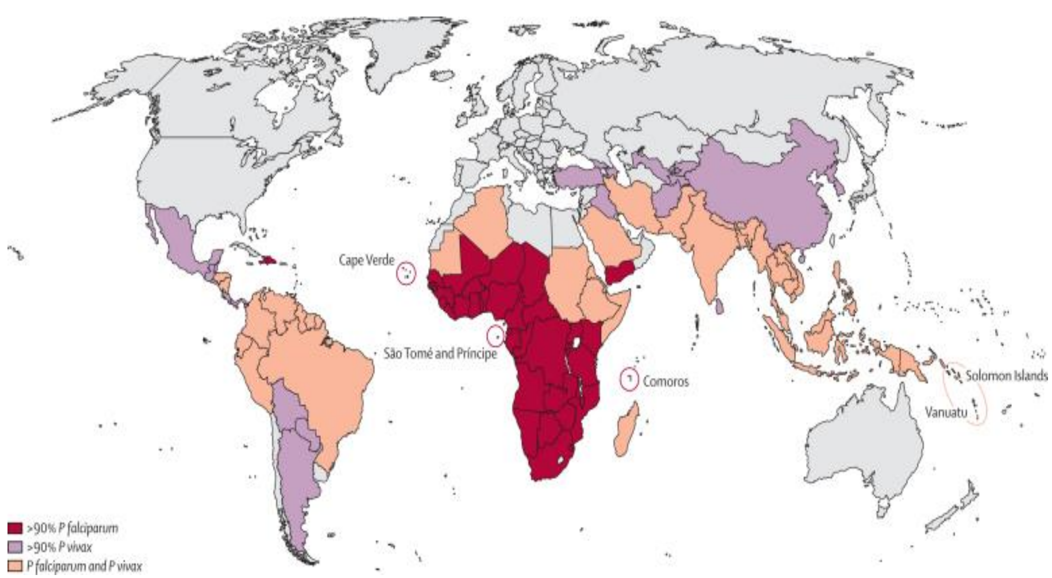


Figure 5: Global categorization of the predominant *Plasmodium* species causing human malaria. Adopted from Feachem et al., (2010)

2.2 Malaria life cycle

The malaria causative agents *Plasmodium* belongs to the phylum Apicomplexa (Mandal, 2014). Apicomplexans are obligate intracellular parasite that infect metazoans and causes a great deal of debilitation to human and animal health (Becker, 2011). The *Plasmodium* parasite is at the center stage of the malaria life cycle. The life cycle of *Plasmodium* involves an alternation of generations between the asexual and sexual forms/generations (Marcus, 2009). The life cycle is also digenetic, as it is completed in two hosts, the primary or definitive host (*Anopheles* mosquito) and the secondary or intermediate host (human).

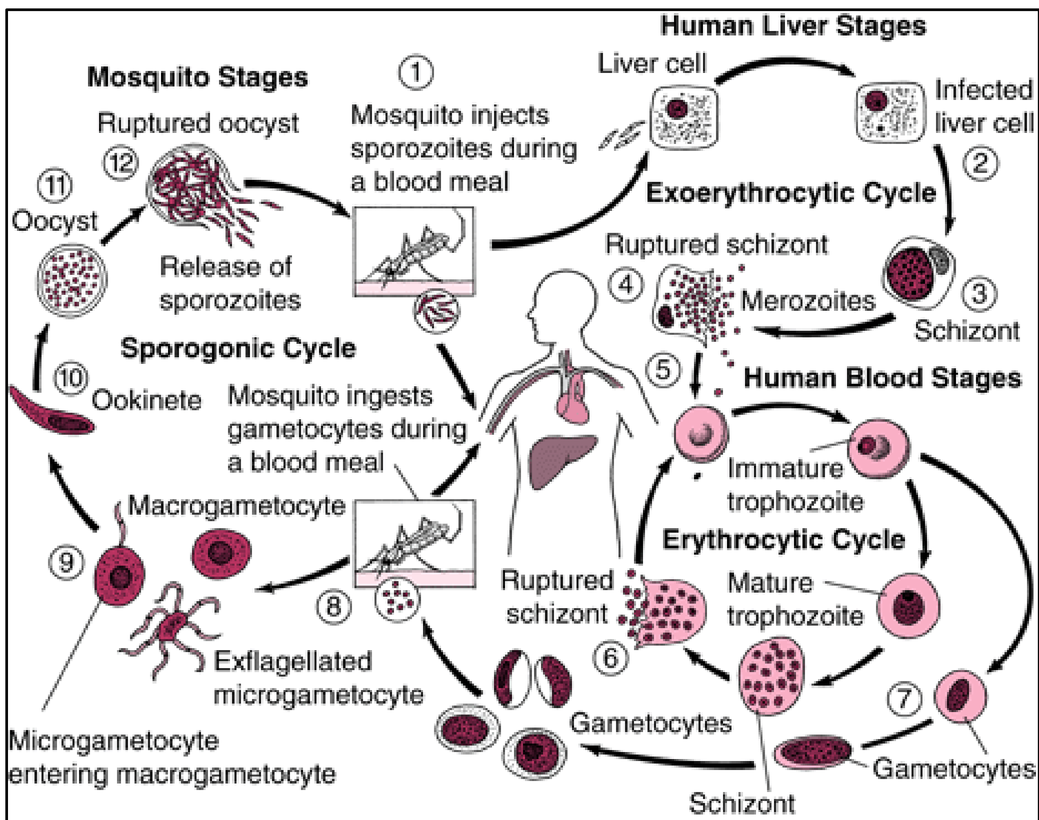


Figure 6: Digenetic *Plasmodium* life cycle, adopted from Vale, Aguiar, & Gomes, (2014)

The sexual stage is completed in the gut of the female mosquito. Figure 6 shows the life cycle, in which infective sporozoites are produced (a stage known as sporogony) and stored in the mosquito salivary glands. There produced Sporozoites are then introduced into a human host via the bite of an *Anophele*s mosquito. The sporozoites travel to the liver, where they undergo schizogony (asexual reproduction by multiple fission) in the pre-erythrocytic and exo-erythrocytic liver phases to produce metacryptomerozoites. The micro metacryptomerozoites then enter the blood stream and immediately invade red blood corpuscles. The micro metacrytomerozoites are then modified into a young round form, known as a trophozoite. The trophozoite is involved in the signet ring stage (Kotpal, 2012).

The ring stage involves the erythrocytic cycle, in which there is continuous infection and bursting of red blood cells. This stage begins with, a young immature trophozoites, which differentiate into gametocytes or matures into a schizont. The schizont releases merozoites which continue the cycle of infecting healthy red blood cells (CDC, 2015). The ring form parasites occasionally develop into male and female gametocyctes, which once ingested by mosquitoes, sexually develops in the mosquito midgut to form ookinete and eventually the infective sporozoites. The sporozoites travel to the salivary gland of the *Anopheles,* where it will be transferred to a human host during feeding(Vale *et al*., 2014).

Furthermore, in the ring stage, proteolysis of haemoglobin into its protein and hematin components is brought about by enzymes. The protein is used as food by the trophozoite, while hematin forms the toxic malarial pigment haemozoin (Kotpal, 2012). Hemozoin is involved in the observed fever during malaria infection(Tiemi Shio *et al*., 2009). The asexual stage eventually comes to an end. Merozoites reinvade the liver and may stay in the liver for a long time (Marcus, 2009). *Plasmodium vivax* and *P. ovale* form resting stages in the liver as

hypnozoites and can cause clinical relapses (Kebede, Assefa, Medhin, Berhe, & Velavan, 2014; Gunn & Pitt, 2012)

The parasite species are identified by morphological characteristics (Lee, Cox-Singh, & Singh, 2009). Furthermore, the morphology of the parasite also depends on the stage in the life cycle (Figure 7).

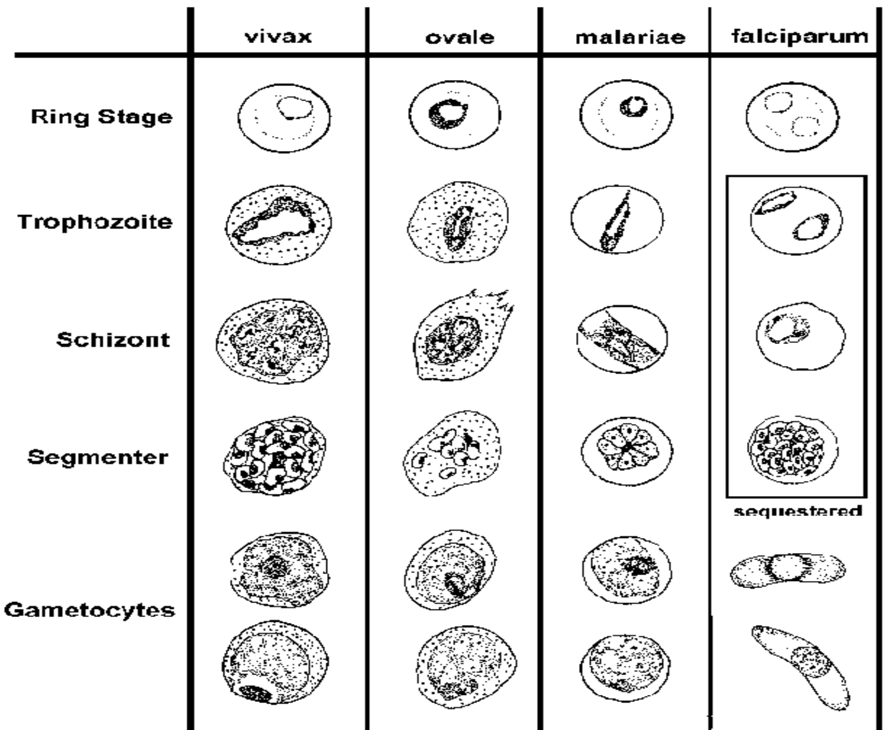


Figure 7: Drawings of the morphology of *Plasmodium* species at different stages of the life cycles. Retrieved from http://www.tulane.edu/

2.3 Distribution, transmission intensity (seasonality) and Endemicity of malaria

The distribution of malaria globally has natural geographic parameters, which are determined largely by latitude, altitude and climate (Feachem & Sabot, 2008). Malaria occurs in all six WHO regions, these regions include the Americas, Africa, Europe, South East Asia, Eastern Mediterranean and Western Pacific. In 2000, the global estimate of cases stood at 227 million

(tolerance: 170 -297 million) and 755,000 deaths (tolerance: 575,000-969,000). Cases and deaths were reduced to 198 million and to 584,000 deaths by 2013, respectively (World Health Organization, 2011, 2014). Over 80% of cases and 90% of deaths occur on continental Africa, mostly in children under 5 years(Coulibaly *et al*., 2013).

Transmission of communicable diseases can either be vertical or horizontal. Horizontal transmission can be directly (sexually), common vehicle (food or water-borne) or vector-borne, such as in the case of malaria (Merrill, 2013). Tumwiine, Mugisha, & Luboobi, (2007) reports that MacDonald (1957) categorized malaria endemicity into 1) **stable endemic**-where populations are continuously exposed to a fairly constant rate of malaria inoculations; 2) **unstable endemic**- exposed to more or less permanent malaria with large fluctuations in the rate at which inoculations occur; and 3) **epidemics**- are an extreme form of unstable malaria, where populations or communities are subjected to transmissions exceeding that which was previously experienced. Infant Parasite Rate (IPR) and Spleen Rate (SR) have been used as a measure of malaria transmission. The spleen rate is no longer a reliable measure of transmission intensity in areas with wide-spread usage of anti-malarial. The most common measure of transmission is the Entomological Inoculation Rate (EIR) or the number of infectious bites per person per year (Bretscher *et al*., 2013; Kelly-Hope & McKenzie, 2009).

Although most malaria endemic settings have seasonal malaria peaks, malaria transmission intensity and seasonality still varies widely across countries (Roca-Feltrer, Schellenberg, Smith, & Carneiro, 2009). There is a wide range of malaria transmission intensities, this range includes areas with low malaria risk and low prevalence of parasites (e.g. sporadic malaria) to areas where continual, repeated exposure to malaria occurs throughout the year (perennial and intense) (e.g. holoendemic malaria)(Moormann *et al*., 2005)

Another measure of endemicity is the *Plasmodium falciparum* parasite rate (PfPR), which is a measure of the proportions of the population carrying asexual stages of the parasite. Depending on the proportion of the PfPR, areas can be categorically classified as being Holoendemic, hyperendemic, mesoendemic and hypoendemic (table 3)

Table 3: Endemicity by *Plasmodium* falciparum Parasite Rate (PfPR) with information from Smith, Guerra, Snow, & Hay,(2007)

Malaria Endemicity	**PfPR**
Holoedemic	>75%
Hyperendemic	50-75%
Mesoendemic	10-50%
Hypoendemic	<10%

The geographic location of countries like Nigeria has a suitable climate to support all year transmission of malaria in most parts of the country(Adigun, Gajere, Oresanya, & Vounatsou, 2015). In Namibia, malaria is overall seasonal and unstable. The Namibia Malaria Indicator Survey (NMIS) 2009 graphic on the distribution of malaria depicts that the southern part of the country is free of indigenous malaria. The north eastern Okavango East and West and Zambezi region has high malaria transmission, the Omusati and other central northern regions are predominantly low malaria transmission areas. Additionally, figure 8 shows that the Omusati

region records less than 1 case/1000 whilst the Zambezi region and Okavango East regions (found in the far north eastern part of Namibia) records between more than 1 case/1000.

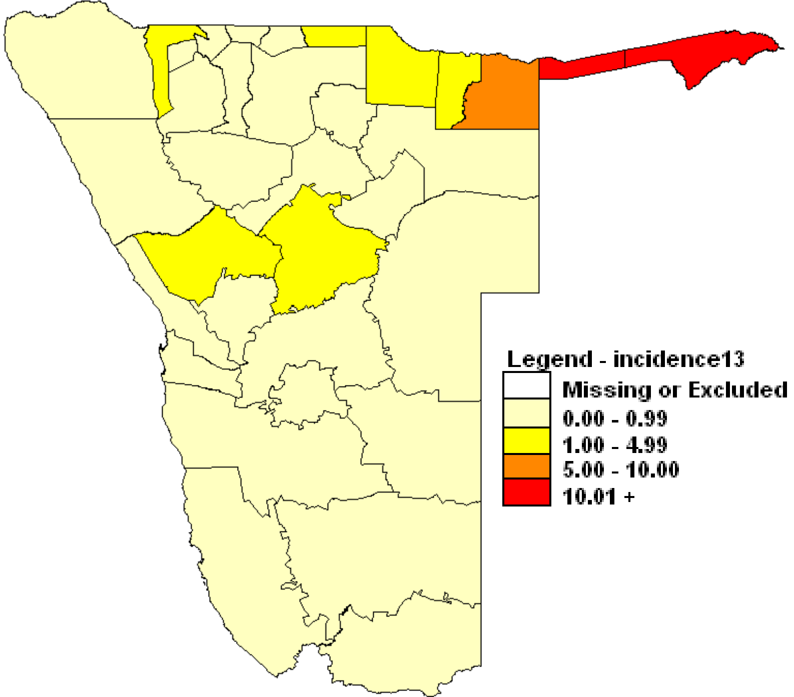


Figure 8: Map of Namibia, showing Malaria incidence by regional stratification. Source: Ministry of Health and Social Services (MoHSS)

2.4 Burden of Malaria: Morbidity and Mortality

Malaria causes major mortality and morbidity in the tropical and subtropical regions, with *P.falciparum* and *P.vivax* contributing the greatest to the global burden of malaria (Mandal, 2014). An estimated 655 000 malaria deaths occurred in 2010, with 91% of these deaths being in sub-Saharan Africa (Kebede, Assefa, Medhin, Berhe, & Velavan, 2014). According to the 2013 WHO estimates, mortality was reduced by 42% globally and by 49% in Africa between 2000 - 2012. A decline in malaria incidence rates by 25% globally and 31% in Africa has also been noticed. These declines are due to intensified intervention strategies. These interventions

included the delivery of an estimated 136 million nets to endemic countries. In addition, in 2012, 135 million (constitutes 4% of global at risk population) were protected by Indoor Residual Spraying (IRS). There was also an increase in the volume of Rapid Diagnostic Tests (RDT), ensuring access to accurate diagnostics and quality assured treatment with Artermisnin-based Combination Therapy (ACT). Surveillance systems were also improved and this has allowed for the detection of 14% more cases in 2012 from 10% in 2010 (World Health Organisation, 2013). Malaria is also ranked among the top 7 leading causes of Disability Adjusted Life Years (DALY's), accounting for 3.3% (82,685,000) DALYS globally (Mori, Ngalesoni, Norheim, & Robberstad, 2014).

2.5 Vector Control Interventions:

2.5.1 Indoor Residual Spraying (IRS)

Indoor Residual Spraying (IRS) is the most broadly used effective primary vector control method, with a large scale impact(Najera & Zaim, 2001). Different types of insecticides are used to control mosquito populations. There different types of insecticides are classified as carbamates (e.g. Bendiocarb), Pyrethroids (e.g. Deltamethrin), Organophosphates and organochlorides (e.g. Dichloro-diphenyl-tricholoroethane DDT))(Berhane *et al*., 2015; Ratovonjato *et al*., 2014; Steinhardt *et al*., 2013). Insecticides have a toxic effect on the mosquitoes and it also have excito-repellant effect, deterring entry and promoting exit out of dwellings.

The introduction of DDT in the 1940's drastically reduced the number of malaria mosquitoes as well as their longevity. The reduction in the number of malaria mosquitoes allowed for the reduction in the burden of disease with some countries, such as the United States of America (USA) eliminating malaria (WHO, 2012a). Indoor Residual Spraying (IRS) is still recommended

for use in high transmission settings, with multiple studies showing its effectiveness in reducing vector densities and malaria transmission (Steinhardt *et al*., 2013). However, in the European Region as countries progress towards elimination, IRS was targeted to smaller populations and thus the population at risk that was protected reached 65%(WHO, 2012c). A 4.5 year study using 3 different insecticides was carried out in a high transmission area of Uganda. IRS was associated with a reduction in malaria morbidity, with the greatest benefits attained with a switch to a carbamate class of insecticide(Kigozi *et al*., 2012). In a study conducted in the Gambia, the carbamate, Bendiocarb was also found to be a viable alternative in settings were resistance has been developed to DDT and pyrethroids (Tangena *et al*., 2013).

In Namibia, DDT, which is an organochloride, is one of the oldest interventions. This intervention has been in use since 1965(Roll Back Malaria, 2010). DDT is suitable for use in traditional huts whilst the pyrethoroid, Deltamethrin, is currently being used as an insecticide in modern housing structures (Mouatcho *et al*., 2009). Both DDT and deltamethrin is sprayed on the walls and surfaces in dwellings (Sadasivaiah, Tozan, & Breman, 2007) to kill mosquitoes as they rest on the sprayed surfaces. There has been a decreasing trend in the use of DDT in the 1980s in favour of pyrethroids since there were some safety and environmental concerns with the use of DDT. However, South Africa has reverted back to the use of DDT following resistance to Deltamethrin (Najera & Zaim, 2001). Similarly in Madagascar, case numbers were significantly reduced with the use of DDT between 1955-1970 (the years of the the Global Malaria Eradication campaign during which *A.funestus* disappeared from many areas). Following the abandonement of this campaing, case numbers had increased in 1986, and was only reduced when DDT was re-introduced for vector control (Ratovonjato *et al*., 2014). DDT has had great impacts in reducing or eliminating malaria as a public health problem in Europe, Latin America,

Russia and some parts of Asia (Tanser, Pluess, Lengeler, & Sharp, 2007). As of 2012, pyrethroids were the most widely used IRS (18/24 countries), whilst 3 countries used Carbamates and 3 used DDT (WHO, 2012c). However, WHO still recommends IRS with DDT in all epidemiologic settings (Sadasivaiah *et al*., 2007).

2.5.2 Insecticide Treated Bed Nets

Distribution of Insecticide Treated Nets (ITN's) to all at risk individuals is being used as an intervention to reduce the burden of malaria. Insecticide Treated Bed Nets (ITNs) is a dual action vector control intervention, which offers a great degree of personal protection by acting as a physical barrier between mosquitoes and people. In addition, the pyrethroid-insecticide on the n*et al*so repels and kills mosquitoes resting on the nets (Sutcliffe & Yin, 2014; Tanser *et al*., 2007).

WHO has also recommended that universal coverage with Long Lasting Insecticidal Nets (LLIN) be achieved and maintained (Nyunt *et al*., 2014). Universal net coverage is defined as a person to net ratio of 2:1 (Kilian, Boulay, Koenker, & Lynch, 2010). LLINs are assumed to maintain efficacy for 3 years, with a study in Benin suggesting LLIN service every 2 years (Gnanguenon, Azondekon, Oke-Agbo, Beach, & Akogbeto, 2014). Rwanda was one of the 1st countries in Africa to achieve universal net coverage. Also, in Rwanda, LLIN net ownership went up from 15% - 82%, achieving a 50% reduction in all case mortalities in children under the age of 5. However, there are numerous studies that have found a large discrepancy between net ownership and use. In Kenya, the difference between ownership and use was 95% vs 59% respectively (Nyunt *et al*., 2014). Although LLIN remains effective, their use by people remains unsatisfactory low due to factors such as lack of nets; lack of advocacy; perceived low malaria in the dry season (Gobena, Berhane, & Worku, 2012)

Furthermore, Rwanda's LLIN distribution program constantly maintains the distribution and replacement of LLINs, in order to guard against the loss of impact (Hakizimana *et al*., 2014). If net replacement is not properly timed, a delay in replacement leads to operational failure whilst premature net replacement is not cost-effective (Gnanguenon *et al*., 2014).

2.5.3 Larviciding

In Gasha Barka and Debub, IRS and ITN have been used as front-line interventions, with larviciding as a supplement (Berhane *et al*., 2015). Larviciding is a technique where the chemical Temephos is applied to water bodies to control anopheline population (MoHSS, 2005). Studies have also been conducted to assess the use of microbes as potential larvicidal agents. In the Kenyan highlands a *Bacillus* based larvicide was applied weekly to vector breeding sites for 19 months and were estimated to reduce the risk of infection by 56% (WHO, 2012a).

Larviciding is appropriate in both urban and peri-urban settings (De Silva & Marshall, 2012). However, in rural settings larviciding is unlikely to be cost effective as there are several water sources. WHO thus recommends that larviciding be used in settings with few waterbodies that can be identified, mapped and treated with ease (WHO, 2012c).

In Namibia, larviciding is carried out during the dry winter months. The larviciding sites are identified by entomological sampling in endemic areas (MoHSS-NVDCP, 2010). Larviciding is ideally carried out before the rainy season, when vector habitats are few and localized (MoHSS, 2005).

2.6 Parasite Control Interventions: Treatment

Presumptive malaria diagnosis based on the symptoms instead of a positive blood slide lead to over diagnosis and unnecessary consumption of costly drugs(Bell & Winstanley, 2004; Bloland, 2001). Antimalarial drugs can be used for treatment or as a prophylaxis(malERA Consultative Group on Drugs, 2011), with gains in reducing malaria morbidity and mortality(Bloland, 2001). Various antimalarials are classified depending on the type of parasite present as well as the stage of infection in the life cycle stage. Drugs can be sporontocidal, schizontocidal, gametocidal or anti-relapse, this depends on them interrupting the sporozoite, schizont, gametocyte or hypnozoite stages respectively. Common drugs include Quinine, amodaquine, primaquine (Bruce-Chwatt, 1962). WHO recommends that the novel and potent class of Artemisnin should be used in combination with partner drugs, a term known as Artemisnin Combination Therapy (ACT) as a first line of treatment for *Plasmodium falciparum* (the majority of infections in Namibia are due to *P.falciparum*)(Plowe, 2007). Resistance to antimalarial has also emerged as a challenge to control efforts (Bloland, 2001).

2.7 Malaria surveillance as an Intervention tool

Surveillance is an important intervention in sustaining the progress made by control programmes as it has the ability to identify outbreaks and prevent resurgence (Kobayashi *et al*., 2012). For cases clustering in a geographic area, geographic based active surveillance will be an effective strategy in preventing further transmission. During Active Case Detection (ACD), fever screening or parasitological screening is done at a community or household level, or in a targeted

population that is considered to be at high risk (Smith Gueye *et al*., 2013). This strategy may aid in detecting both symptomatic and asymptomatic infections. Hence, both parasitological and entomological surveillance are crucial to identifying parasites and vectors respectively. They are also crucial in detecting resistance, thus ensuring that malaria elimination programs are informed.

2.8 Malaria Risk factors

Regardless of the transmission intensity, it is important to determine and understand the individual and household risk factors that would allow for the better targeting of interventions (Winskill, Rowland, Mtove, Malima, & Kirby, 2011). Equally, an assessment of the potential impact of global climate change on the incidence of malaria gives support for an increased risk to malaria due to the expansion of the areas suitable for malaria transmission. Thus, factors such as the local environmental conditions, socio-economic developments and malaria control programs need not to be disregarded in the assessment of the risk and potential to significantly increase the number of years of healthy life lost (Martens, Niessen, Rotmans, & McMichael, 1995).

Risk factors for malaria can be classified into 3 major categories, namely, (1) Biological; (2) Environmental factors; (3) Human-related factors. Some of the risk factors are sometimes classified into more than 1 category. For this reason, the risk factors will not be discussed under any particular sub-heading. Risk factors influence the parasite density (Kimbi, 2012); longevity of infective *Anopheles* mosquitoes; human-vector contact and subsequently the number of human infections (Ndoen, Wild, Dale, Sipe, & Dale, 2012). Knowledge of the risk factors play an important role in efforts of trying to reduce malaria morbidity and mortality or better in realizing the goals of eliminating malaria

Breeding sites: The proximity of humans settlements to vectors have previously been used in studies in an attempt to identify individual and household level risk factors associated with malaria transmission(Chirebvu, Chimbari, & Ngwenya, 2014). Also, *Anopheles* species larvae was sampled from breeding sites in a study by Chirebvu, Chimbari, & Ngwenya (2014) on the assessment of risk factors associated with malaria transmission in northern Botswana. These authors found that the presence of mosquito breeding sites in close proximity to human settlements exacerbated the problem of mosquito biting, which increases the risk of malaria as a result of the accumulation and proliferation of breeding sites, following a rainy season. Furthermore, breeding sites may also a form due to a lack of proper municipal waste collection or water drainage systems (Rosas-Aguirre *et al*., 2015), whilst flooding may actually result in epidemics (Chirebvu *et al*., 2014; Ding *et al*., 2014).

Insecticide resistance: Whilst, there has been considerable reduction in malaria morbidity, experts fear that decreased susceptibility of mosquitoes to ITN and IRS may compromise the effectiveness of malaria control efforts(Kleinschmidt *et al*., 2015; Mnzava *et al*., 2015). Areas such as KwaZulu-Natal have driven *Anopheles funestus* to near extinction when DDT was in use as an insecticide. The switch to pyrethroid was accompanied by an increase in case numbers because *A.funestus* developed resistances against pyrethroids. The reversion to DDT saw the reduction of case numbers (Kleinschmidt *et al*., 2015). In Bioko Island, Equatorial Guinea, deltamethrin resistance was seen in *A. gambiae* species that harboured a knockdown resistance (*kdr)* mutation associated with pyrethroid resistance. It is also worth noting, that there is minimal evidence on the impact of insecticide resistance due to confounding. Henceforth, resistance monitoring is of the utmost importance in IRS based control programmes to avoid program failure (Sharp, Ridl, Govender, Kuklinski, & Kleinschmidt, 2007).

Immunity: Malaria clinical immunity has an effect on the control of parasites. Specifically, maintaining parasitemia below the critical threshold level, thus in effect, preventing the induction of malaria symptoms (Dodoo *et al*., 2002). Immunity is determined by the transmission intensity or malaria endemicity in an area. Immunity has also been associated with the age and exposure as a measure of blood stage infections, rather than EIR. In a model to measure, the protective immunity against severe disease symptoms, Gupta, Snow, Donnelly, Marsh, & Newbold (1999) found that, a single infection is sufficient to confer strong protection against severe malaria and at least 3 infections are necessary in low transmission. These authors also found that infants in a high transmission area have high immunity due to maternal antibodies which are gradually lost by the 6 months, with a more acute decline between 0.5 – 1 year.

Age and Gender: In a study carried out in rural villages of Tanzania, children between the ages of 5–13 years were found to be at an increased risk of malaria, with the odds being less for females when compared to males. The same study also suggested that declines in malaria transmission have the potential to shift the age categories to older children (Winskill *et al*., 2011). Similar trends, where also found in Cambodia, whereby *Plasmodium spp* (with the exceptions of *P.f and Pv)* tended to be more prevalent amongst the 5-14 age group, with no difference between gender though(Sluydts *et al*., 2014). Another study has found a positive correlation between age and disease severity, particularly, in people aged ≤40(Schwartz, Sadetzki, Murad, & Raveh, 2001).

Occupation and Socio-Economic: Malaria is also referred to as the disease of the poor or a disease of poverty. At the same time measuring the socio-economic status of an individual or household can be challenging. Researchers have often relied on household ownership of assets rather than collecting income or expenditure data. Levels of education, occupation and residence,

being rural or urban have also been used as proxy for Socio-Economic Status (SES). Occupational attributes act as a risk factor in some instances-for example agricultural workers and the people from areas to which they migrate to are also at an increased risk of malaria(Worrall, Basu, & Hanson, 2003). Additionally, socio-economic factors such as construction of material walls, roof and floor of houses, main source of drinking water and the toilet facilities. Overall, the risk of malaria is generally higher for households in the lower socio-economic bracket (Ayele, Zewotir, & Mwambi, 2012). Contrastingly, Somi *et al*., (2008) found no association between malaria and SES.

Migration/Travel: Migrant plantation workers are at an increased risk of malaria. Noteworthy, in a population of migrant workers in Myanmar there were other challenges such as universal net coverage and utilization of Insecticide Treated Bed Nets (ITNs) (Nyunt *et al*., 2014). Human migration has been associated with border malaria within Southeast Asia. In another study in Myanmar by Li *et al*., (2013), the authors found that people that have reported travel are most likely to have had previous infections. When malaria infections are acquired in an endemic area and diagnosed in a non-endemic area it is termed as "imported malaria or importation"(Fonseca, Dias, Baptista, & Torgal, 2014). It was also found that imported malaria were more common amongst the working class male that have travelled for business purposes (Li *et al*., 2013). In Europe, 80% of infections are caused by *Pf* with 90% being imported from sub-Saharan Africa by people visiting their friends and family (Behrens, Neave, & Jones, 2015; Broderick *et al*., 2015).

Health Access: Prompt access to effective antimalarials is important in the fight against malaria. Global and national health policies need to be continually adopted with the changing dynamics of the malaria disease. Policies may lead to malaria episodes not being treated properly. The

PROMPT ACCESS programme in Tanzania strategies involved seeking to improve care seeking at community level and to strengthen the quality of care at health facilities (Hetzel *et al*., 2007). Poor access to public health facilities is an impediment to effective treatment (World Health Organization, 2005). A practical example seen in Tanzania, were the quickest and first response to malaria was self-treatment from home with drugs from a private drug retail store. This practice had led to ineffective treatment and increased drug resistance due to sub-standard drug regimens and poor prescribing practices. Hamel, Odhacha, Roberts, & Deming (2001) found that carers and health workers were both major providers of treatment to febrile children, with 43% of the children receiving antimalarials at home; and 47% receiving it at a health facility. Carers provided prompt treatment usually on the first day of symptoms, with children that have gone to health facility waiting for at least 2 days before treatment is sought. The carers administered the 1st line of treatment at the time, but oftenly administered it in sub-therapeutic or potential toxic dose levels (Hamel *et al*., 2001). There are several other factors that acted as obstacles to health access-these are**:** cost, absence of trusted medical professionals, unavailability of diagnostic instruments, long waiting time, and distance to health facility (Hetzel *et al*., 2007).

2.9 Detection and Diagnosis of Malaria parasitaemia

Malaria diagnosis is important in both control and elimination settings. Despite its importance, malaria diagnosis rates tend to be low, particularly in African countries (Lourenço, Kandula, Haidula, Ward, & Cohen, 2014). Accurate, rapid and differential detection of malaria parasites is a pre-requisite to the institution of proper therapy. Centre for Disease Control (CDC) also recommends that differential diagnosis should be considered for all febrile patients that have travelled to endemic areas or patients that have fevers of unknown origin (Mangold *et al*., 2005); In Namibia, national guidelines recommend that all febrile cases should be parasitological

confirmed, in order to identify symptomatic and asymptomatic parasite carriers and subsequently eliminate the foci of transmission (Ministry of Health and Social Services, 2010). It is important to use accurate diagnostic tools in the identification of *Plasmodium* infections during field or community based surveys. This diagnostics includes the conventional Microscopy; Rapid Diagnostic Tests, which are gaining popularity. In comparison to microscopy and RDTS, molecular diagnosis using various Polymerase Chain Reaction (PCR) techniques has been used as the reference or gold standard in the detection of PCR (Fançony, Sebastião, Pires, Gamboa, & Nery, 2013).

2.9.1 Microscopy

Light microscopy (LM) is the gold standard for *Plasmodium* detection, this is a procedure that relies on giemsa staining and viewing of thin blood slides for parasite detection. This procedure is laborious i.e. preparation, reading and interpreting of slides is time-consuming. It has been shown in previous studies that regardless of the microscopists experience, errors and misdiagnosis are inevitable particularly in the case of low parasitaemia and mixed infections (Mangold *et al*., 2005). Light microscopy with an experience person can however be used to detect parasites even when RDT results were negative and *vice versa* (Bell, 2002). Microscopy can be a highly useful tool for diagnosis. Paradoxically, with expertise, it can detect up to 50 parasites per µl (0.001% parasitemia) and identify plasmodia in 98% of the cases (Portero *et al*., 2010).

In order to enhance detection of parasites, fluorescent dyes with an affinity for nucleic acids were used. There two commonly used dyes are Acridine Orange (AO) and benzothiocarboxypurine (BCP), both are excited at 490 nm to exhibit an apple green and yellow colour. In addition, Rhodamine-123 is another dye that detects viable parasites as it relies on a functional parasite

membrane for uptake. AO is faced with the drawback of non-specificity as it binds both cellular and parasite nucleic acid. AO has a varying and wide range of specificity (41 -93% at 0.002% parasitemia) and over 93% specificity for Pf. Specificity of AO was also found to be below 53% in non-Pf species (Moody, 2002).

2.9.2 Serological: Immunochromatographic test (ICT)

Serological approaches detect and quantify either antibodies or antigens in samples. Thus Enzyme-linked Immunosorbent Assay (ELISA) principle is founded on the immunology principle of antigen-antibody specific binding. The 3 types of ELISA vary mainly in the manner in which the antigen is captured, the use of one (primary) or two (both primary and secondary) antibodies. **Direct ELISA** makes use of a single enzyme conjugated primary antibody; **Indirect ELISA** involves the capturing of an antigen by a primary antibody, followed by a secondary antibody conjugated to an enzyme for colourimetric detection of the antigen in the presence of the substrate (Gan & Patel, 2013) (Figure 9). Finally, **Capture Assay** or **Sandwhich ELISA** is the principle by which the malaria RDT operates and is described in the subsequent paragraphs.

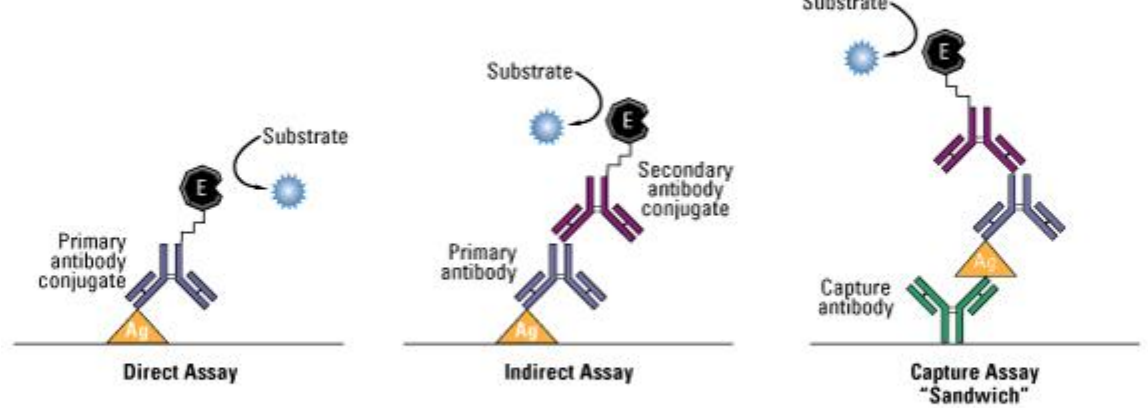


Figure 9: Different types of ELISA and the enzyme-substrate reaction to produce a visible colour change. Source: http://www.lifetechnologies.com Accessed: August, 24, 2015.

Antibody detection is often used as measure of transmission intensity or past exposures to a disease (Baidjoe *et al*., 2013) and can thus not be relied upon for malaria diagnosis in a clinical setting (Walker *et al*., 2014). This measure is attained through indirect ELISA for Immunoglubulin G (IgG) that recognizes Apical Membrane Antigen -I (AMA-I) and Merozoite Surface Protein-I-$_{19 \text{ or } 42}$ (MSP-I_{19}) or (MSP-$I_{42)}$ (Williams *et al*., 2009).

Henceforth, immunochromatographic cassettes using conjugated monoclonal antibodies immobilized on a nitrocellulose strip are used to detect malaria parasitic antigens, as an alternative to microscopy. The cassette or dipstick is comprised of two sets of Antibodies (Abs). These are the capture and detection Antibodies; the capture Abs is immobilized, whilst the detection Ab is conjugated to an indicator. When parasitized blood is placed in a well with a lysing agent, it leads to the release of the parasites antigen after red blood cell rapturing. The released parasite protein/antigen passes through the tube by capillary action and by the aid of the added buffer. The antigens are then captured by the immobilized labelled antibody to form a complex. The antibody-indicator complex produces a visible line(Murray, Gasser, Magill, & Miller, 2008). It is the accumulation of microscopic dye particles on the thin band that produces the visible line to confirm the presence of parasites (Figure 10, A-C).

RDT cassettes are being widely used since they allow for rapid and inexpensive diagnosis (Fançony *et al*., 2013). Williams *et al*. (2009) have also cited ease of use and robustness as attributes for the popularity of RDTs.

RDTs sensitivity and specificity have been evaluated in different settings of varying endemicity and predominant species(Yan *et al*., 2013). Rapid malaria diagnostic test (MRDT) needs to have

certain attributes, these attributes differ according to the regional malaria epidemiology. For instance, in Africa, for an RDT to aid in reducing mortality through proper diagnosis and subsequent therapeutic care, the RDT must be able to detect the predominant *Plasmodium falciparum* parasites with the test results being rapidly available(Murray *et al*., 2008). Antibodies are used to target two common proteins, namely, the Histidine Rich Protein-2 (pHRP-2) and the parasite Lactose dehydrogenase (pLDH) or Aldolase. The former being specific for *P.falciparum* and the latter being for all *Plasmodium* species (Aydin-schmidt *et al*., 2013; Yan *et al*., 2013). In Namibia, the Carestart HRP-2/pLDH (Pf/PAN) RDT is being used as a Point of Care (POC) diagnostic test. In China, these devices were found to have a specificity and sensitivity similar to that of Microscopy, with the added advantage of its use in the diagnosis of local and imported malaria across the border with ease (Xiaodong *et al*., 2013).

RDTs were found to have a sensitivity and specifity of slightly above 80% for *Plasmodium* species and approximately 70% for *Plasmodium falciparum* respectively. The sensitivity of the ICTs increases with higher parasitemia (Portero *et al*., 2010). WHO reports that RDTs reach 100% sensitivity when parasitemia is above a 100 parasites per microliter (Mangold *et al*., 2005),. The sensitivity can also be reduced to 60% below a 100 parasites per microliter (Xiaodong *et al*., 2013).

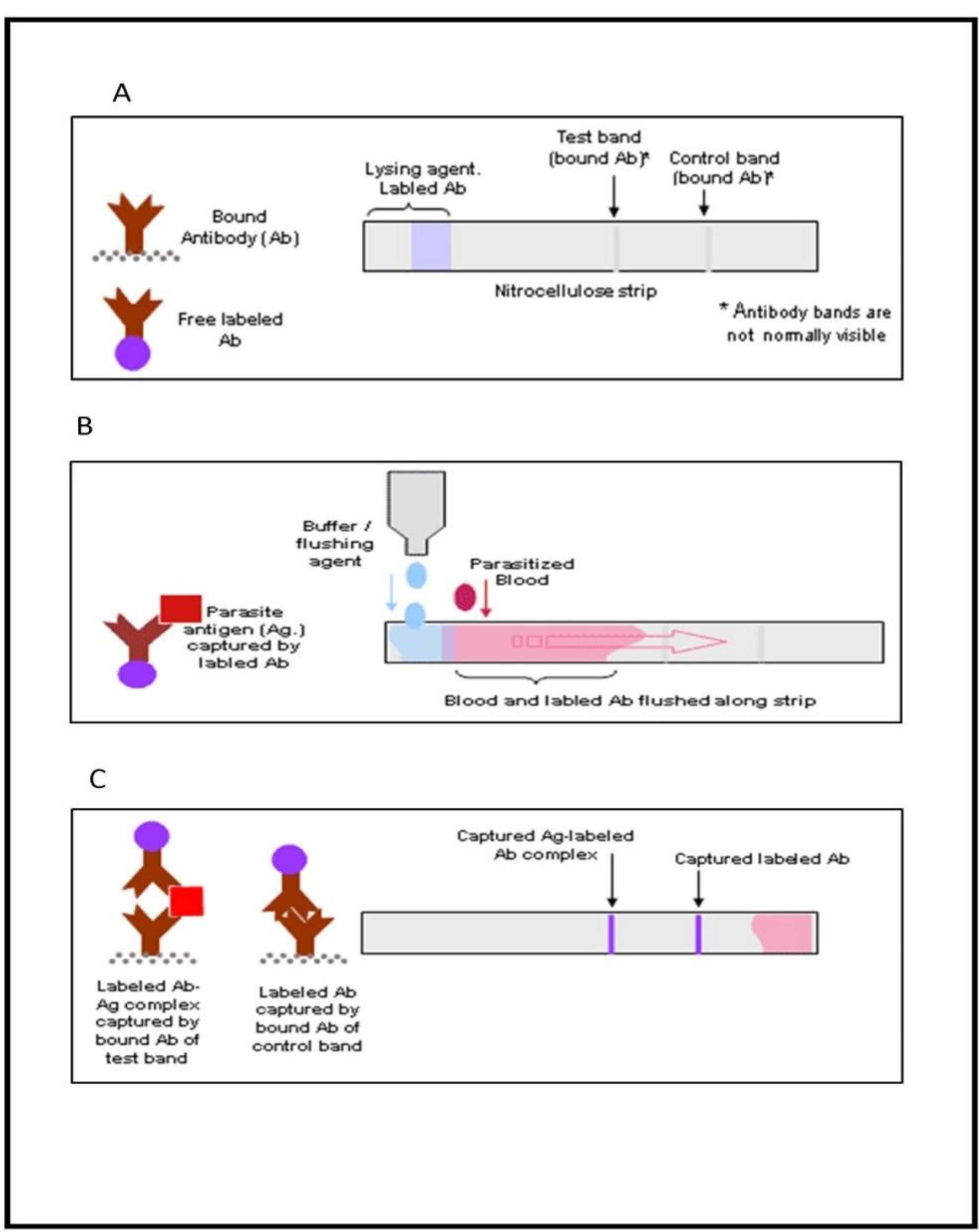


Figure 10: A-C: Mechanism of Action of Immunochromatographic test (ICT) or RDT in detecting *Plasmodium* spp. Schematic adopted from Malaria Rapid Diagnostic Tests (2015).

WHO recommended that all cases should be parasitological confirmed in order to initiate treatment (Ghayour Najafabadi *et al*., 2014; Mubi *et al*., 2013). Parasite antigen targeting RDTs were introduced in 2010 in Namibia, as a clinical diagnostic test (Ministry of Health and Social Services, 2010). Sub-patent infections or low parasitemia (Mosha *et al*., 2013) and the prozone effect leads to false negative results in RDTs, whilst persistent antigenicity from previous infections leads to false positive results (Fançony *et al*., 2013). HRP-2 is associated with RDT false positive rates when compared to pLDH which clears out faster after anti-malarial treatment(Xiaodong *et al*., 2013).

2.9.3 Molecular detection and identification

In recent years, molecular detection of *Plasmodium* with higher sensitivity is gaining importance due to its capacity for accurate detection when compared to either microscopy or immunochromatographic methods of detection(Mangold *et al*., 2005). Methods such as the Polymerase Chain Reaction (PCR) are capable of detecting few parasites/µl (low parasite densities) in blood and will become important for elimination programmes (Rosas-Aguirre *et al*., 2013). Molecular tools are highly accurate and sensitive in detecting low parasite densities and identifying the species(Li *et al*., 2014), but a point of care diagnostic with the same accuracy does not exist in a health setting despite the limitations of PCR for routine clinical usage.

The methods, principle and application of PCR is described in detail by Joshi & Deshpande, (2010). In summary, the overall aim of PCR is to amplify Deoxyribonucleic Acid (DNA). Double stranded DNA (dsDNA) is denatured into two separate single stranded DNA (ssDNA) strands, where one will serve as a template in the amplification reaction or synthesis of new dsDNA. A primer (short DNA fragment) anneals to the complementary template strand, onto

which *Taq* DNA polymerase (isolated from: *Thermus aquaticus*)(Roayaei & Galehdari, 2008) attaches any of the four nucleotides for the synthesis of a new strand. The three major steps of **Denaturation, Annealing and Extension** in DNA amplification for conventional PCR are carried out at different temperatures. The amplification products are visualized by gel electrophoresis for the presence or absence of bands on a gel (Joshi & Deshpande, 2010). On the other hand, LAMP occurs at isothermal temperature and uses *Bst* Polymerase with high strand displacement activity, isolated from *Bacillus staerothermophilus* (Hasan, Al, & Arjunan, 2014).

To date, several methods for the detection of malaria have been developed and have been evaluated in field settings, including the reported molecular techniques of nested PCR (P. Li *et al*., 2014); multiplex PCR(Harris *et al*., 2010) and the novel Loop-mediated Amplification-PCR or LAMP(FIND, Eiken Chemicial, & Hospital For Tropical Diseases, 2012; Mohon *et al*., 2014; Paris *et al*., 2007). Tests were carried out with different types of blood samples, mainly whole blood obtained by venipuncture (Saiwichai, Maneepak, Songprakhon, Harnyuttanakorn, & Nithiuthai, 2009)or Dry Blood Spot (DBS) filter papers (Hue, Phong, Chan, Hoan, & Thuy, 2011) as sources of DNA. Other contributors to molecular detection of *Plasmodium* also evaluate urine and saliva as source of parasite DNA(Estévez *et al*., 2011; Ghayour Najafabadi *et al*., 2014).

Nested PCR (nPCR) is completed in two steps, a genus-specific (primary round) and species-specific (secondary round). The former step targets the mitochondrial DNA (mtDNA) (figure 13) whilst the latter identifies the species-specific *Plasmodium* DNA by amplifying the 18S ribosomal RNA (rRNA) region of the parasite (Mekonnen, Aseffa, Medhin, Berhe, & Velavan, 2014). In the nPCR protocol by Snounou & Singh (2002) and Li *et al*., (2014), the

primary round PCR targets the small subunit RNA (ss RNA) instead of the mtDNA reported previously.

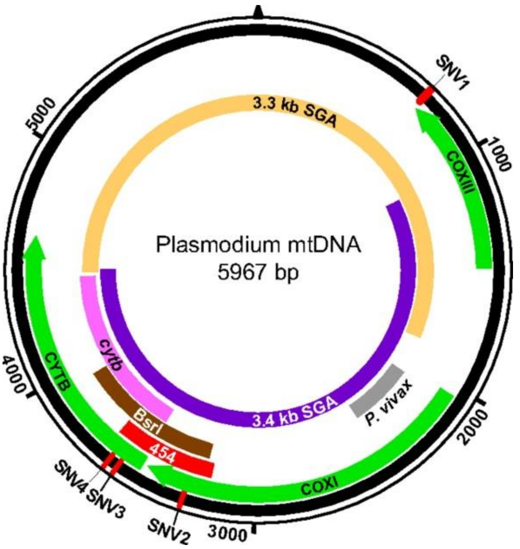


Figure 11: Schematic illustration of *Plasmodium* mitochondrial genome adopted from Sundararaman et al.,(2013)

PCR is more sensitive when DNA is obtained from whole blood rather than Dry Blood Spots (DBS) by finger prick. DBS however have practical applications for resource limited settings, as it can be stored at room temperature. Furthermore, DBS do not require large amounts of blood and the method by which blood is obtained is less invasive when compared to venipuncture for whole blood (Strøm, Moyo, Fataki, Langeland, & Blomberg, 2014). Haanshuus *et al*., (2013) compared the sensitivity of using two different amplification targets, in a single-amplification PCR. The first amplification target is the 18S ssRNA and the second being the mitochondrial DNA target. These authors have found 97% sensitivity in a single-amplification PCR targeting mtDNA, higher than that of 18S ssRNA and the reference nPCR described by the genus and

species specific nPCR by Singh *et al*., (1999). The single-amplification PCR can also be used for species identification by introducing an additional sequencing step (Haanshuus *et al*., 2013).

Like Microscopy, PCR is also labour intensive, with a long turnaround time; (Farcas, Zhong, Mazzulli, & Kain, 2004). The high sensitivity of PCR also makes it prone to contamination due to pre and post-test sample handling(Farcas *et al*., 2004) where even trace amounts of DNA can produce misleading results or false positives. Additional limitations includes, non-specific annealing of PCR to sequences that are similar, but not necessarily identical to the target DNA (Lilit & Avashia, 2013). Real-Time quantitative PCR (RT-PCR) mitigates against some of these limitations. This type of PCR detects and quantifies PCR amplicons after each cycle. The real time quantification is achieved by the use of DNA binding dyes such as SYBR, hybridization probes or fluorescent probes (Farcas *et al*., 2004).

Loop-mediated isothermal Amplification (LAMP) is a novel nucleic acid based detection technique that needs to be further optimized and validated as a malaria diagnostic tool. The Eiken PAN or Pf LAMP tubes are pre-packaged with vacuum dried reagents that are stable at ambient temperature (Polley et al., 2013). PCR results are influenced by primer mismatch, annealing temperature and number of cycles (Sipos, Szekely, Palatinszky, Revesz, & Nikolausz, 2007). Unlike conventional PCR which requires cycling through different reaction conditions (temperature and time), the LAMP reaction is performed at an isothermal temperature of 65°C Since the LAMP technique occurs at isothermal temperature, non-specific binding does not occur (Lau, et al., 2011) and the LAMP reaction does not need expensive thermocycler and can be performed with a water bath or heating block. *Bst* polymerase is robust and have strand displacement activity (Mohon *et al*., 2014). *Bst* polymerase also tolerates PCR inhibitors (Mori

& Notomi, 2009), thus further conferring it better performance over PCR. LAMP produces highly specific results within an hour, making results readily available (i.e. fast turnaround time).

The LAMP reaction employs the use of 2 outer primers (F3, B3) and 2 inner primers (FIP, BIP). These four primers, recognizes 6 separate regions of the target DNA, conferring the LAMP method its high specificity. Visualization of amplicons by gel electrophoresis is not necessary, as positive results are either detected by turbidity measure or by fluorescence. Calcein is used for fluorescence and manganese ions bound to pyrophosphate to form the insoluble Magnesium pyrophosphate (a byproduct of the LAMP reaction) to denote successful amplification (Mori & Notomi, 2009). One particular shortcoming with LAMP is that it is highly prone to contamination. Cross contamination occurs as tubes are opened and closed, which may result in false positive results. Hence separate rooms are used for LAMP setup and analysis.

2.10 Conclusion to Literature Review

There has been a global declining trend of malaria infections. However, the goal of elimination remains far-fetched in the absence of country specific or country tailored elimination programs. This is because malaria disease epidemiology is different across different endemicity settings. Unknown risk factors which allows for the continuous human-infectious vector contact and on-going transmission is one of the challenges that countries aiming for elimination need to consider. Accurate and sensitive molecular diagnostic techniques become increasingly important as the routinely used point of care diagnostics fail to detect low density infections as well as clusters or hotspots of infection. Identification of risk factors and accurate diagnosis are great tools for community-oriented interventions as well as the clinical management of all infections.

CHAPTER 3: MATERIALS AND METHODS

3.1. Research design

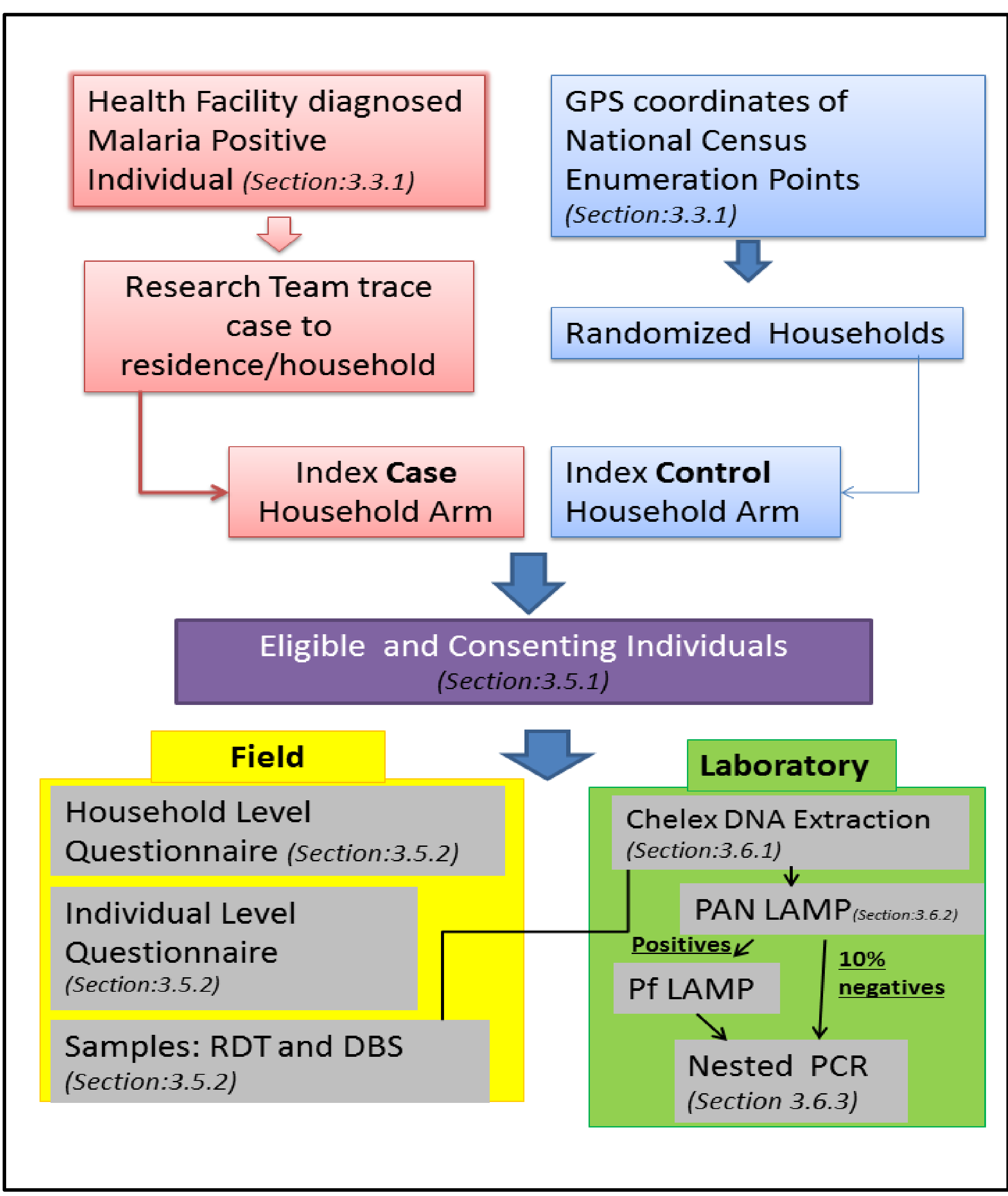


Figure 12: Process flow chart of the research design.

Figure 12 is a process flow chart, indicating the classification of households as a case or control; structure or nature of the interview conducted; the collection of samples; and the downstream molecular assays carried out on field collected blood samples.

3.2. Study Area and Population

The study was carried out in the Omusati region (figure 13), located in northern Namibia. The Omusati region is located in a malaria endemic zone, sharing borders with other malarious regions of Oshana, Kunene, and Ohangwena region (Ministry of Health and Social Services, 2009). Politically, the Omusati region is comprised of 12 constituencies, with major towns, namely, Outapi (Regional capital), Okahao, and Ruacana. The town is administratively divided into the Outapi, Oshikuku, Tsandi and Okahao Health Districts, totaling 6 health centres; 39 clinics and 94 outreach points (Omusati Regional Council, 2010).

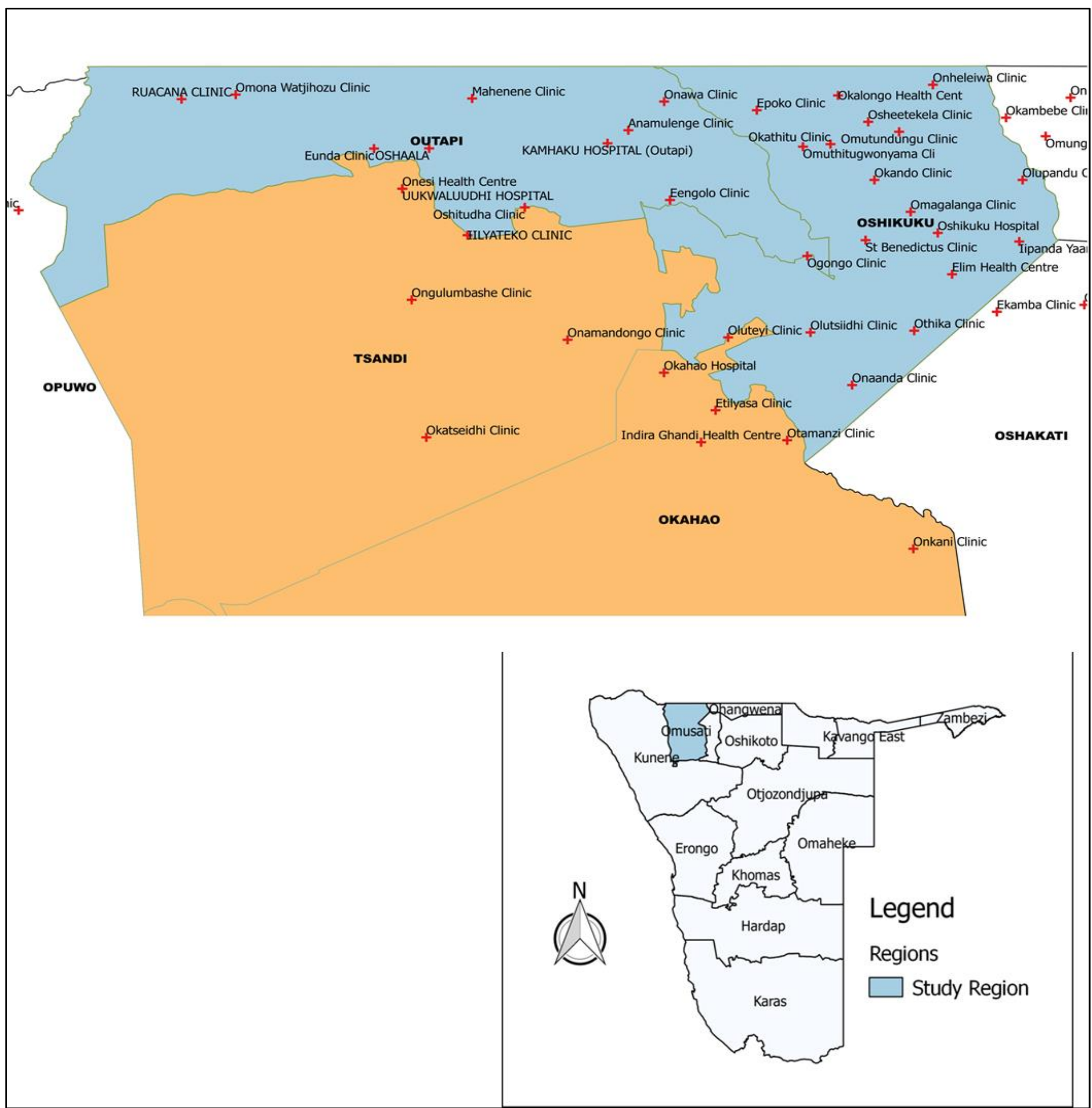


Figure 13: Map of Namibia showing Omusati Region and the study health districts and health facilities (red crosses). Erastus Haindongo (2015)

In addition, the region has a catchment population of 277 713, the Outapi and Oshikuku health districts were the only two districts that were included in the study. These two districts are the largest by population size, making up ~70.5% of the catchment population. The population size

of Oshikuku and Outapi Health Districts are 114,318 and 81,613 respectively (Circular Omusati Regional Office , 2014). Hence, the study population comprised of all individuals leaving in households within the confines of the two study districts, for interview and blood sample provision.

Ninety-seven percent of people in Omusati live in rural areas, with an average household size of 5.2 people and population density of 9.1 people per km^2(Namibia Statistics Agency, 2011). The region is comprised of 46,698 households; these households are traditionally known as homesteads amongst the Owambo people. Homesteads consist of a fenced cluster of huts and open areas (figure 14). Each hut or structure serves its own function- these functions range from sleeping, cooking, receiving guest and storage(Namibia Statistics Agency, 2011; Wilhelm, 2012).

Figure 14: Typical Oshiwambo homestead. Source: www.serasphere.net

These districts were selected because their populations are representative i.e. being both peri-urban and rural. Both the study health districts also shares their northern borders with the moderate-high malaria transmission Cunene Provinces of Southern Angola, allowing distance

from the border to be considered as a factor in transmission of malaria. Both health districts are at the risk of flooding, which affects the malaria transmission cycle, following the cessation or recession of flood waters. (Office of the Prime Minister: Directorate of Emergency Management).

3.3. Sample size and sampling

An unmatched case-control study was conducted. Assuming that case-controls have a 10% and 20% prevalence of a particular exposure, yields an Odds ratio of 2.25 at 95 Confidence Interval. For a 1:2 ratio of cases and controls, 158 cases and 316 controls would be required to detect the difference with 80% power.

A mixed-method sampling strategy was employed. The case sample was comprised of the entire population of all traceable incident cases that had a test positive RDT/microscopy from all health facilities (i.e. clinics, health centres and district hospitals) in the Oshikuku and Outapi Health Districts of the Omusati Region. On the other hand, National Census Enumeration area point (GPS) data was randomized and conveniently visited (i.e. pre-selected points in the vicinity of visited case households were investigated at the same time).

3.3.1 Inclusion and exclusion Criteria

In this study, a case index household was classified as such, if a resident person tests malaria positive by RDT or Microscopy at a health facility in any of the two study districts in the region. The cases were reported from the 23 clinics, 4 health centers and the 2 district hospitals in the study districts. This method or tool of investigation that relies on health facility based cases to recruit all the other members of the same household as a case, regardless of symptoms is

commonly known as Reactive Case Detection (RACD) (Sanders, Gueye, Phillips, & Gosling, 2012).

The comparison, control index household on the other hand, was one of the many randomly selected points from the National Census Enumeration Areas (EA's) in the study region. These enumeration areas are comprised of geographic co-ordinates representing homesteads. There was no knowledge of the malaria situation of these households prior to investigation. All persons, household members and guests that had spent the night prior to the study visit in a given case or control household were eligible for inclusion in the study.

3.4 Ethical Clearance

The project was part of a broader study which has been issued a research permit by Ministry of Health and Social Service (MoHSS) Bio-medical Research Ethics Committee, London School of Hygiene and Tropical Medicine (LSHTM) Ethics Committee and the University of California San Francisco (UCSF) Institutional Review Board. Furthermore, UNAM's Research and Publications Office (RPO) accepted the permits in 2012.

The research was sub-divided into a field and laboratory component. The procedures in each component are sequentially outlined in separate sub-themes below.

3.5. Field Procedures

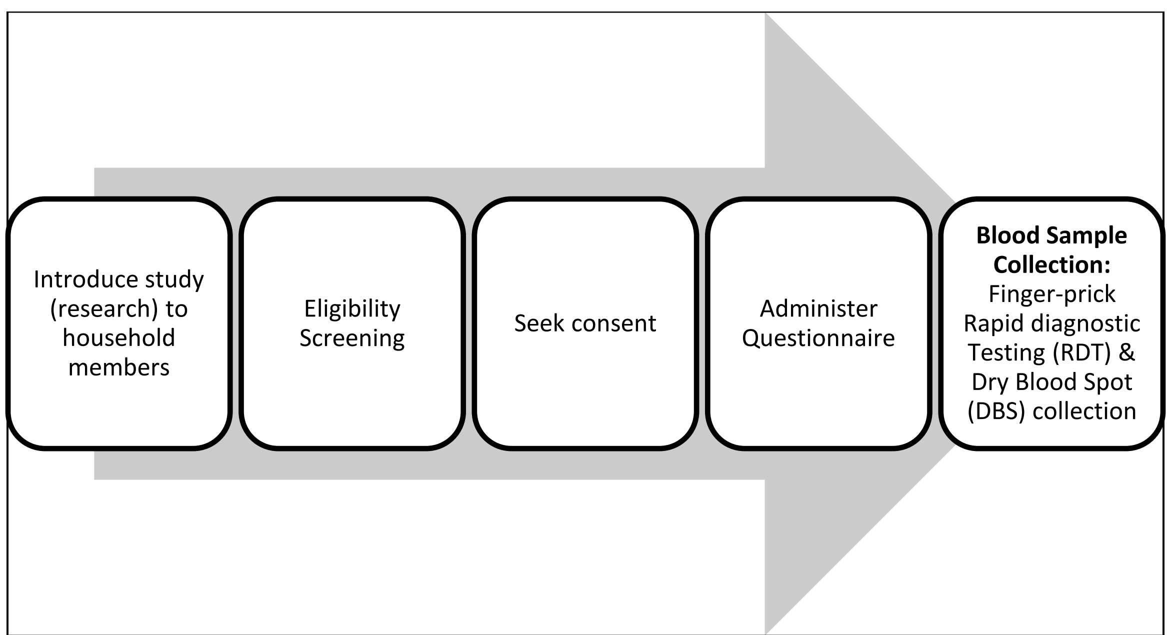


Figure 15: Diagram showing a process flow chart of the field procedure

A health-facility and community-based study was carried out in the Omusati Region between January–May 2014. Figure 15, shows the manner in which the research was conducted in the field. First, the study was introduced to all household members of all ages and sexes before recruitment. People were informed that participation is voluntary and should they decide to participate, they will be required to give consent by signing the study consent forms. Participants were also informed that an RDT will be performed and Dried Blood Spot (DBS) will be collected; required to respond to questions related to malaria risk as well as an inspection of their sleeping structures.

3.5.1. Eligibility and Consent

The names of all members that had spent the night prior to visitation by the field team were recorded. Afterwards, the researcher read the consent form to household members. There was one consent form for every individual, people that are legally defined as adults (people above the age of 18) signed their own consent forms, whereas an adult was required to provide signatory consent for children below the age of 18 (See Appendix 1-2, for typical English and Oshiwambo consent forms).

3.5.2 Questionnaire: Determination of Risk factors of transmission

A questionnaire that was pre-programmed on a Nexus (Android platform) tablet was used to capture the information from respondents. The questionnaire was adopted from the National Malaria Indicator Survey 2009 (NMIS 2009) and modified by the MRC (UNAM) and Malaria Elimination Initiative (MEI) UCSF teams. Questions pertaining to the demographics (age and gender), net ownership and usage, presence of a breeding site, travel in the past 6 weeks, outdoor nocturnal behavior, household spraying, and treatment seeking behavior following the self-reported history of fever were elicited from all eligible study participants in case and control households. Net characteristics (condition, source, etc) and sleeping structure characteristic (construct and materials used) were also investigated. The responses were selected from the available options and in some instances free text was entered. All the data collected was uploaded to a secure server/cloud. (See appendix 5 for full questionnaire).

3.5.3 Blood sample collection and storage

(i) Rapid Diagnostic Tests (RDTs) and Dry Blood Spots (DBS)

Due to ethical considerations, the drawing of blood from humans was performed by a registered nurse. Before the collection of blood samples, pre-printed self-adhesive triplicate bar codes were used to label the consent form, RDT and DBS. The codes served as unique identifiers for every study participant.

An RDT test was performed by a nurse, according to the Malaria *Carestart* HRP-2/pLDH combo manufacture's guidelines (See appendix 4). Briefly a finger or heel prick using a needle or lancet was done to draw blood from a person. A few microliters of blood was drawn up into the dropping pipette, the collected blood sample was then placed into the sample well of the RDT cassette. Subsequently, a buffer was added to the assay well and the results were read through the screen.

Four blood spots were also collected on the Whatmann filter paper, air-dried and folded close afterwards. The RDT and its corresponding DBS were stored in a zip lock bag. A desiccant was placed inside the zip lock bag and sealed, ensuring that there is no air in the bag. The bags were then stored in a freezer at -20 degrees Celsius (°C) in Oshakati and occasionally transported to the laboratory in Windhoek with continued storage at the same temperature.

3.6. Laboratory Procedures

The RDTs cassettes and DBS samples collected in the field were used as sources of *Plasmodium* DNA to comparatively assess the sensitivity and specificity as well as concordance between RDTs, LAMP and nPCR.

The extractions and amplifications were performed in different sections. A de-contamination practice involving the wiping of laboratory bench surfaces and pipettes was routinely carried out before and after laboratory work was carried out. Firstly, the benches and transfer pipettes were

sprayed with 10% bleach followed by blotting with toilet paper; secondly, the benches and pipettes were then sprayed with water 2 times, paper towel was again used to blot the surfaces and pipettes. A clean pair of gloves was used for the bleach wash and another pair for the water washes. To avoid cross-contamination, there was no cross transfer of pipettes between the extractions and amplification areas.

3.6.1 Extraction of DNA by Chelex-100 method

DNA was extracted from both filter papers and RDTs using a modified chelex extraction protocol by Cox-Singh *et al*. described by Strøm, Tellevik, Hanevik, Langeland, & Blomberg, (2014). For every sample, a punch was used to remove filter paper from one DBS, in addition an RDT strip removed from an RDT and cut into 4 pieces; the filter paper and the RDT strips were each placed in separate 1.5 ml centrifuge tubes. The surgical blade used for cutting was sterilized using 70% ethanol and then distilled water before being dried off with paper towel. The sterilizing of the blade was done between successive samples. A volume of 50µl of 10% Saponin and 1ml of 1x Phosphate Buffer Saline (PBS) was pipetted to each centrifuge tube containing a cut out sample piece. The tubes were briefly vortex and placed in the refrigerator at 4°C for overnight incubation. The following day, tubes were centrifuged for 5 seconds at 8000 rpm using a 24 well centrifuge (Eppendorf Centrifuge 5424). The PBS-saponin solution was aspirated; followed by a wash using PBS to get rid of all residual saponin, red blood cells and antibodies. In the wash step, 1ml PBS was added to the centrifuge tubes and incubated at 4°C for 15-30 minutes. After incubation, the PBS was aspirated off. The DNA was released from the filter paper by adding 150µl of 20% chelex solution. The tubes were then placed on a heating block pre-heated to 98°C for 10 minutes. The tubes were then removed for a 5 minute centrifugation step at 8000 rpm. The supernatant eluate was transferred to another set of tubes, avoiding the

transfer of chelex beads. A final 10 minute centrifugation was done; the supernatant eluate was again transferred to another set of tubes, again avoiding the transfer of chelex beads. The tubes containing the eluates were stored at -20°C until such a time when LAMP or PCR was performed.

3.6.2 Loop-mediated Isothermal Amplification (LAMP)

The PAN-LAMP kit specific for all *Plasmodium species* (PAN-LAMP) was used to amplify *Plasmodium* DNA from DBS's and RDT's.

Equal 15 µl volumes of the eluate from DBS's/RDT's and nuclease free water was added to the PAN-LAMP tubes containing vacuum dried reagents (*Bst* DNA Polymerase, dNTPs, Magnesium sulfate, Calcein, Manganese chloride and primers) that were vacuum dried in the cap of each tube. Then, 30µl of Negative Control Mal (NC Mal) and 30µl Positive Control Mal (PC Mal) provided within the kit were added to tubes. The negative and positive controls were added to the 1st and last reaction tube for all amplification runs. The reaction tubes were then inverted for two minutes to collect the solution in the cap. The strip of reaction tubes were then inverted 5 times to thoroughly mix the solution with the vacuum dried reagents. A PCR-thermo-cycler (Applied Biosystems 2720 Thermal Cycler) was used for the reaction. The thermo-cycler reaction condition was set at 65°C for 45 minutes. After 45 minutes, the tubes were placed under Ultraviolet (UV) light at λ=366nm and 254nm, to observe the change in turbidity and fluorescence, i.e. *Plasmodium* positive tube fluoresce bright green and negative samples remain green with no fluorescence. The samples that were positive by PAN-LAMP were subjected to *Pf*-LAMP to confirm if they were *Plasmodium falciparum* positive. The procedure for *Pf*-LAMP is similar to that of PAN-LAMP.

3.6.3 Nested Polymerase Chain Reaction (nPCR)

Nested PCR consist of two PCR rounds, a genus-specific (primary round) and a species-specific (secondary round) PCR, which respectively amplifies All *Plasmodium species* DNA and differentiate species by amplicon size on a gel.

The genus-specific primary (1°) round PCR was run using primers:

Forward CytB_1 (5'-TTTAGCAAGTCGATATACACCAGA-3') and;

Reverse CytB_2 (5'-CTTTAACTTGCCAACTCCCTATCA-3') targeting the Cytochrome B region. A volume of 20µl was aliquoted from a tube containing master mix into PCR tubes. The reaction mixture was comprised of 1 µM of each primer; 200µM dNTPs; 3.0 mM $MgCl_2$; 2U Taq (Invitrogen Regular); 1 x PCR buffer and 8.1 µl of nuclease-free water. After the addition of the reaction mixture to the tubes, 5µl of the eluate was added to the tube, totaling a final volume of 25µl per PCR tube.

For the primary round PCR, **Initial denaturation** was done for 5 minutes (mins) at 94°C, then **40 PCR cycles** of 30 seconds (s) for 94°C (denaturation); 90s at 52.5°C (annealing) and 90s at 68°C (extension). **Final elongation** was then done at 68°C for 10 mins. Holding was done at 4°C (Figure 21). This reaction program was carried out in the same thermo-cycler used for LAMP.

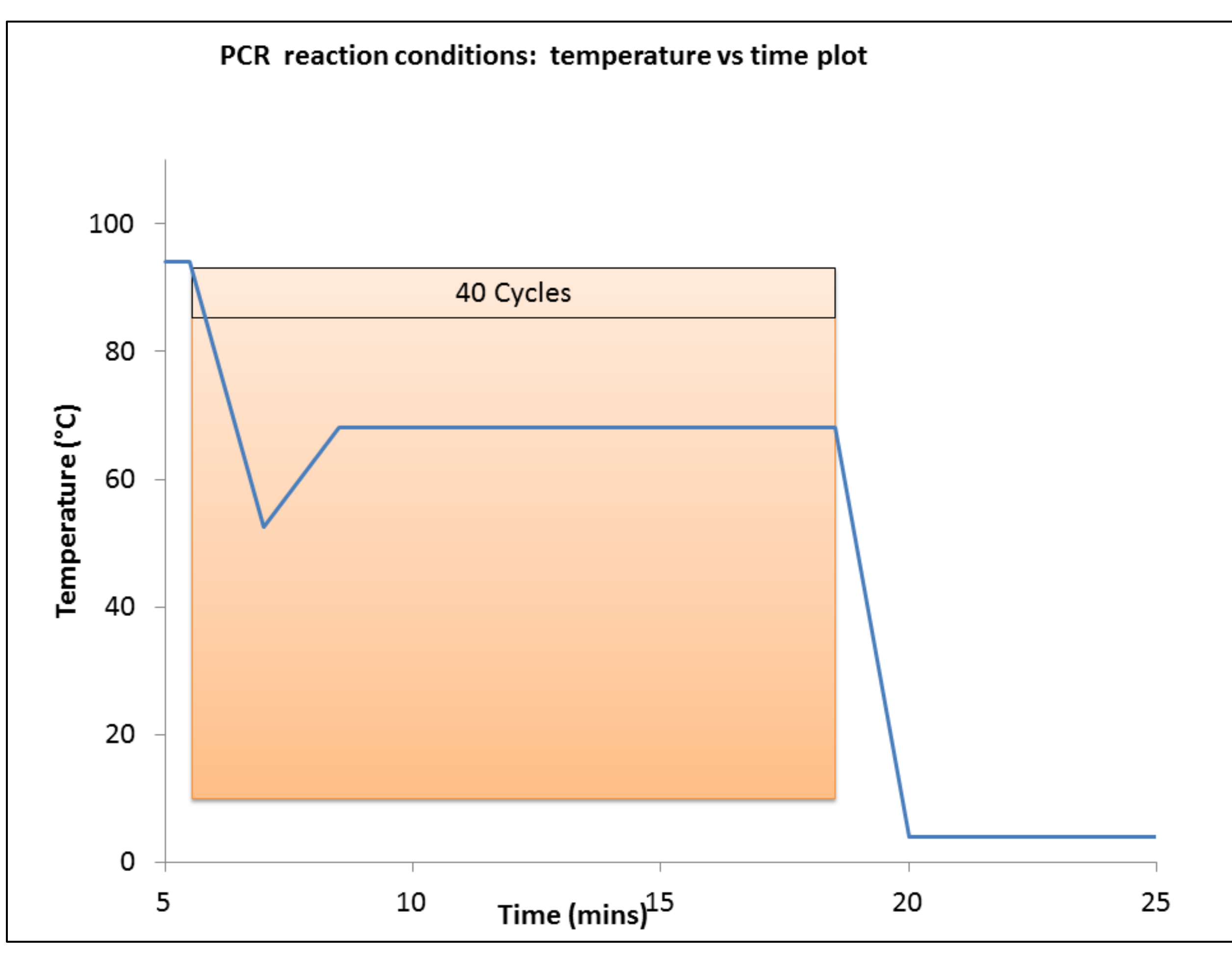


Figure 16: *Plasmodium* Genus specific PCR reaction conditions curve

The 2nd round reaction mixture was comprised of 1 µM NCB1 primer (5'-GAGAATTATGGAGTGGATGGT-3') and 1 µM NCB2 primer (5'-TGGTAATTGACATCCAATCC-3'); 200µM dNTPs; 2.5 mM $MgCl_2$; 2U Taq (Invitrogen Regular); 1 x PCR buffer and 8.3 µl of nuclease-free water. A volume of 2µl of the 1° product was transferred to 10µl of nuclease-free water in a PCR tube, to make a final dilution of 1:5. Five microliters of the diluted product was then transferred to a PCR tube containing the reaction mixture for the 2nd round amplification. Figure 16 shows the 2nd round reaction conditions, which

begins with an **Initial denaturation** at 94°C for 5 minutes, followed by **40 PCR cycles** of 30 seconds carried out at: 60°C for 90s; 72°C for 90s and **final elongation** at 72°C for 10 minutes.

3.6.4 Cytochrome B digestion

An endonuclease restriction enzyme, AluI that recognizes AG | CT TC | GA was used to digest the amplicons. This allowed for restriction band patterns for species determination to be produced on a gel. The incubation step involved the mixing of the 2° round PCR product with 1µl of AluI+ 5µl of AluI buffer and subsequently incubated at 37°C for 4 hours.

3.6.5 Gel electrophoresis and Visualization

A 2.5% agarose gel was prepared from LE Agarose dissolved in TBE Buffer and 10µl of Green nucleic acid stain for every 100 ml of molten Agarose-TBE solution for visualization. Eight microliters of DNA Ladder was loaded into the 1st well, 2µl of 6x loading dye and 8µl nested PCR product. The gel was then run at 110 volt for 1.5 hours. The gel was placed on a UV tray in the Gel Doc EZ Imager for band visualization using computer aided software Image Lab Version 5.0

3.7. Data Analysis

The responses to the questionnaire was uploaded to a secure online server or cloud and exported as .csv files. Frequency counts and percentages were calculated using the R software (v 3.2.0). Cross frequency tables were created in excel. Maps and the GPS points of the visited households were overlayed on a map using Quantum GIS Desktop 2.8.2. Odds Ratio (O.R) was calculated as a measure of the risk of association. A 95% Confidence interval for all the measures were also given. The chi square test was used to compare the sums of the responses for categorical

variables between individuals in case and control households. A multi-variate logistic regression model was run for explanatory variables that were statistically significant at $\alpha=0.05$. A comparison of the sensitivity, specificity, Positive Predictive Value and Negative Predictive Value (NPV) of RDTs and LAMP was calculated using PCR as the reference standard.

CHAPTER 4: RESULTS

A total of 69 health facility malaria-RDT individual cases were traced to 59 households, classified as case index households. The case households were comprised of 566 individuals. In the comparison control group, the study recruited 352 individuals from 76 unmatched, randomly selected households. The locations of the study households are shown on the map below (Figure 22). 94% of cases occurred towards the northern border with Angola between latitude -17.700 and between longitudes 14.500 and 15.500.

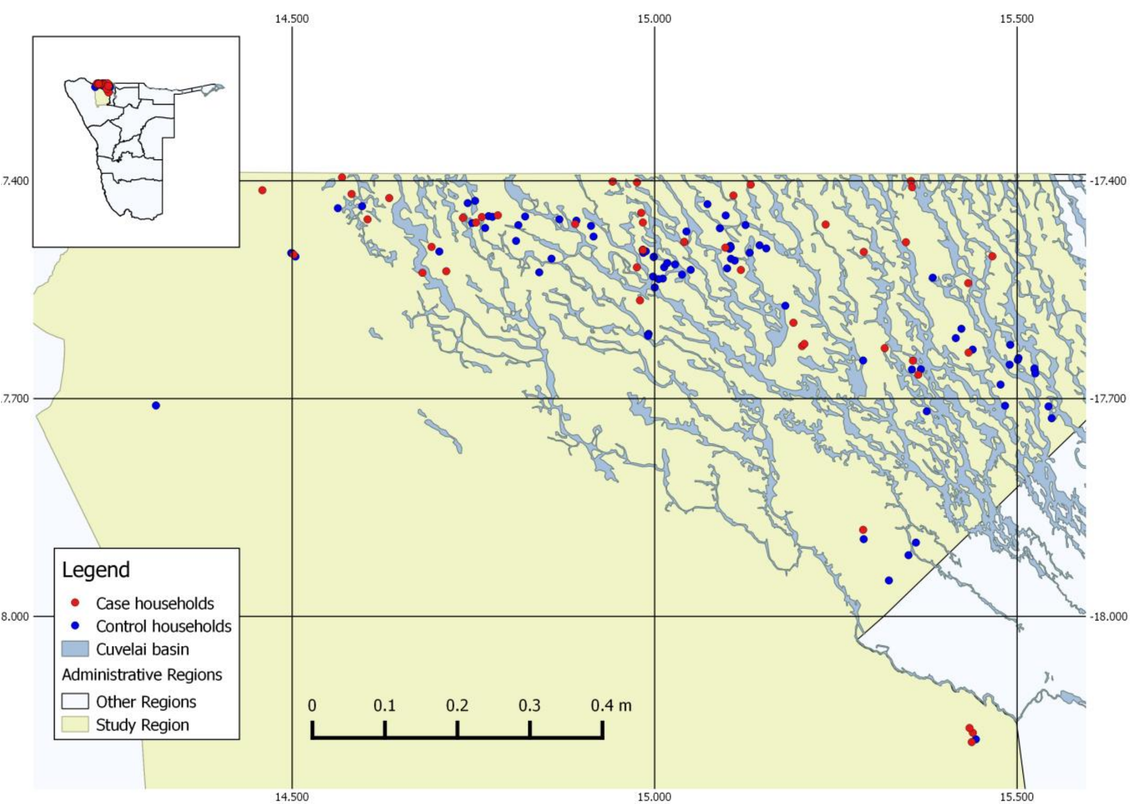
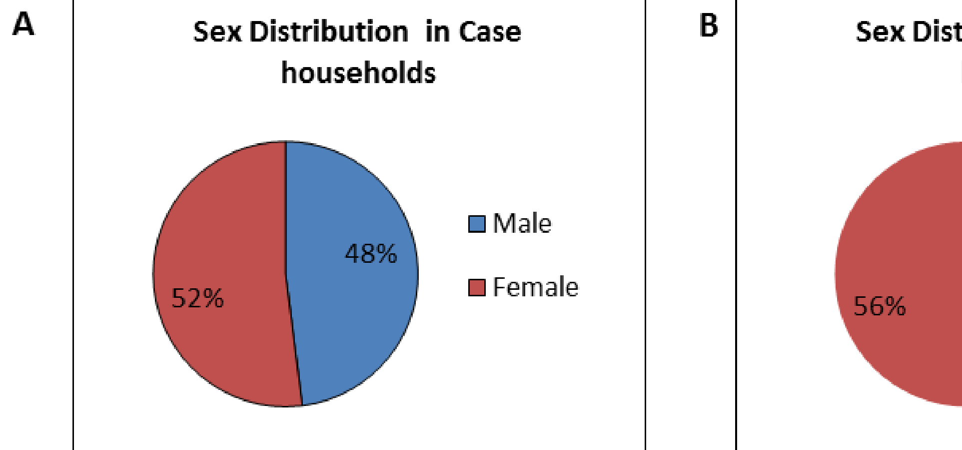


Figure 17: Location of case (red dots) and control (blue dots) of the visited households. Map created by Erastus H. Haindongo (2015)

There were 9 and 13 individual refusals from case and control households respectively. A total of 190 individuals did not provide either RDT or DBS as they were not available on the 1st and 2nd visits despite another member of the household consenting and answering the study questionnaire on their behalf.

4.1 Demographics of study participants

The sex ratio of males:females in case households were 48:52 and 44:56 in control households (Figure 18). The median age in case households were 14 (range: 0.03 – 94 years) and 19 (range: 0.08 – 110 years) for control households. Furthermore, categorizing the ages in the population pyramids (Figure 19) it was found that the greatest proportion of individuals in control households were young people between the ages of 6 – 14. On the other hand, the greatest proportion of individuals in case households were older than those in control households, this were individuals in the age group 16-35, comprising 32.1%.



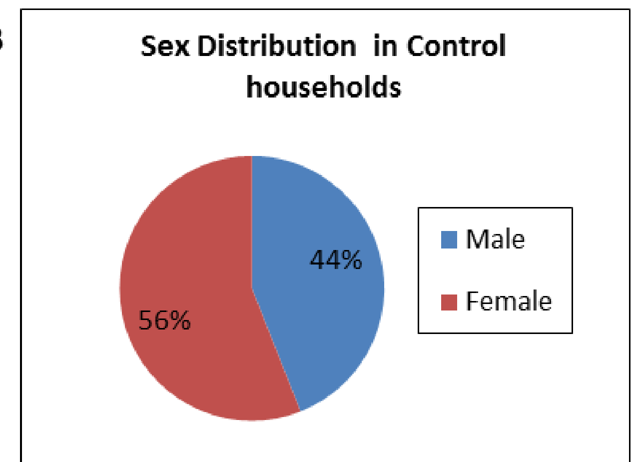


Figure 18: Pie charts showing the distribution of males and females in Case (A) and Control (B) households.

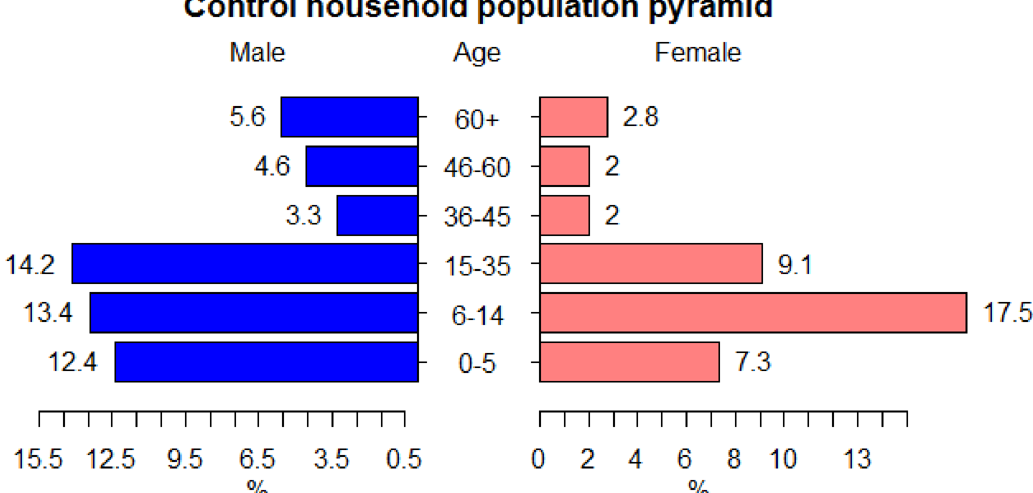


Figure 19: Population pyramids in Case (below) and control (above) household by age and gender

4.2 Risk factors for malaria transmission

4.2.1 Risk factor by age group

The odds of being from a malaria index household has been positively associated with people in the age group 14 – 35 (O.R=1.24, CI=0.37-4.26) and strongly associated within the age group of

35 – 45 (O.R= 15.06, CI=2.73-382.35, p-value <0.001). On the other hand children between the age of 5 -14 significantly have a reduced odds of being from a case index household (O.R=0.021, CI: 0.077-0.55, p-value=0.01) (Table 4).

Table 4: Univariate analysis for risk of association by age

Age Category	Case (%)	Controls (%)	Univariate Odds Ratio	95% CI	p-value
(0,5]	19.81132	20.967742	1		
(5,14]	23.89937	32.795699	0.21	0.077-0.55	0.001 *
(14,35]	32.38994	24.731183	1.24	0.37-4.36	0.77
(35,45]	8.490566	5.6451613	15.06	2.73-382.35	<0.001 ***
(45,60]	5.974843	6.9892473	0.635	0.28-1.40	0.317
(60,110]	9.433962	8.8709677	0.97	0.27-3.55	1.00
NA[1]	1.257862	6.1827957	0.56	0.16-1.87	0.361

[1]proportion of people with missing ages |Significance level: '*'0.05; '**' 0.01; '***' 0.001

4.2.2 Total number of nets owned and usage

In this study net ownership, usage and characteristic were investigated. The results are shown in table 7 and 8. From the density histograms (Figure 20) indicating the distribution of nets per household, it was found that in case households at least 40% of the households have reported to own 0-1 nets, whilst only about 8% of the households own at least 10 nets. In control households however, over 50% of the households have reported not to own between 0-1 net. The majority of households in both case and controls own between 1 -3 nets. However, there was an increased risk of malaria to households owning less than 5 nets (O.R=3.89, C.I: 0.47 – 161.29, p-value=0.05).

Although case households own more nets by absolute number, according to the universal net coverage definition, the low net density of 0.54 net/per 2 people in case households and 0.74 net per 2 people in control households (0.74) is indicative of insufficient nets.

Table 5: Indicating the total net count, distribution and general use of nets amongst children and adults

Variable	Case n(%)	Control n(%)
Total Number of Nets	153	131
Net density	0.27	0.37
CHW participation[1]	208 (79.69)	109 (53.96)
Children that don't use nets	173 (66.80)	113 (58.55)
Adults that don't use nets	163 (62.45)	81 (40.1)

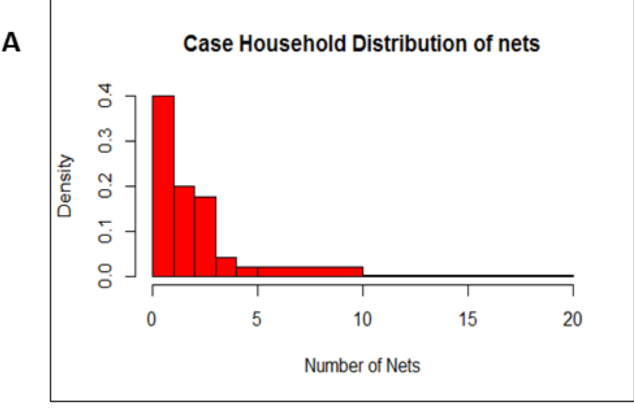
[1]demonstrating appropriate net hanging

Table 6: Univariate analysis of association and frequency distribution of nets in case and control households

Number of nets	Case (%)	Control (%)	O.R (95% C.I)	p-value
0 - 5	62.15	48.7	3.89 (0.47-161.29)	0.050 *
5-10	12.93	3.91	1.41 (0.16-79.74)	0.233
10-15	2.21	0	0 (0.03-133.92)	
15-25	5.05	1		

significance level: '*'0.05; '**' 0.01; '***' 0.001

A



B

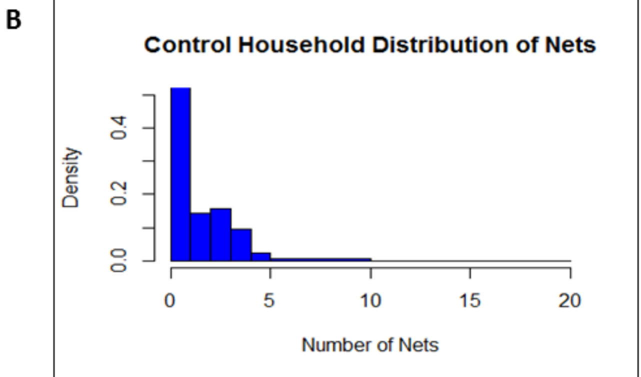


Figure 20: Distribution of the number of nets per household in Case (A) and control (B) households

Low net usage (O.R=1.4, CI=1.04-1.8, p-value=0.01) was found to be the predisposing risk factor for malaria transmission in the study districts. Furthermore, in table 5 above, a comparison of the proportion of non-net usage amongst adults and children in case and control households found an increased risk to malaria amongst adults that have reported non-net usage (O.R=2.38, CI: 1.41-4.37, p-value=0.001).

4.2.3 Source, condition and treatment of Nets

This study also reports on the findings of the characteristics of nets used by individuals. These characteristics included aspects such as the net condition; the source of nets (where it was obtained); and pre- and post-insecticide treatment of the net. It was found that 91% of the nets in case households were in an average – poor condition, compared to the 29.42% in control households that had nets in a similar condition. Nets with average and poor condition were significantly associated with the risk of malaria, O.R= 31.68, p-value=<0.01 and O.R=9.72, p-value=<0.001 respectively (Table 7).

Figure 32 depicts the source of nets in case and control households. Retail shops (31.15%) and Government clinic/hospital (27.05%) are the main source of nets for case households, whilst control households have reported equal proportions of 25.83 for other sources such as obtaining the net from a family member and retailer shops as the main source of their nets.

An investigation into the net status inside the sleeping structure (i.e. status include whether the net was hanged, taken off, still in bag or not observed) found a reduced risk of malaria with the practice of taking the net off during the day (i.e. not hanged during the day) (O.R=0.11, p-value=0.05).

Table 7: Indicating the proportions and univariate association of net condition and status of net in sleeping structure.

Variable	Case n(%)	Control n (%)	Odds Ratio (O.R)	95%C.I	p-value
Net Condition					
Good condition (no holes)	11 (9.02)	84 (70.59)	1		
Average condition (a few small holes)	86 (70.5)	18 (15.13)	31.68	15.52-76.06	<0.01 **
Poor condition (2-3 torch sized holes visible)	25 (20.5)	17 (14.29)	9.72	4.50-25.45	<0.001 ***
Net Status in Sleeping Structure					
Net hanging	120 (98.36)	98.36 (85.71)	1		
Net (not) used or still in bag	0	1 (0.84)	0.26	0.05-10.1	0.77
Net taken off during the day	2 (1.64)	15 (12.61)	0.11	0.03-0.71	0.005 *
Not observed	0	1 (0.84)	0.27	0.05-10.1	0.77

Significance level: ‘*’0.05; ‘**’ 0.01; ‘***’ 0.001

4.2.4 Presence of Mosquito breeding site

In this study, it was found that 76.7% of the case households were located in close proximity to mosquito breeding sites, more than control households which reported 60.25%. The odds of being a case household was significantly associated with the presence of mosquito breeding sites (O.R=2.21, C.I:1.21- 4.15, p-value=0.01).

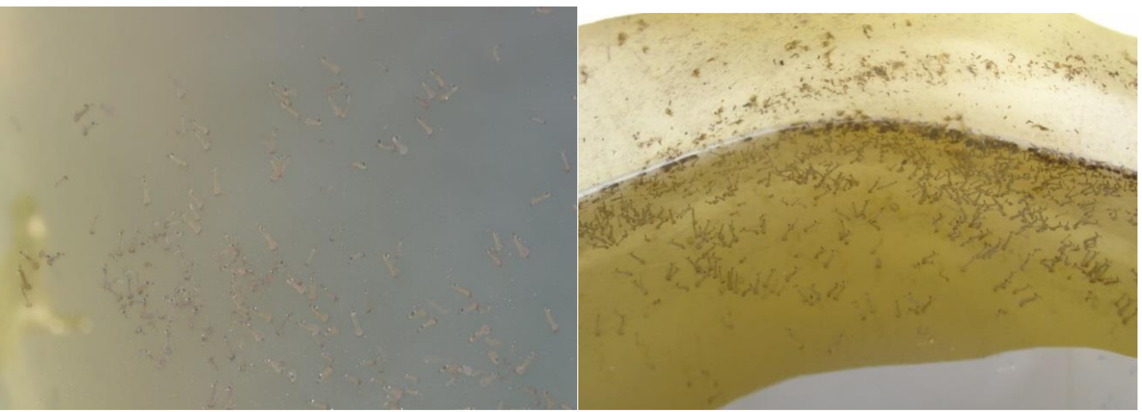


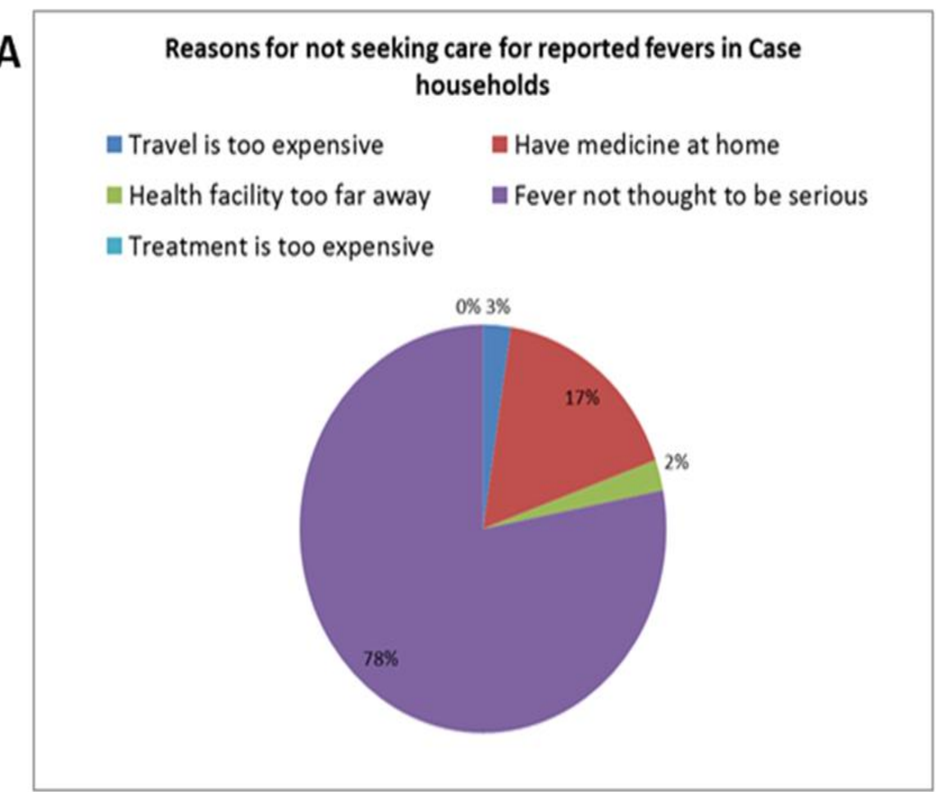
Figure 21: Images showing larvae in an uncovered container of water at a house in the Oshikuku district. Erastus Haindongo (2014)

4.2.5 History of fever, treatment seeking-behaviour, nocturnal behaviour and travel

The treatment seeking behavior of the respondents was assessed by capturing information on the history of fever and the subsequent care they sought the last time they had a fever, as well as the reasons to why they had sought such kind of care. Univariate analysis by Odds Ratio is shown in table 10 below.

Having had a fever within the past two days (OR=1.23), sleeping outside (O.R=2.76), and being outside at night (O.R=1.29) was positively associated with being from a malaria index household. These were however not statistically significant. i.e. p-value>0.05.

Care for fevers was chiefly sought from government hospitals or clinics, these comprised 98.6% and 96.1% of individuals in case and control households respectively that have reported to have had fever. The risk of malaria was reduced when treatment for fever was sought. Not seeking care for fevers due to distance to health facility (O.R=6.26, p-value=0.01) and the perception that fevers were not serious (O.R=3.03, p-value :< 0.001) was associated with an increased risk to malaria (Figure 22) (Table 8).



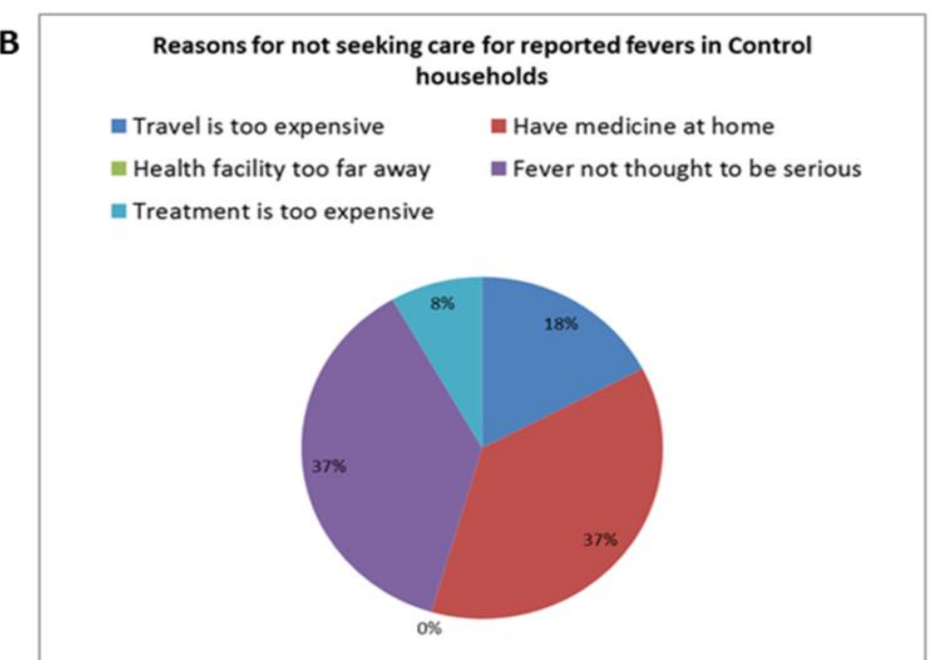


Figure 22: Reasons for not seeking care for fevers in study household, case households (A) and control households (B)

The travel locations outside the study health district to which the respondents have been to within the past 6 weeks was also recorded, as a proxy for the risk of malaria importation between health districts, regions and countries. In this study, travel was thus not associated with the risk of malaria.

Table 8: Univariate analysis for Individual risk factors

Variable	Odds Ratio (95% CI)	p-value
Net usage[1]		
Yes	*1*	
No	*1.4 (1.05 – 1.8)*	0.01 **
Fever in the past 2 days		
Yes	*1.23 (0.6-2.4)*	0.53
No	*1*	
Slept outside		
Yes	*2.76 (0.13-58.05)*	0.51
No	1	
Outside at night[2]		
Inside	1	
Outside	1.29 (0.88 – 1.91)	0.19
Don't 'know	1.6 (0.18-46.24)	0.69
Last Fever Treated		
Yes	1	
No	0.86 (0.32-2.37)	0.81
No fever	0.67 (0.36-1.24)	0.22
Treatment not sought		
Travel is too expensive	*1*	
Health facility too far away	*6.26 (1.003-209.47)*	0.01 **
Have medicine at home	*2.124 (0.64-12.05)*	0.13
Fever not thought to be serious	*9.70 (3.03 -49.97)*	<0.001 ***
Treatment is too expensive	*0.3 (0.067-9.52)*	0.682
Travelled Outside District		
No	*1*	
Yes	*0.71 (0.45 -1.12)*	0.14

[1]Net usage the night prior to visitation [2]Outside between (10 PM-06 AM) due to several activities| significance level: '*'0.05; '**' 0.01; '***' 0.001

4.2.6 Indoor Residual Spraying: Vector Control

The study also aimed comparing whether there is a higher risk of malaria in non-sprayed households, information was therefore collected on the spraying of sleeping structures, as well as the reasons for not spraying. Figure 23 showing the proportions of households sprayed. Spraying was 1.85 times higher in case households. The association to the risk of malaria in non-sprayed households was weak (O.R=0.46, CI: 0.23-1.03, p-value=0.05).

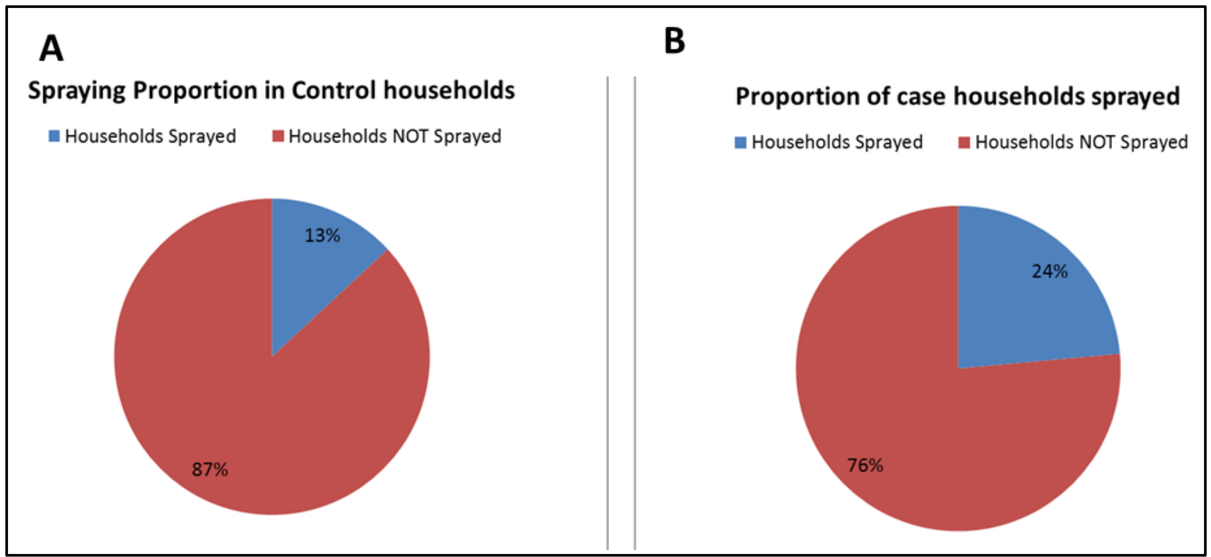


Figure 23: Proportions of Indoor Residual Spraying in case (B) and control (A) households

Figure 24 shows the reason/s why sleeping structures were not sprayed. It was found that the main reason for not spraying was chiefly due to sprayers not reaching or visiting the households. In essence, case households were visited 2.63 times more than control households, correlating with the higher spray coverage found for case households (Figure 24).

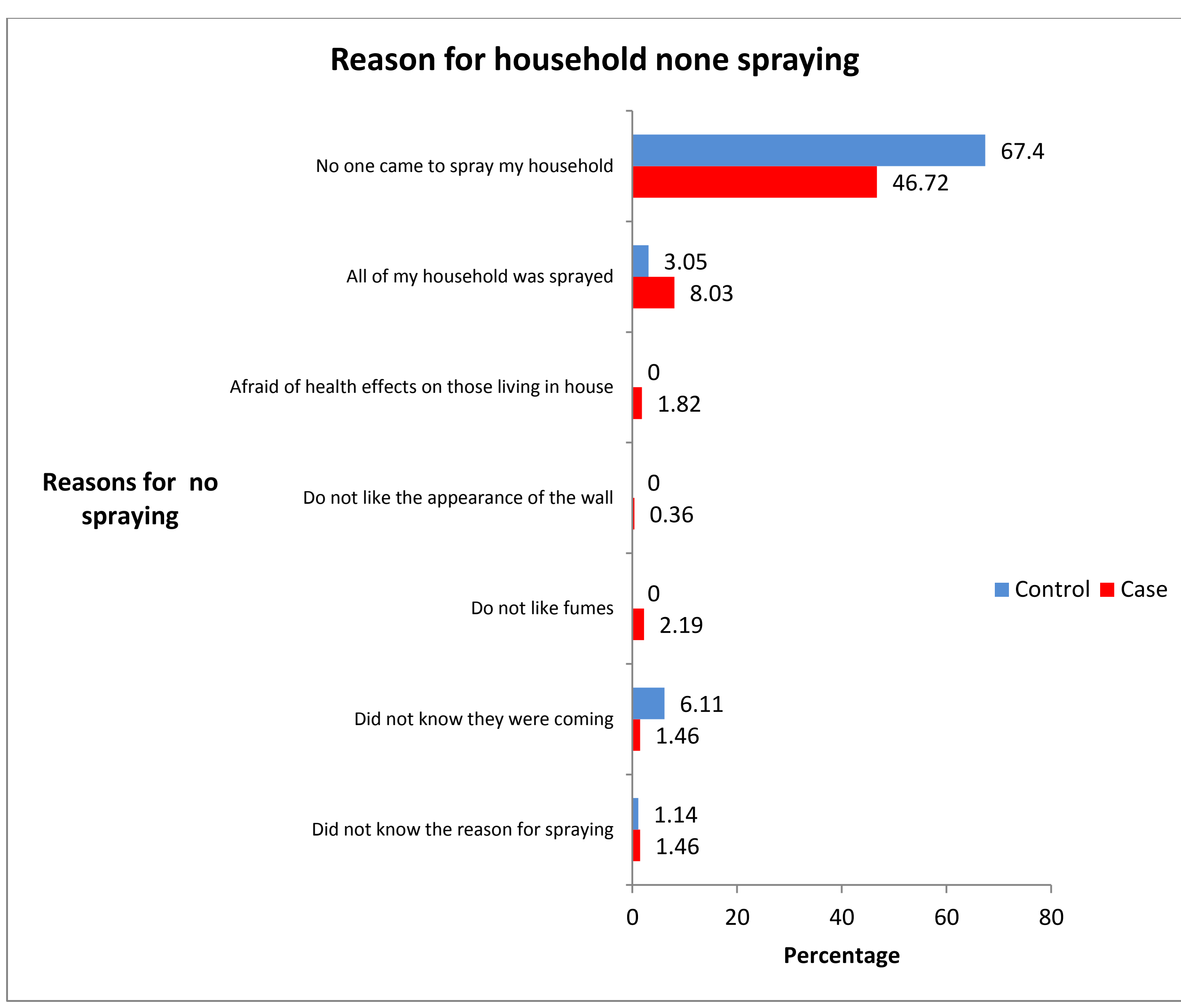


Figure 24: Response to why sleeping structures were not sprayed

4.2.7 Socio-Economic Indicators

The difference in the proportion of the assets owned by households was compared using χ^2, the asset ownership data served as a proxy for the Socio-Economic Status (SES) of the different households. The bar graph (figure 25) shows that from the 12 types of assets investigated, control households have reported to own more assets than case households. A significant

difference was found for owning a mobile phone, refrigerator, stove and a car/truck (p-value<0.05) (Table 9).

Table 9: Chi square and p-values for types of assets found in households

Type of Asset	Chi.square	p-value
Electricity	2.8984	0.08867
Radio	1.2711	0.2596
Television	2.8984	0.08867
Mobile phone	5.4915	0.01911 **
Non-mobile phone	0.29064	0.5898
Refrigerator	6.2845	0.01218 **
Stove	7.9177	0.004895 *
Bicycle	1.0436e-29	1
Motor Cycle/Scooter	0.31043	0.5774
Car Truck	4.5596	0.03274 *
Donkey	0.93876	0.3326
Tractor	0.082428	0.774
Degree of freedom (d.f)=1		

Significance level: ‘*’0.05; ‘**’ 0.01; ‘***’ 0.001

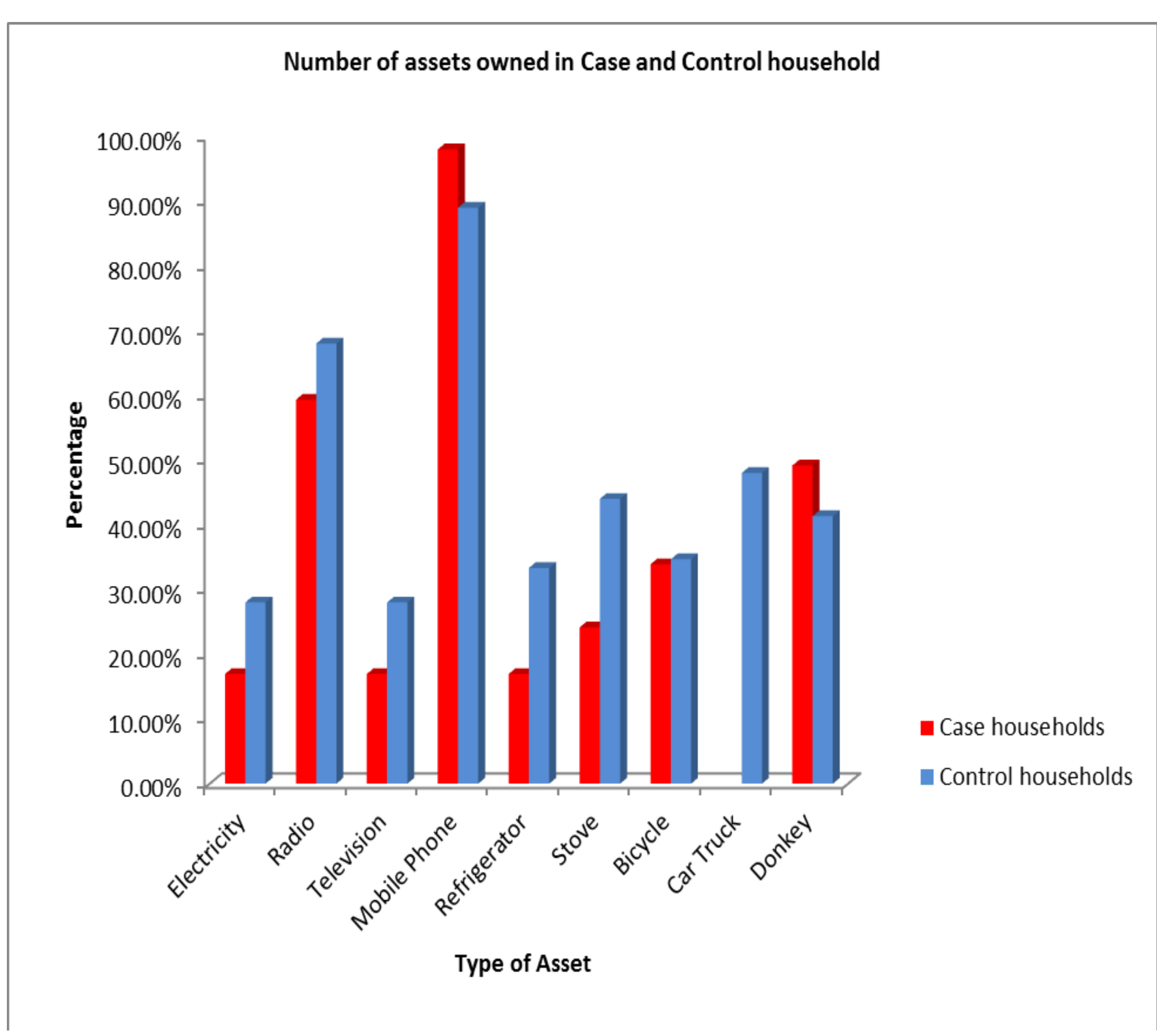


Figure 25: Proportions of Assets owned in case and control household

Table 10– 12 shows information on the main source of fuel and water that households utilize for cooking and drinking respectively, as well as information on the type of toilet facilities in households. None of these variables were significantly associated with an increased risk of malaria.

Table 10: Univariate measure of association for type of toilet facilities in a household

Variable	Case n(%)	Control n(%)	O.R	95% CI	p-value
Composite toilet	0	8 (2.03)	1		
Flush/pour flush toilet	44 (14.19)	36 (9.11)	2.85	0.3-178.76	0.09
Ventilated improved pit latrine	33 (10.65)	161 (40.76)	0.52	0.06-30.5	0.47
Pit latrine with slab	14 (4.52)	36 (9.11)	0.91	0.11-66.13	0.33
No toilet facility/bush/field	219 (70.65)	154 (38.99)	3.58	0.43-194.09	0.06

Table 11: Univariate measure of association for main source of drinking water

Variable	Case n(%)	Control n(%)	O.R	95% C.I	p-value
Borehole	0	2 (0.51)	1		
Piped into yard/standpipe	185 (57.45)	285 (72.15)	0.8	0.016-40.74	0.92
Public tap/standpipe	29 (9.01)	31 (7.84)	1.17	0.02-65.06	0.94
Protected well	0	10 (2.53)	0.20	0.001-28.21	0.51
Unprotected well	9 (2.8)	2 (0.51)	5.6	0.04-760.46	0.47
Dam	5 (1.55)	1 (0.25)	6.324	0.02-1986.40	0.51
Pond	70 (21.74)	49 (12.41)	1.79	0.034-93.97	0.77
Canal/irrigation channel	21 (6.52)	13 (3.3)	2.02	0.03-123.97	0.74

Table 12: Univariate measure of association for main source of fuel

Variable	Case n(%)	Control n(%)	O.R	95% C.I	p-value
Coal	0	5 (1.27)	1		
Electricity	36 (11.4)	13 (3.29)	2.25	0.37-83.59	0.17
Wood	262 (83.98)	249 (63.04)	1.11	0.2-27.92	0.44
Gas	14 (4.49)	118 (29.88)	0.12	0.021-4.027	0.57
Paraffin	0	9 (2.28)	0.13	0.022-18.18	0.80

4.2.8 Sleeping structure characteristics

The study investigated the sleeping room construct of household members with regards to the type of material used in the construction of the door, windows, wall and floor, as well as the presence of eaves between the roof and wall (See appendix 7). The information on the construct of a 140 sleeping structures in case households and 153 control households was collected in this study. For the different room constructs (types of floor, wall, and door material) there was no associative risk factor to malaria found. An association to the risk of malaria was found with structures that were never re-plastered (O.R=1.55, p-value=0.277) and those plastered “less than a year ago” (O.R=1.29, p-value=0.357), these association was however not significant.

4.3 Detection of *Plasmodium* infections by RDT, LAMP and nested PCR

4.3.1 Samples

Finger-prick blood samples were collected on RDT and DBS filter paper from case and control households for each individual. There were a total of 556 individuals in case households with 459 consenting to provide blood samples. In control households there were 384 individuals and 271 study participants who provided blood samples.

4.3.2 Comparison of the detection of *Plasmodium* infections by LAMP, nested PCR and RDT in case and control households.

There were 40 positives by RDT in case households (shown in figure 28) and there were no RDT positives in any of the control households investigated. Figure 26 shows a positive and negative RDT performed in the field, whereas figure 27 shows the collection of DBS.

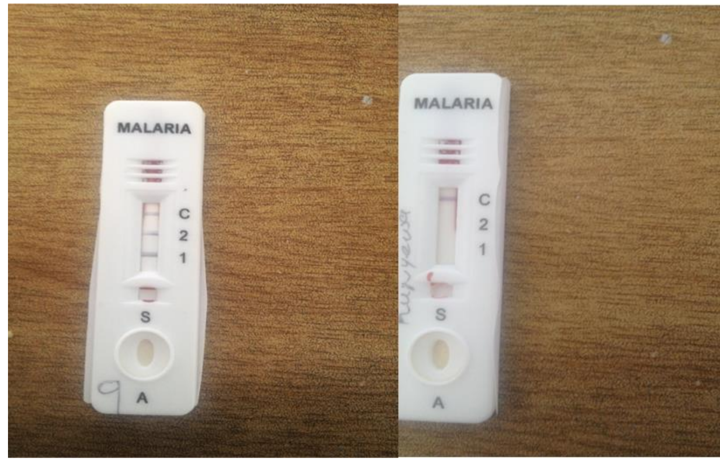


Figure 26: Image of RDT with mixed infection (3 lines) (R) and Negative (only control line) (L). Erastus Haindongo (2015)

Figure 27: A nurse performing DBS in Katima Mulilo. Erastus Haindongo (2015)

Figure 28, LAMP and nested PCR detected 25 and 13 positives respectively from the 730 individual samples. Figure 29 and 30 shows positive samples by LAMP and n-PCR respectively. From the 25 LAMP positives, 22 (88%) were from case households and 3 (12%) were from control households. From the 13 n-PCR positives, 11 were from case households and the remaining 2 were from control households.

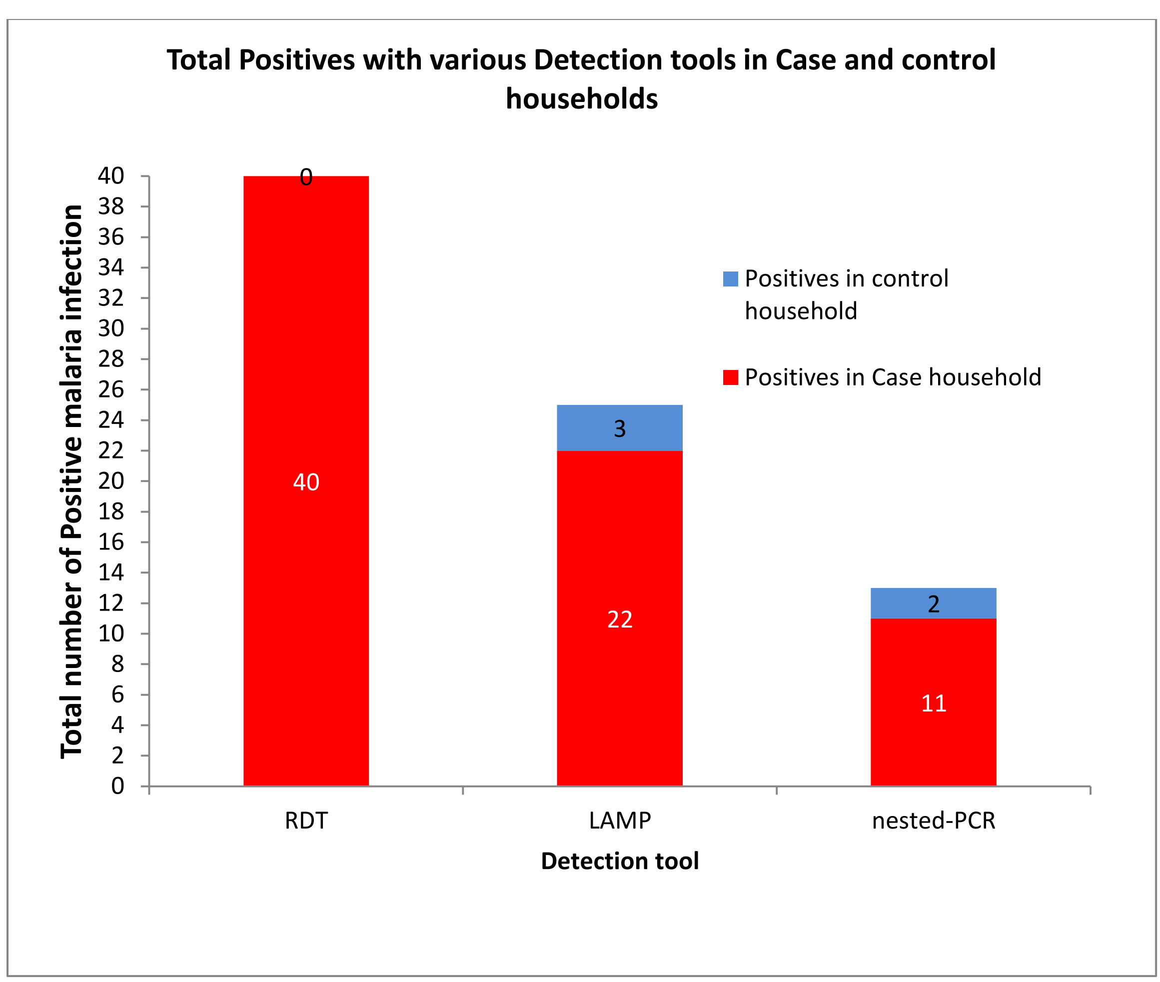


Figure 28: Total *Plasmodium* positivity by RDT, LAMP and nested-PCR

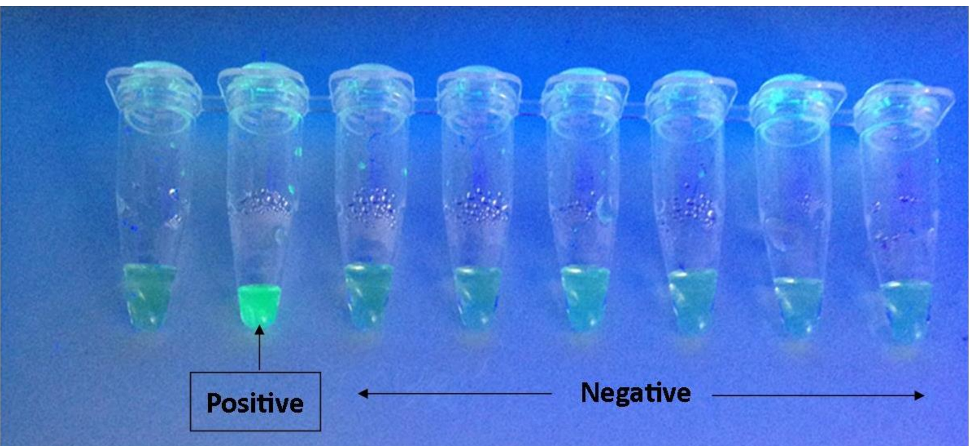


Figure 29: Representative image of LAMP tubes under UV light- tube no 2 is positive (green fluorescence) and the rest are negative (no fluorescence). Source: Erastus Haindongo (2015)

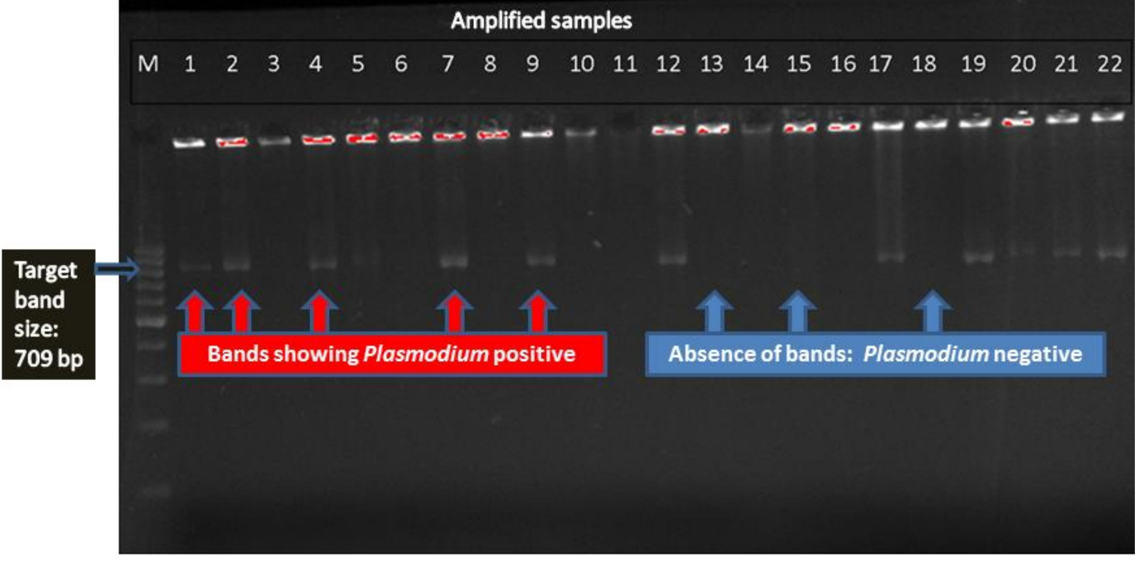


Figure 30: A representative gel image of a nested PCR product. Bands in lane 1,2,4,5,7,9,12,17,19,20,21,22 indicative of positives.

4.3.2 Comparison of symptomatic cases (index cases) and asymptomatic (secondary cases)

From the 22 LAMP positives from case households, 15 LAMP positives were from RDT positive indexes samples. Only 7 out of the 10 secondary RDT positive samples from case

households were positive by LAMP. In case households, LAMP detected 50% less and 30% less infections in RDT positive index and secondary infections respectively.

Furthermore, from the 22 LAMP positives from case household samples only 11 (50%) case household samples were positive by n-PCR. 2 out of 3 LAMP positives from control household samples were confirmed by the reference n-PCR method. In essence, an aggregate 52% of the LAMP positives were confirmed by nested-PCR (Figure 31). *i.e.* LAMP detected 48% (1.92 x) more positives than n-PCR. The general trend seen is that of positivity reducing from RDT to LAMP to nested-PCR for index cases, asymptomatic/secondary cases and control household samples.

A univariate analysis was carried out on several individual risk factors and a multiple logistic regression was performed on the variables that have reached statistical significance by univariate analysis. A Generalized Linear Model (GLM) was performed for the predictor variables age, sex and net usage that have reached statistical significance (i.e. p-value ≤ 0.05). Age was associated with malaria, p-value=0.00318.

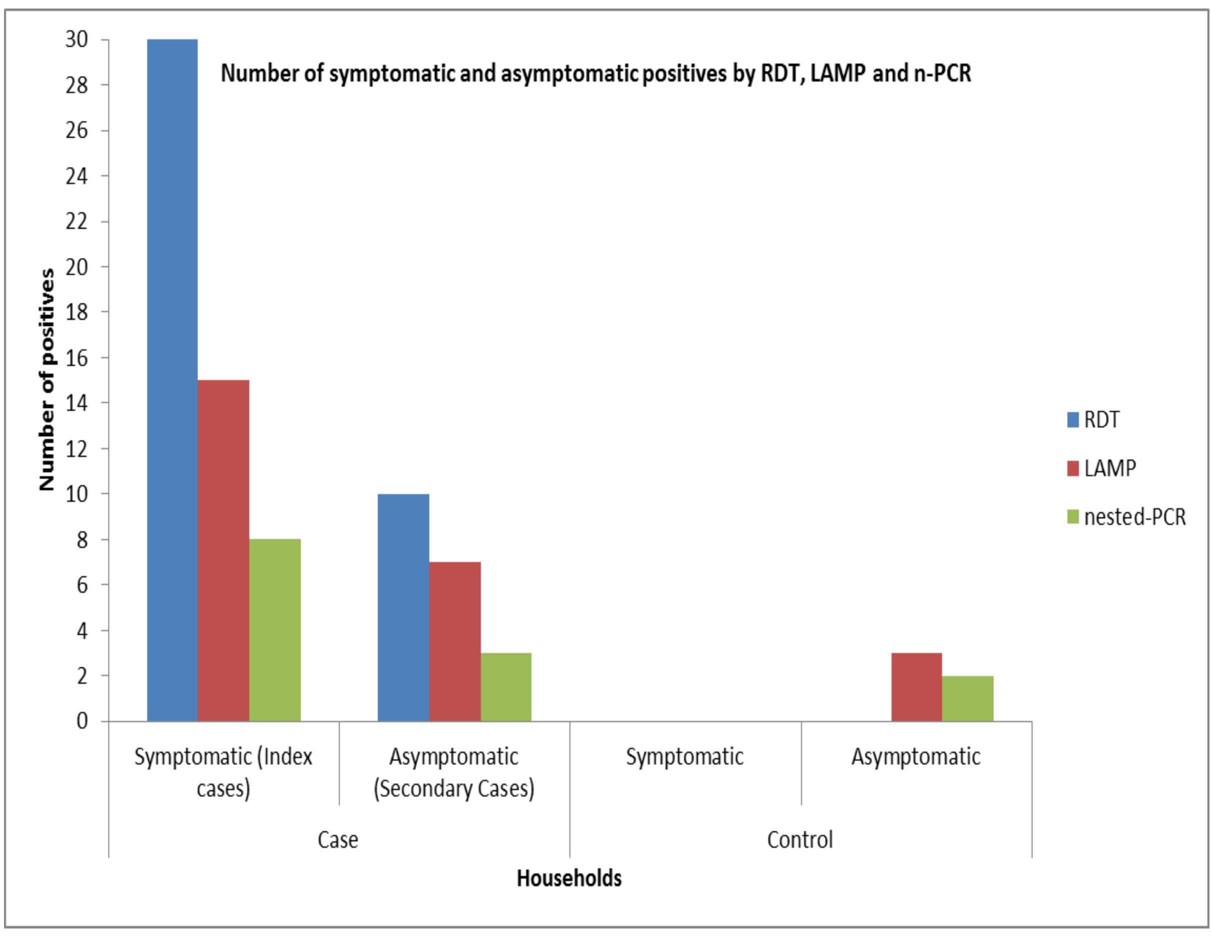


Figure 31: Results from diagnostic tests (RDT, LAMP and nested PCR) for case and control households.

The positives samples species were determined by *Plasmodium falciparum* specific LAMP and Cytochrome B digestion. All species were confirmed to be *Plasmodium falciparum*.

Using PCR as a reference standard (table 13) LAMP was more sensitive and specific than RDTs. LAMP also had a higher Positive Predictive Value (PPV) than that of RDTs. RDT found higher malaria prevalence than LAMP (3.20% vs 1.75%). Furthermore, the Positive Likelihood ratio was higher with LAMP than with PCR (60.75% vs14.56%).

Table 13: Sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) using PCR as the reference standard

	LAMP % (95% CI)	**RDT % (95% CI)**
Sensitivity	100 (75.29 -100)	76.47 (50.10-93.19)
Specificity	97.89 (97.14 – 99.15)	95.88 (92.45 – 96.51)
PPV	52 (31.31 - 72.20)	35.14 (18.57 – 49.13)
NPV	100 (99.49 - 100)	99.19 (97.93 – 99.78)
Positive Likelihood Ratio	60.75 (34.66 – 106.47)	14.56 (9.26 – 22.18)
Disease Prevalence	1.75% (0.94% - 2.98%)	3.20 (1.88 – 5.08)

CHAPTER 5: DISCUSSION

In a country like Namibia that has undergone a changing epidemiology of malaria from high transmission to low transmission in recent years, it becomes increasingly important to identify the risk factors of transmission, which will inform country elimination programs on the interventions that will need strengthening for elimination to be achieved.

Bed net usage is used a key determinant of the impact of ITN. In this study, net usage the previous night was low in case households, this was associated with a 40% increased risk of malaria (O.R =1.4, p-value=0.01). Littrell *et al*., (2013) in the RACD study in northern Senegal also found the prevalence to be higher among those that have reported non-net usage the night prior to visitation. In separate studies in several African countries by Baume & Marin, (2007) and Ndjinga & Minakawa (2010) found net usage to be between 15-55% of the available nets. Kateera *et al*., (2015) reported reasons such as seasonality for non-net usage, whereby net usage was higher during the wet season with the increased mosquito density and low in the dry hot season due to heat discomfort. Hence, the season during which information on net usage was collected is important for informed decision making. Bauch *et al*. (2013) cited social and cultural factors (understudied) to be reasons for non-net usage and adherence to preventive behaviour. Pulford *et al.* (2011) reported on the importance of establishing whether sub-optimal net utilization is due to unavailability or a failure to utilize the available nets, this is mainly important as it will allow for an informed and appropriate response to be undertaken.

An investigation into household net ownership revealed that there was an insufficient number of nets in both case and control households. Case households recorded a higher total number of nets however, the average household size (9.59 people per household) was double than that of control households (4.57 people per household). This size is almost double that of the 5.2 people per

household reported for Omusati region in the 2011 Population and Housing Census(National Statistics Agency, 2012). A calculation of the quotient between total number of nets and total individuals found a net density (or net distribution per 2 persons) of 0.54 and 0.74 in case and control households respectively. Net density was higher in control households, which may contribute to the absence of infections in control households. The net distribution in both households falls short of the recommended 1 net per 2 people in order to achieve universal net coverage (WHO, 2014). In this study, households with 5 nets or less were also found to be at risk of malaria (O.R=3.89, p-value=0.05). This finding suggests that assuming an average household size of 9.59 people observed for case households, at least 10 or more nets would be required to ensure sufficient nets for at risk households. Despite net distribution in Zanzibar, there were still not enough nets, as the distribution did not take household size into account. As a consequence, the adults were left unprotected as they opted that the younger children use the nets instead (Bauch *et al*., 2013). Low net usage in this study is linked to insufficient nets. There seem to be a general willingness for net usage by household members had net coverage been high. Some studies have reported disparities between net ownership and usage. These disparities often included high net coverage with low net usage (Kateera, Ingabire, *et al*., 2015). In both case and control households, children and adults both reported not to have enough nets as the main reason for non-net usage. Other studies have cited reasons such as heat discomfort, perceived low mosquito density and sleeping away from home (Pulford *et al*., 2011).

An investigation into the involvement of Community Health Workers (CHW) in demonstrating proper net usage revealed that CHW's were more involved in case households than in control households. Hence, the impact of CHWs in malaria prevention is not evident in the findings of the study undertaken. CHW's plays an important role before, during and after net distribution

campaigns. CHWs are involved in the dissemination of malaria related information as well as the demonstration of net hanging (UNHCR, 2010).

The practice of sleeping outside and/or being outside at night due to other reasons was higher in malaria case households compared to control households, this difference was however not significant. Chirebvu, Chimbari, & Ngwenya (2014) associated history of malaria episodes to late outdoor activities (O.R=7.016). Activities such as sleeping outdoors at night contribute to high malaria rates. This is more so in settings where the *Anopheline* vector exhibits both exophagic and exophillic behaviours. Protection against infectious bites remains minimal, even in the presence of full IRS and ITN coverage due to outdoor nocturnal activities (Monroe *et al*., 2015). In a study in Bioko Island, no difference was found between indoor and outdoor biting of *Anopheles gambiae s.s* (Reddy *et al*., 2011). This highlights the importance of protective measures both in and outdoors.

Furthermore, in this study the presence of water bodies around households was significantly associated with the risk of malaria or being a case household. The risk of being from a case household was doubled (O.R=2.21, p-value=0.01) in the presence of water bodies. Identifying breeding sites allows for the targeting of aquatic larval stages of the vector, which suppresses vector density and as such complements IRS and ITN (Lwetoijera *et al*., 2014). People settle closer to ponds or dig wells as a source of water for themselves and their livestock. Using the collected GPS coordinates of households to overlay them on a map of the hydrographic network (figure 17) reveals the spatial location of many case households are located in close proximity to water bodies. Malaria *Anopheline* vectors are known to prefer clean water for breeding (De Silva & Marshall, 2012). A GIS approach using remote sensing data to map vector transmitted diseases in Malaysia (a country in pre-elimination phase) showed that *Anopheline* and *Culicine*

larval breeding sites were located 100 – 400m from human settlements (Ahmad *et al*., 2011). Distance from a low lying area with frequent standing water was found to be the strongest predictor of malaria incidence (Staedke *et al*., 2003).

In this study, the under 5 year olds were less at risk, with the older age groups of between 14 -45 being at an increased risk of infection. In high endemic areas, pregnant women and children below the age of 5 were most at risk of the disease (Mawili-Mboumba *et al*., 2013). Control strategies were thus mainly aimed at these age groups(Pemberton-Ross, Smith, Hodel, Kay, & Penny, 2015). Averting infections in one age group is likely to shift the risk to other age groups, this is due to changes in exposure and delays in acquisition of immunity and blood stage malaria. Other studies have reported on the "peak shift" phenomenon where there is a shift to the younger age groups when transmission intensity increases due to acquired immunity with age (Carneiro *et al*., 2010). In Gabon, malaria prevalence decreased between the years 2008-2011, with the risk of infection increasing with increasing age, with noticeable reductions in the below 5 (Mawili-Mboumba *et al*., 2013). The finding of this study is consistent with that of the changing epidemiology of infectious disease. Both human activity and vector breeding habitats play a role in the mosquito-human exposure. The shift to the older age group in the Namibian setting, is likely due to behavioural patterns such as playing and working close to *Anopheles* vectors, especially at dusk when the vectors are more active(Kateera, Mens, *et al*., 2015). Given that transmission is stable in Namibia, Pemberton-Ross *et al*., (2015) reports that a decrease in transmission results in a decrease in the challenge to immunity; decrease in the acquisition of natural immunity and thus decay in the pre-existing immunity which shifts the risk of acquisition to the older aged groups. Serological studies have also agreed with the age shift to older

populations as transmission decreases, antibodies wane with the absence of repeated exposure to parasites (Kobayashi *et al*., 2012).

Fever-screening remains important for malaria case management, as by Namibia's National Malaria Policy (2015), all patients presenting with a history of fever (i.e. an axillary body temperature above 37°C) should be administered a malaria-RDT (MoHSS, 2005). Parasitological diagnosis of fevers is important for case management (Elmardi *et al*., 2011), as relying on the presence of fever can have serious problems, as fever-symptoms are associated with numerous other Neglected Tropical Diseases (Noden & van der Colf, 2013). Having reported fever within the past two days was higher in case households (O.R=1.23, 95%CI: 0.6-2.4). Moreover, having no fever and not seeking treatment for fever was not associated with the risk of malaria. *i.e*. control households did not seek treatment for their fevers reporting reasons such as fevers not perceived to be serious or having medicine at home. The self-reported fevers for which treatments was not sought, could have most likely been due to other ailments other than malaria. In Somali, over 80% of the fevers were linked to cough, running nose and sore throat (Youssef, Alegana, Amran, Noor, & Snow, 2010). Almost 70% of the individuals in case households have stated fever not thought to be serious as the reason for not seeking treatment. This can present challenges to early diagnosis of malaria cases and may potentially result in adverse symptoms or complicated malaria. In a study in Sudan on self-reported fever, Elmardi *et al*. (2011) found the proportions of fevers that are likely to be due to malaria low, with the highest infection prevalence in individuals that have reported fever on the day of visitation, followed by those that have reported fever within 2 weeks when compared to those that had no fever.

Human movement creates hotspots of infections and can thus be used to estimate the import of infections into low endemic areas (Le Menach *et al*., 2011). Travel was found to be low within

case household, thus rendering it protective and suggesting that most of the infections are due to on-going local transmission. At this point, it is worth noting that some cases were not traceable due to missing or wrong information provided. These untraceable cases were suspected to be from the high endemic regions of Angola. The practice of providing false information was done to avoid the high hospital fees. These practices have the potential to increase transmission as these people sometimes overnight at households where they have family members. From the 89 individuals that have reported travel within 6 weeks prior to visitation, about 30% (n=26) had reported travelled to Angola, to areas with an endemicity class of 5-40%. Also of those that have undertaken local trips, 24% had travelled to areas with an endemicity class of <1% and the rest to areas with an endemicity class of 5-40%. This data suggest that travel to areas with a high endemicity contributes to the risk of malaria infection. In order to accurately measure importation data such as the date of onset of symptoms and mode of travel can provide useful information of parasite incubation time. People crossing the border by walking are more likely to not cross through the formal border crossing points. A study in Bioko Island found that children that have travelled to the mainland within 8 weeks were at an increased risk of infection (Bradley *et al*., 2015). Travel to Sub Saharan Africa is responsible for at least 90% of the infections in Italian travelers (Romi *et al*., 2015). Travelers from high endemic areas are usually asymptomatic. This is due to the absence of infections caused by repeated exposure, followed by the loss of immunity and subsequent disease onset (D'Ortenzio *et al*., 2008).

An investigation of the sleeping structure construct did not find any particular type of material to contribute to the risk of malaria. This is because the construct of the houses in both case and control households were generally made up of the same construct. It was generally noted that the greatest proportion of sleeping structures were traditional huts, with a few being modernized

structures. Obaldia (2015) reported that there the type of housing construct had an increasing risk of association to malaria acquisition, where poor wall material was linked to higher infection prevalence. Chirebvu *et al*. (2014) study found an association between traditional huts and history of malaria episodes; this is because traditional huts normally have large eaves. In this study, 65.87% of the sleeping structures in case households had eaves, this was comparable to 68.7% in control households. Eaves were therefore not associated to the risk of malaria. The finding of this study suggests that perhaps a lot of the infections happen outdoors rather than in doors.

Ratovonjato *et al*., (2014) found that IRS with DDT and pyrethroid greatly decrease vector-human contact, as well as a decrease in the plasmodial index. Unexpectedly, Indoor Residual Spraying was higher for sleeping structures in case households. Henceforth, spraying was associated to the risk of malaria. The finding that IRS is associated with an increased malaria risk suggests that the *Anopheline* species could have developed resistance to the insecticide or it has changed resting behaviour. In this case, individuals in sprayed structures would still be left at high risk of malaria. However, in the absence of reported resistance to DDT in Namibia, there is a lack of evidence to support the development of resistance to DDT claim. This finding does not show the impact of IRS in reducing malaria, thus factors such as the spraying quality and actual coverage need to be further explored. Whilst the impact of IRS was not evident in the findings of this research, it was demonstrated in Botswana, where the epidemiology of malaria changed after conducting IRS and ITN in districts that reported high malaria cases. In the same study in Botswana, Simon *et al*., (2013) reported that IRS was responsible for the initial decline in cases.

The condition of the nets in case households were found to be in a poorer condition when compared to those in control households. Nets in poor condition with holes were strongly

associated with case households (p-value<0.01). A cross sectional survey in Ethiopia, a malaria eliminating country, found that non-net usage was due to torn, old and dirty nets (Batisso *et al*., 2012). Similarly, Ngondi *et al*. (2011) found net utilization to reduce with increasing age of the net and with unsafe nets(>5 holes). In a high transmission country of Benin, the blood feeding rate of *Anopheles gambiae* was higher for untreated nets with large holes. In addition, the level of protection was the same for treated nets regardless of their physical condition (Asidi, N'Guessan, Akogbeto, Curtis, & Rowland, 2012). Contrary to equal levels of protection found with treated ITNs, the cross-sectional study by Nonaka *et al* (2014) in Niger (a high burden country) found that ITN have reduced protection, with children under 5 years reporting a past malaria episode. Although the findings of this study have associated poor physical net condition with an increased risk of malaria, surveillance studies on vector populations, density and resting and feeding behaviour will be important in determining the impact of ITNs.

Furthermore, the government clinics/hospitals provided the majority of nets in case households, this could be the reason why most of these nets were factory treated. An observation was made that people in case and control households had little knowledge that post-insecticide net treatment was possible. Knowledge of this is very crucial as it will allow people to treat their nets after obtaining, thus maintaining bed nets dual protection against vectors by acting as a physical barrier and a repellant/insecticide (Sutcliffe & Yin, 2014; Tanser et al., 2007).

In developing countries, there has been a lack of attention given to health inequities particularly in the context of infectious diseases such as malaria (Dickinson, Randell, Kramer, & Shayo, 2012). Worall et. al. (2003) in the review on socio-economic status and malaria reported that level of education, income/expenditure and occupation may also be used as measures of SES but they are often intricately linked and have disadvantages. In this study however, SES was

measured using the asset ownership index. One notable problem with using the asset index is that of asset varies across surveys and even when surveys use the same assets, they are often not comparable (Worrall et al., 2003).

In this study, there were proportional differences in the ownership of various assets between case and control households. Control households reported higher proportions or ownership for at least ¾ of the 12 different assets. The difference was only significant for owning a mobile phone, refrigerator, stove and donkeys (4 assets). Therefore, this result suggests that higher SES is protective against malaria. Notably, radio and TV perhaps gives household members access to malaria prevention information.

Baragatti *et al*., (2009) a study in Burkina Faso agrees with the findings of this study that a relationship of higher SES is significantly associated with a lower risk of infection (O.R=0.75). In a systemic-review and meta-analysis, this relationship of SES to malaria infections was also found amongst children (Tusting *et al*., 2015). Similarly, a cross-sectional survey in India found more malaria cases among poor people. These malaria cases reduced with an increasing monthly income (Yadav, Dhiman, Rabha, Saikia, & Veer, 2014). Contrary to these, in a study comparing malaria in urban-rural South east Nigeria it was found that there was more self-reported malaria amongst those in a higher SES quintile. One reason for this finding could be that people in higher SES have a high perception of illness, with the poor, less likely to associate symptoms of malaria with the disease. Thus, people in higher SES could have over-reported, with the contrary that those with lower SES could have under-reported (Onwujekwe *et al*., 2009).

There are some studies that have reported that malaria is a disease of the poor. Further support for this is seen as the disease is concentrated in poorest continents and countries (Ayele et al.,

2012). This is more pronounced at a macro-level, considering that about 60% of the deaths are from 20% of the world's poorest populations. However at a micro-scale (household and individual) studies have produced mixed results (Sonko *et al*., 2014). Several scholars have hypothesized that socio-economic standing influences household construct/quality, type of toilet facility and source of water and fuel used for drinking and cooking respectively (Obaldia, 2015; Sonko *et al*., 2014). Besides, a study by Krezanoski, Tsai, Hamer, Comfort, & Bangsberg (2014) revealed that whilst a higher SES standing may suggest the ability to be able to afford protective measures such as bed net ownership, this was indeed not the case and found it to be related to the household member's knowledge of malaria instead.

Control households scored higher in the ownership of most of the studied assets. However, access to electricity was positively associated with case households. Access to electricity is indicative of higher socio-economic standing, allowing people in households to use other assets such as televisions, radio, mobile phones etc. but has no directly known effect on malaria preventive measures as the use of electrically operated coils and repellants are not so common in rural settings. It would be expected that if a household has access to electricity it would mostly likely be using electrically operated indoor stoves. In rural northern, Namibia, electricity is mostly used to operate appliances such as TV and the charging of mobile phones but hardly for cooking and heating. The majority of case households have correspondingly reported to use wood as their main source of fuel for cooking. The odds of being a case household was higher with the use of wood as the main source of fuel. Individuals in case households may spend time in the fields or bushes collecting wood during the peak mosquito biting times. In the rural setting, cooking with firewood is mostly done outdoors, whilst gas and paraffin on the other hand was found to have a protective effect against malaria because these stoves are generally used in

indoors. A substantial number of the population in developing countries relies on solid fuel such as wood for cooking and heating (Adah *et al*., 2010). In Namibia, the mosquito vectors are *Anopheles arabiensis, A. gambiae* and *A. funestus.* The former is the main vector and the populations of the latter two have been greatly reduced over the years (Kamwi, 2005; MoHSS-NVDCP, 2010; Smith Gueye *et al*., 2014). Fornadel, Norris, Glass, & Norris (2010) in a study in Macha, Zambia found *Anopheles arabiensis* to have outdoor resting and biting behaviour, biting occurred before sunset and during sunrise to circumvent the effects of ITN. Hence, activities that require individuals to be outdoors especially at sunset when families are preparing their dinner exposes individuals to mosquito bites which have the potential to cause disease.

Accurate malaria diagnosis is important in order to identify transmission foci and to optimally design and target interventions. For countries approaching elimination, the need for parasitological confirmation by RDT, microscopy or molecular methods becomes important as not all fevers are attributable to malaria (Lourenço et al., 2014). In Namibia, HRPII/pLDH RDTs are routinely being used as a malaria point of care diagnostics (MoHSS, 2005). However, RDTs give false negative results in low endemic settings. It was found that this false negatives were due to a low parasitaemia which results in infections being missed as the parasitaemia is below the detection threshold (Laban *et al*., 2015). Many studies have found that LAMP and PCR often detects more infections when compared to the point-of-care RDTs (Cook *et al*., 2015; Golassa, Enweji, Erko, Aseffa, & Swedberg, 2013). In the research setting, the high sensitivity and specificity of PCR has rendered it the gold standard (Strøm, Tellevik, Hanevik, Langeland, & Blomberg, 2014). At low transmission, infections are reported to be mostly asymptomatic with low density parasitemia that falls within the detection threshold of nested PCR and LAMP (Imwong *et al*., 2014). LAMP is a novel technique that is cost-effective and can be carried out

with ease in the field-setting (Oriero *et al*., 2015), but however needs to be compared to the gold standard PCR in order to be validated for use as a detection tool.

Generally, DNA for PCR analysis was extracted from whole blood, but a convenient way of storing finger prick dried blood spots on filter paper is being used to collect and transfer specimens for DNA extractions (Strøm, Tellevik, et al., 2014). MRDTs have also been used as sources of DNA (Papa Mze *et al*., 2015; Veron & Carme, 2006).

In this study the Chelex-100 method was used to extract DNA from both RDTs and DBS. LAMP and nPCR was performed using the extracted DNA. Strøm *et al*., (2014) in a study in which they compared 4 methods of extracting DNA from DBS, found that the Chelex, QIAamp DNA minikit and Instagene matrix to have performed well, as it resulted in a limit of detection of between 0.5 – 2 parasites/μl.

Only 7 out the 26 RDT positive index cases that reported to have been treated for malaria were positive by nPCR. There is considerable inter-individual variability in parasite clearance since it is a function of the host, parasite and drug factors (White, 2011). Aydin-schmidt *et al*., (2013) in a study in children under 5 looking at the usefulness of RDTs for the assessment of parasite clearance after ACT administration found the median clearance times of 28 days (range: 7 to >42) and 7 days (range: 2 to 14) days for HRP2 and LDH-based RDTs. RDT false positives are due to persistent antigenicity. Due to the persistence of antigens, HRP2 RDT is not suitable for treatment follow-up(Kattenberg *et al*., 2012). Hence, the clearance of parasite coupled with persistent antigenemia is a probable reason for the discordance between RDT and PCR positivity. RDTs further detected 10 secondary cases. From 10 secondary infections by RDT only 7 and 3 were confirmed by LAMP and PCR. This cannot be reconciled with the findings

from other studies except that since RDTs can give positive results for up to 30 days. This study captured information on the history of malaria in the past 14 days, hence information on the history of malaria within 30 days would probably have been useful in discerning the discordance between the three methods. Furthermore, LAMP has detected 6 additional secondary infections; only 4 were confirmed by PCR. This suggests that LAMP is more sensitive and superior to PCR. These results also demonstrate that RDTs do miss infections in low prevalence settings.

A sensitivity and specificity of 76.47% and 95.88% was found for RDT in this study. Other scholars have reported values in similar ranges 62.3 – 97.1 and 87.4 - 96.9 for sensitivity and specificity respectively (Faye *et al*., 2013; Ojulong, Kh, & Sn, 2011). The calculated sensitivity is an indication that RDTs have a low true positivity rate and are associated with a high false positivity rate due to the persistence of HRPII antigens. The calculated specificity rate is also slightly lower, and this could be due to the low parasite density common in low endemic settings or the prozone effect.

The low PPV (35%) found for RDTs is likely due to the large proportion of false positives found and because of using nPCR as the reference standard. Collecting the RDT and DBS on the day of diagnosis or before treatment is initiated, is crucial if qualitative comparison to molecular diagnostics are to be made in order to accurately evaluate the diagnostic. Many studies report on a PPV of between 79% - 94% for RDT using microscopy as the gold standard (Bharti *et al*., 2008; Hawkes *et al*., 2014; Moges *et al*., 2012). In a study in Uganda across sites with varying transmission found a low PPV of 20% at a site with low transmission. In Sudan a low PPV of 11.1% for RDTs when compared to PCR was also found (Kashif *et al*., 2013). The PPV varies with age, parasite densities, and season. The PPV found for RDTs cannot be fully relied on as it

does not take into account the history of malaria beyond 30 days for which antigens persist resulting in false positive results.

Analysis found LAMP to have a high sensitivity and specificity. This high sensitivity and specificity of 100% and 97.4% agrees with earlier findings by (Cook *et al*., 2015; Vallejo, Martínez, González, Arévalo-Herrera, & Herrera, 2015). The diagnostic accuracy of LAMP was found to be comparable to that of nPCR and RT-PCR (Polley et al., 2013; Vallejo et al., 2015). LAMP has high specificity and sensitivity, as amplification only occurs when the 6 regions on the target DNA are recognized by 2 inner and outer primers (Hsiang, Greenhouse, & Rosenthal, 2014). Nested PCR on the contrary, targets the mitochondrial Cytochrome B region with the use of 2 primers in each of the 2 rounds of PCR (Baidjoe *et al*., 2013). The difference in the number of primers and target regions confers LAMP superiority over n-PCR, thus the observed number of LAMP positives that were deemed false are most likely true positives, since nested PCR was used as a reference. Another reason for these positives, could be due to the high risk of contamination associated with LAMP (Morris *et al*., 2015). In this study a low PPV of 52% for LAMP was found. Sema *et al*., (2015) reports a PPV of 52% for *Plasmodium* and a low PPV of 27% for *Plasmodium falciparum*. In the same study by Sema *et al.,* (2015), the agreement of PCR-LAMP had gone from substantial to moderate by the calculated Kappa values of 0.776 and 0.557 for *Plasmodium* and *Plasmodium falciparum* respectively.

CHAPTER 6: CONCLUSION AND RECOMMENDATION

This study recognizes the usefulness and robustness of RACD as a surveillance tool to identify challenges to elimination efforts. It revealed that transmission is mainly (95.6% of infections) confined in the rural areas of Outapi and Oshikuku health districts. Many of the infections occurred around/in households located 34 km's away from the northern Namibian border. These findings provide information on the grid, in which interventions should be strengthened.

The risk factors for infection were also determined in households identified by RACD. The lack or insufficient number of nets together with low net usage was a predisposing risk factor. Hence, this finding supports the need for mass net distribution that takes into account household size to achieve universal net coverage coupled with strategies such as Information, Education and Communication (IEC) to encourage net usage. The age group of 35 – 45 had a higher risk of malaria and this could be linked to social behaviour that involves being outside at night. Households located in close proximity to water bodies were found to have a higher risk of infection. Water bodies serves as breeding sites for mosquitoes that have the potential to maintain high mosquito densities and as such sustain the transmission of infections. Thus targeted larvaciding to water bodies harboring larval populations would be necessary in interrupting transmission. Low Socio-Economic Status was associated with an increased risk to malaria.

The findings with the use of LAMP and nested-PCR, provides moderate support for their use alongside RACD in the detection of symptomatic and asymptomatic infections in a low transmission setting of Namibia especially for detection of secondary cases. They were more sensitive than RDTs and their predictive values highlight the need for more sensitive diagnostics

which can accurately detect asymptomatic infections, potential reservoirs for onward transmission.

It is therefore, recommended that programmes or countries in the pre-elimination phase adopt RACD in combination with LAMP as a strategy to detect and clear out all residual asymptomatic infections in order to prevent the spread of infections, as well as outbreaks. Future work should include entomological studies to detect larval populations in communities, followed by subsequent larvaciding. Retrospective or baseline studies will also be important in understanding the shifts in the transmission patterns.

REFERENCES

Adah, O., Shown, L., Yusuff, O., Envauladu, E., Banwat, M., Dhakin, A., … Bupwatda, P. (2010). Indoor air pollution in rural settings in Plateau State, Nigeria. *Jos Journal of Medicine*, *5*(1), 30–33. http://doi.org/10.4314/jjm.v5i1.62022

Adigun, A. B., Gajere, E. N., Oresanya, O., & Vounatsou, P. (2015). Malaria risk in Nigeria: Bayesian geostatistical modelling of 2010 malaria indicator survey data. *Malaria Journal*, *14*(1), 1–8. http://doi.org/10.1186/s12936-015-0683-6

Ahmad, R., Ali, W. N., Nor, Z. M., Ismail, Z., Hadi, A. a, Ibrahim, M. N., & Lim, L. H. (2011). Mapping of mosquito breeding sites in malaria endemic areas in Pos Lenjang, Kuala Lipis, Pahang, Malaysia. *Malaria Journal*, *10*(1), 361. http://doi.org/10.1186/1475-2875-10-361

Asidi, A., N'Guessan, R., Akogbeto, M., Curtis, C., & Rowland, M. (2012). Loss of household protection from use of insecticide-treated nets against pyrethroid-resistant mosquitoes, Benin. *Emerging Infectious Diseases*, *18*(7), 1101–1106. http://doi.org/10.3201/eid1807.120218

Aydin-schmidt, B., Mubi, M., Morris, U., Petzold, M., Ngasala, B. E., Premji, Z., … Mårtensson, A. (2013). Usefulness of Plasmodium falciparum -specific rapid diagnostic tests for assessment of parasite clearance and detection of recurrent infections after artemisinin-based combination therapy, 1–11. http://doi.org/10.1186/1475-2875-12-349

Ayele, D. G., Zewotir, T. T., & Mwambi, H. G. (2012). Prevalence and risk factors of malaria in Ethiopia. *Malaria Journal*, *11*(1), 195. http://doi.org/10.1186/1475-2875-11-195

Baidjoe, A., Stone, W., Ploemen, I., Shagari, S., Grignard, L., Osoti, V., … Bousema, T. (2013). Combined DNA extraction and antibody elution from filter papers for the assessment of malaria transmission intensity in epidemiological studies. *Malaria Journal*, *12*(1), 272. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3750228&tool=pmcentrez&rendertype=abstract

Baragatti, M., Fournet, F., Henry, M.-C., Assi, S., Ouedraogo, H., Rogier, C., & Salem, G. (2009). Social and environmental malaria risk factors in urban areas of Ouagadougou, Burkina Faso. *Malaria Journal*, *8*, 13. http://doi.org/10.1186/1475-2875-8-13

Batisso, E., Habte, T., Tesfaye, G., Getachew, D., Tekalegne, A., Kilian, A., … Lynch, C. (2012). A stitch in time: a cross-sectional survey looking at long lasting insecticide-treated bed net ownership, utilization and attrition in SNNPR, Ethiopia. *Malaria Journal*, *11*(1), 183. http://doi.org/10.1186/1475-2875-11-183

Bauch, J. a, Gu, J. J., Msellem, M., Mårtensson, A., Ali, A. S., Gosling, R., & Baltzell, K. a. (2013). Perception of malaria risk in a setting of reduced malaria transmission: a qualitative study in Zanzibar. *Malaria Journal*, *12*, 75. http://doi.org/10.1186/1475-2875-12-75

Baume, C. a., & Marin, M. C. (2007). Intra-household mosquito net use in Ethiopia, Ghana, Mali, Nigeria, Senegal, and Zambia: Are nets being used? Who in the household uses them? *American Journal of Tropical Medicine and Hygiene*, *77*(5), 963–971. http://doi.org/77/5/963 [pii]

Behrens, R. H., Neave, P. E., & Jones, C. O. (2015). Imported malaria among people who travel to visit friends and relatives: is current UK policy effective or does it need a strategic change? *Malaria Journal*, *14*(1), 1–6. http://doi.org/10.1186/s12936-015-0666-7

Bell, D., & Winstanley, P. (2004). Current issues in the treatment of uncomplicated malaria in Africa. *British Medical Bulletin*, *71*, 29–43. http://doi.org/10.1093/bmb/ldh031

Berhane, A., Mihreteab, S., Ahmed, H., Zehaie, A., Abdulmumini, U., & Chanda, E. (2015). Gains attained in malaria control coverage within settings earmarked for pre-elimination: malaria indicator and prevalence surveys 2012, Eritrea. *Malaria Journal*, *14*(1), 467. http://doi.org/10.1186/s12936-015-0992-9

Bharti, P. K., Silawat, N., Singh, P. P., Singh, M. P., Shukla, M., Chand, G., … Singh, N. (2008). The usefulness of a new rapid diagnostic test, the First Response Malaria Combo (pLDH/HRP2) card test, for malaria diagnosis in the forested belt of central India. *Malaria Journal*, *7*, 126. http://doi.org/10.1186/1475-2875-7-126

Bloland, P. B. (2001). *Drug resistance in malaria*.

Bousema, T., Griffin, J. T., Sauerwein, R. W., Smith, D. L., Churcher, T. S., Takken, W., … Gosling, R. (2012). Hitting hotspots: spatial targeting of malaria for control and elimination. *PLoS Medicine*, *9*(1), e1001165. http://doi.org/10.1371/journal.pmed.1001165

Bousema, T., Okell, L., Felger, I., & Drakeley, C. (2014). Asymptomatic malaria infections: detectability, transmissibility and public health relevance. *Nat Rev Micro*, *12*(12), 833–840. Retrieved from http://dx.doi.org/10.1038/nrmicro3364

Bradley, J., Monti, F., Rehman, A. M., Schwabe, C., Vargas, D., Garcia, G., … Kleinschmidt, I. (2015). Infection importation: a key challenge to malaria elimination on Bioko Island, Equatorial Guinea. *Malaria Journal*, *14*(1), 46. http://doi.org/10.1186/s12936-015-0579-5

Bretscher, M. T., Supargiyono, S., Wijayanti, M. a, Nugraheni, D., Widyastuti, A. N., Lobo, N. F., … Drakeley, C. J. (2013). Measurement of Plasmodium falciparum transmission intensity using serological cohort data from Indonesian schoolchildren. *Malaria Journal*, *12*, 21. http://doi.org/10.1186/1475-2875-12-21

Broderick, C., Nadjm, B., Smith, V., Blaze, M., Checkley, a., Chiodini, P. L., & Whitty, C. J. M. (2015). Clinical, geographical, and temporal risk factors associated with presentation and outcome of vivax malaria imported into the United Kingdom over 27 years: observational study. *Bmj*, *350*(apr16 13), h1703–h1703. http://doi.org/10.1136/bmj.h1703

Bruce-Chwatt, L. J. (1962). Classification of antimalarial drugs in relation to different stages in the life-cycle of the parasite: commentary on a diagram. *Bulletin of the World Health Organization*, *27*(27), 287–290.

Carneiro, I., Roca-Feltrer, A., Griffin, J. T., Smith, L., Tanner, M., Schellenberg, J. A., … Schellenberg, D. (2010). Age-patterns of malaria vary with severity, transmission intensity and seasonality in sub-Saharan Africa: A systematic review and pooled analysis. *PLoS ONE*, *5*(2). http://doi.org/10.1371/journal.pone.0008988

Chirebvu, E., Chimbari, M. J., & Ngwenya, B. N. (2014). Assessment of risk factors associated with malaria transmission in tubu village, northern botswana. *Malaria Research and Treatment*, *2014*, 403069. http://doi.org/10.1155/2014/403069

Cook, J., Aydin-schmidt, B., González, I. J., Bell, D., Edlund, E., Nassor, M. H., … Björkman, A. (2015). Loop-mediated isothermal amplification (LAMP) for point-of-care detection of asymptomatic low-density malaria parasite carriers in Zanzibar, 1–6. http://doi.org/10.1186/s12936-015-0573-y

Cotter, C., Sturrock, H. J., Hsiang, M. S., Liu, J., Phillips, A. a, Hwang, J., … Feachem, R. G. (2013). The changing epidemiology of malaria elimination: new strategies for new challenges. *The Lancet*, *382*(9895), 900–911. http://doi.org/10.1016/S0140-6736(13)60310-4

Coulibaly, D., Rebaudet, S., Travassos, M., Tolo, Y., Laurens, M., Kone, A. K., … Doumbo, O. K. (2013). Spatio-temporal analysis of malaria within a transmission season in Bandiagara, Mali. *Malaria Journal*, *12*(1), 82. http://doi.org/10.1186/1475-2875-12-82

D'Ortenzio, E., Godineau, N., Fontanet, A., Houze, S., Bouchaud, O., Matheron, S., & Le Bras, J. (2008). Prolonged Plasmodium falciparum infection in immigrants, Paris. *Emerging Infectious Diseases*, *14*(2), 323–6. http://doi.org/10.3201/eid1402.061475

De Silva, P. M., & Marshall, J. M. (2012). Factors contributing to urban malaria transmission in sub-saharan Africa: a systematic review. *Journal of Tropical Medicine*. http://doi.org/10.1155/2012/819563

Dickinson, K. L., Randell, H. F., Kramer, R. a., & Shayo, E. H. (2012). Socio-economic status and malaria-related outcomes in Mvomero District, Tanzania. *Global Public Health*, *7*(June 2015), 384–399. http://doi.org/10.1080/17441692.2010.539573

Ding, G., Gao, L., Li, X., Zhou, M., Liu, Q., Ren, H., & Jiang, B. (2014). A mixed method to evaluate burden of malaria due to flooding and waterlogging in Mengcheng County, China: a case study. *PloS One*, *9*(5), e97520. http://doi.org/10.1371/journal.pone.0097520

Dodoo, D., Omer, F. M., Todd, J., Akanmori, B. D., Koram, K. a, & Riley, E. M. (2002). Absolute levels and ratios of proinflammatory and anti-inflammatory cytokine production in vitro predict clinical immunity to Plasmodium falciparum malaria. *The Journal of Infectious Diseases*, *185*(7), 971–979. http://doi.org/10.1086/339408

Elmardi, K. a, Noor, A. M., Githinji, S., Abdelgadir, T. M., Malik, E. M., & Snow, R. W. (2011). Self-reported fever, treatment actions and malaria infection prevalence in the northern states of Sudan. *Malaria Journal*, *10*(1), 128. http://doi.org/10.1186/1475-2875-10-128

Estévez, P. T., Satoguina, J., Nwakanma, D. C., West, S., Conway, D. J., & Drakeley, C. J. (2011). Human saliva as a source of anti-malarial antibodies to examine population exposure to Plasmodium falciparum. *Malaria Journal*, *10*(1), 104. http://doi.org/10.1186/1475-2875-10-104

Fançony, C., Sebastião, Y. V, Pires, J. E., Gamboa, D., & Nery, S. V. (2013). Performance of microscopy and RDTs in the context of a malaria prevalence survey in Angola: a comparison using PCR as the gold standard. *Malaria Journal*, *12*, 284. http://doi.org/10.1186/1475-2875-12-284

Farcas, G. a, Zhong, K. J. Y., Mazzulli, T., & Kain, K. C. (2004). Evaluation of the RealArt Malaria LC Real-Time PCR Assay for Malaria Diagnosis Evaluation of the RealArt Malaria LC Real-Time PCR Assay for Malaria Diagnosis. *Journal of Clinical Microbiology*, *42*(2), 636–638. http://doi.org/10.1128/JCM.42.2.636

Faye, B., Nath-Chowdhury, M., Tine, R. C., Ndiaye, J. L., Sylla, K., Camargo, F. W., … Gaye, O. (2013). Accuracy of HRP2 RDT (Malaria Antigen P.f®) compared to microscopy and PCR for malaria diagnosis in Senegal. *Pathogens and Global Health*, *107*, 273–8. http://doi.org/10.1179/2047773213Y.0000000102

FIND, Eiken Chemicial, & Hospital For Tropical Diseases. (2012). *Manual of Standard Operating Procedures for malaria LAMP DNA extraction methods*.

Fonseca, A. G., Dias, S. S., Baptista, J. L., & Torgal, J. (2014). Imported malaria in portugal 2000-2009: a role for hospital statistics for better estimates and surveillance. *Malaria Research and Treatment*, *2014*, 373029. http://doi.org/10.1155/2014/373029

Fornadel, C. M., Norris, L. C., Glass, G. E., & Norris, D. E. (2010). Analysis of Anopheles arabiensis blood feeding behavior in southern Zambia during the two years after introduction of insecticide-treated bed nets. *The American Journal of Tropical Medicine and Hygiene*, *83*(4), 848–53. http://doi.org/10.4269/ajtmh.2010.10-0242

Gan, S. D., & Patel, K. R. (2013). Enzyme immunoassay and enzyme-linked immunosorbent assay. *The Journal of Investigative Dermatology*, *133*(9), e12. http://doi.org/10.1038/jid.2013.287

Ghayour Najafabadi, Z., Oormazdi, H., Akhlaghi, L., Meamar, A. R., Nateghpour, M., Farivar, L., & Razmjou, E. (2014). Detection of Plasmodium vivax and Plasmodium falciparum DNA in human saliva and urine: loop-mediated isothermal amplification for malaria diagnosis. *Acta Tropica*, *136*, 44–9. http://doi.org/10.1016/j.actatropica.2014.03.029

Gnanguenon, V., Azondekon, R., Oke-Agbo, F., Beach, R., & Akogbeto, M. (2014). Durability assessment results suggest a serviceable life of two, rather than three, years for the current long-lasting insecticidal (mosquito) net (LLIN) intervention in Benin. *BMC Infectious Diseases*, *14*(1), 69. http://doi.org/10.1186/1471-2334-14-69

Gobena, T., Berhane, Y., & Worku, A. (2012). Low long-lasting insecticide nets (LLINs) use among household members for protection against mosquito bite in Eastern Ethiopia. *BMC Public Health*, *12*(1), 914. http://doi.org/10.1186/1471-2458-12-914

Golassa, L., Enweji, N., Erko, B., Aseffa, A., & Swedberg, G. (2013). Detection of a substantial number of sub-microscopic Plasmodium falciparum infections by polymerase chain reaction: a potential threat to malaria control and diagnosis in Ethiopia. *Malaria Journal*, *12*(1), 352. http://doi.org/10.1186/1475-2875-12-352

Gupta, S., Snow, R. W., Donnelly, C. a, Marsh, K., & Newbold, C. (1999). Immunity to non-cerebral severe malaria is acquired after one or two infections. *Nature Medicine*, *5*(3), 340–343. http://doi.org/10.1038/6560

Haanshuus, C. G., Mohn, S. C., Mørch, K., Langeland, N., Blomberg, B., & Hanevik, K. (2013). A novel, single-amplification PCR targeting mitochondrial genome highly sensitive and specific in diagnosing malaria among returned travellers in Bergen, Norway. *Malaria Journal*, *12*, 26. http://doi.org/10.1186/1475-2875-12-26

Hakizimana, E., Cyubahiro, B., Rukundo, A., Kabayiza, A., Mutabazi, A., Beach, R., … Karema, C. (2014). Monitoring long-lasting insecticidal net (LLIN) durability to validate net serviceable life assumptions, in Rwanda. *Malaria Journal*, *13*, 344. http://doi.org/10.1186/1475-2875-13-344

Hamel, M. J., Odhacha, a., Roberts, J. M., & Deming, M. S. (2001). Malaria control in Bungoma District, Kenya: A survey of home treatment of children with fever, bednet use and attendance at antenatal clinics. *Bulletin of the World Health Organization*, *79*(11), 1014–1023. http://doi.org/10.1590/S0042-96862001001100004

Harris, I., Sharrock, W. W., Bain, L. M., Gray, K., Bobogare, A., Boaz, L., … Cheng, Q. (2010). A large proportion of asymptomatic Plasmodium infections with low and sub-microscopic

parasite densities in the low transmission setting of Temotu Province, Solomon Islands: challenges for malaria diagnostics in an elimination setting. *Malaria Journal*, *9*, 254. http://doi.org/10.1186/1475-2875-9-254

Hasan, A., Al, A., & Arjunan, S. (2014). Molecular characterization of thermostable DNA polymerase of Bacillus stearothermophilus spp isolated from soil in Bangalore , India Pelagia Research Library Pelagia Research Library. *European Journal of Experimental Biology*, *4*(4), 67–72.

Hawkes, M., Conroy, A. L., Opoka, R. O., Namasopo, S., Liles, W. C., John, C. C., & Kain, K. C. (2014). Use of a three-band HRP2/pLDH combination rapid diagnostic test increases diagnostic specificity for falciparum malaria in Ugandan children. *Malaria Journal*, *13*(1), 43. http://doi.org/10.1186/1475-2875-13-43

Hetzel, M. W., Iteba, N., Makemba, A., Mshana, C., Lengeler, C., Obrist, B., … Mshinda, H. (2007). Understanding and improving access to prompt and effective malaria treatment and care in rural Tanzania: the ACCESS Programme. *Malaria Journal*, *6*, 83. http://doi.org/10.1186/1475-2875-6-83

Hsiang, M. S., Greenhouse, B., & Rosenthal, P. J. (2014). Point of Care Testing for Malaria Using LAMP, Loop Mediated Isothermal Amplification. *The Journal of Infectious Diseases*, *210*, 1–3. http://doi.org/10.1093/infdis/jiu253

Hue, N. T., Phong, P. T., Chan, N. D. H., Hoan, N. K. H., & Thuy, H. T. T. (2011). An Efficiency Human Genomic DNA Extraction from Dried Blood Spots. *Procedia Environmental Sciences*, *8*, 179–185. http://doi.org/10.1016/j.proenv.2011.10.029

Imwong, M., Hanchana, S., Malleret, B., Rénia, L., Day, N. P. J., Dondorp, A., … White, N. J. (2014). High-throughput ultrasensitive molecular techniques for quantifying low-density malaria parasitemias. *Journal of Clinical Microbiology*, *52*(9), 3303–3309. http://doi.org/10.1128/JCM.01057-14

Joshi, M., & Deshpande, J. . (2010). Polymerase Chain Reaction : Methods , Principles and. *International Journal of Biomedical Research*, *5*, 81–97. Retrieved from www.ssjournals.com

Kamwi, R. (2005). *Malaria Situation in Namibia: A study of vector species and effectiveness of the past and current control strategies in selected parts of Namibia*.

Karunaweera, N. D., Galappaththy, G. N., & Wirth, D. F. (2014). On the road to eliminate malaria in Sri Lanka: lessons from history, challenges, gaps in knowledge and research needs. *Malaria Journal*, *13*(1), 59. http://doi.org/10.1186/1475-2875-13-59

Kashif, A. H., Adam, G. K., Mohmmed, A. a, Elzaki, S. E., AbdelHalim, A. M., & Adam, I. (2013). Reliability of rapid diagnostic test for diagnosing peripheral and placental malaria in an area of unstable malaria transmission in Eastern Sudan. *Diagnostic Pathology*, *8*, 59. http://doi.org/10.1186/1746-1596-8-59

Kateera, F., Ingabire, C. M., Hakizimana, E., Rulisa, A., Karinda, P., Grobusch, M. P., … Mens, P. F. (2015). Long-lasting insecticidal net source, ownership and use in the context of universal coverage: a household survey in eastern Rwanda. *Malaria Journal*, *14*(1), 390. http://doi.org/10.1186/s12936-015-0915-9

Kateera, F., Mens, P. F., Hakizimana, E., Ingabire, C. M., Muragijemariya, L., Karinda, P., … van Vugt, M. (2015). Malaria parasite carriage and risk determinants in a rural population: a malariometric survey in Rwanda. *Malaria Journal*, *14*(1), 16. http://doi.org/10.1186/s12936-014-0534-x

Kattenberg, J. H., Tahita, C. M., Versteeg, I. a. J., Tinto, H., Traoré-Coulibaly, M., Henk, D. F., … Mens, P. F. (2012). Antigen persistence of rapid diagnostic tests in pregnant women in Nanoro, Burkina Faso, and the implications for the diagnosis of malaria in pregnancy. *Journal of Tropical Medicine and International Health*, *17*(5), 550–557. http://doi.org/10.1111/j.1365-3156.2012.02975.x

Kelly-Hope, L. a, & McKenzie, F. E. (2009). The multiplicity of malaria transmission: a review of entomological inoculation rate measurements and methods across sub-Saharan Africa. *Malaria Journal*, *8*, 19. http://doi.org/10.1186/1475-2875-8-19

Kigozi, R., Baxi, S. M., Gasasira, A., Sserwanga, A., Kakeeto, S., Nasr, S., … Dorsey, G. (2012). Indoor residual spraying of insecticide and malaria morbidity in a high transmission intensity area of Uganda. *PLoS ONE*, *7*(8). http://doi.org/10.1371/journal.pone.0042857

Kilian, A., Boulay, M., Koenker, H., & Lynch, M. (2010). How many mosquito nets are needed to achieve universal coverage? Recommendations for the quantification and allocation of long-lasting insecticidal nets for mass campaigns. *Malaria Journal*, *9*(1), 330. Retrieved from http://www.malariajournal.com/content/9/1/330

Kimbi, H. K. (2012). Environmental Factors and Preventive Methods against Malaria Parasite Prevalence in Rural Bomaka and Urban Molyko, Southwest Cameroon. *Journal of Bacteriology & Parasitology*, *04*(01), 1–5. http://doi.org/10.4172/2155-9597.1000162

Kleinschmidt, I., Mnzava, A. P., Kafy, H. T., Mbogo, C., Bashir, A. I., Bigoga, J., … Donnelly, M. J. (2015). Design of a study to determine the impact of insecticide resistance on malaria vector control: a multi-country investigation. *Malaria Journal*, *14*(1), 282. http://doi.org/10.1186/s12936-015-0782-4

Kobayashi, T., Chishimba, S., Shields, T., Hamapumbu, H., Mharakurwa, S., Thuma, P. E., … Moss, W. J. (2012). Temporal and spatial patterns of serologic responses to Plasmodium falciparum antigens in a region of declining malaria transmission in southern Zambia. *Malaria Journal*, *11*(1), 438. http://doi.org/10.1186/1475-2875-11-438

Krezanoski, P. J., Tsai, A. C., Hamer, D. H., Comfort, A. B., & Bangsberg, D. R. (2014). Household malaria knowledge and its association with bednet ownership in settings without large-scale distribution programs: Evidence from rural Madagascar. *Journal of Global Health*, *4*(1), 010401. http://doi.org/10.7189/jogh.04.010401

Laban, N. M., Kobayashi, T., Hamapumbu, H., Sullivan, D., Mharakurwa, S., Thuma, P. E., … Moss, W. J. (2015). Comparison of a PfHRP2-based rapid diagnostic test and PCR for malaria in a low prevalence setting in rural southern Zambia: implications for elimination. *Malaria Journal*, *14*(1), 25. http://doi.org/10.1186/s12936-015-0544-3

Le Menach, a., Tatem, a. J., Cohen, J. M., Hay, S. I., Randell, H., Patil, a. P., & Smith, D. L. (2011). Travel risk, malaria importation and malaria transmission in Zanzibar. *Scientific Reports*, *1*, 93. http://doi.org/10.1038/srep00093

Lee, K.-S., Cox-Singh, J., & Singh, B. (2009). Morphological features and differential counts of Plasmodium knowlesi parasites in naturally acquired human infections. *Malaria Journal*, *8*, 73. http://doi.org/10.1186/1475-2875-8-73

Li, N., Parker, D. M., Yang, Z., Fan, Q., Zhou, G., Ai, G., … Wang, Y. (2013). Risk factors associated with slide positivity among febrile patients in a conflict zone of north-eastern Myanmar along the China-Myanmar border. *Malar J*, *12*(1), 361. http://doi.org/10.1186/1475-2875-12-361

Li, P., Zhao, Z., Wang, Y., Xing, H., Parker, D. M., Yang, Z., … Fan, Q. (2014). Nested PCR detection of malaria directly using blood filter paper samples from epidemiological surveys. *Malaria Journal*, *13*(1), 175. http://doi.org/10.1186/1475-2875-13-175

Lilit, G., & Avashia, N. (2013). Polymerase Chain Reaction. *Journal of Investigative Dermatology*, *133*(6), 392–395. Retrieved from http://dx.doi.org/10.1016/B978-0-12-374984-0.01186-4

Littrell, M., Sow, G. D., Ngom, A., Ba, M., Mboup, B. M., Dieye, Y., … Steketee, R. W. (2013). Case investigation and reactive case detection for malaria elimination in northern Senegal. *Malaria Journal*, *12*(1), 331. http://doi.org/10.1186/1475-2875-12-331

Lourenço, C., Kandula, D., Haidula, L., Ward, A., & Cohen, J. M. (2014). Strengthening malaria diagnosis and appropriate treatment in Namibia : a test of case management training interventions in Kavango Region. *Malaria Journal*, *13*, 508.

Lwetoijera, D. W., Harris, C., Kiware, S. S., Dongus, S., Devine, G. J., McCall, P. J., & Majambere, S. (2014). Effective autodissemination of pyriproxyfen to breeding sites by the exophilic malaria vector Anopheles arabiensis in semi-field settings in Tanzania. *Malaria Journal*, *13*(1), 161. http://doi.org/10.1186/1475-2875-13-161

malERA Consultative Group on Drugs. (2011). A research agenda for malaria eradication: drugs. *PLoS Medicine*, *8*(1). http://doi.org/10.1371/journal.pmed.1000402

Mandal, S. (2014). Epidemiological aspects of vivax and falciparum malaria: global spectrum. *Asian Pacific Journal of Tropical Disease*, *4*(Suppl 1), S13–S26. http://doi.org/10.1016/S2222-1808(14)60410-2

Mangold, Manson, Koay, Stephens, Regner, Thomson, … Kaul. (2005). Real-time PCR for detection and identification of Plasmodium spp. *Journal of Clinical Microbiology*, *43*(5), 2435–2440. http://doi.org/10.1128/JCM.43.5.2435

Mawili-Mboumba, D. P., Bouyou Akotet, M. K., Kendjo, E., Nzamba, J., Medang, M. O., Mbina, J.-R. M., & Kombila, M. (2013). Increase in malaria prevalence and age of at risk population in different areas of Gabon. *Malaria Journal*, *12*(3), 1–7. http://doi.org/10.1186/1475-2875-12-3

Mboera, L. E. G., Mazigo, H. D., Rumisha, S. F., & Kramer, R. a. (2013). Towards malaria elimination and its implication for vector control , disease management and livelihoods in Tanzania. *MalariaWorld Journal*, *4*(19), 18–20.

Mekonnen, S. K., Aseffa, A., Medhin, G., Berhe, N., & Velavan, T. P. (2014). Re-evaluation of microscopy confirmed Plasmodium falciparum and Plasmodium vivax malaria by nested PCR detection in southern Ethiopia. *Malaria Journal*, *13*, 48. http://doi.org/10.1186/1475-2875-13-48

Mnzava, A. P., Knox, T. B., Temu, E. A., Trett, A., Fornadel, C., Hemingway, J., & Renshaw, M. (2015). Implementation of the global plan for insecticide resistance management in malaria vectors: progress, challenges and the way forward. *Malaria Journal*, *14*(1), 1–9. http://doi.org/10.1186/s12936-015-0693-4

Moges, B., Amare, B., Belyhun, Y., Tekeste, Z., Gizachew, M., Workineh, M., … Kassu, A. (2012). Comparison of CareStart™ HRP2/pLDH COMBO rapid malaria test with light microscopy in north-west Ethiopia. *Malaria Journal*, *11*(1), 234. http://doi.org/10.1186/1475-2875-11-234

Mohon, A. N., Elahi, R., Khan, W. a, Haque, R., Sullivan, D. J., & Alam, M. S. (2014). A new visually improved and sensitive loop mediated isothermal amplification (LAMP) for diagnosis of symptomatic falciparum malaria. *Acta Tropica*, *134*, 52–7. http://doi.org/10.1016/j.actatropica.2014.02.016

MoHSS. (2005). *National Malaria Policy*. Windhoek.

MoHSS-NVDCP. (2009). *Namibia Malaria Incidator Survey 2009*.

MoHSS-NVDCP. (2010). *Malaria Strategic Plan (2010-2016)*.

Monroe, A., Asamoah, O., Lam, Y., Koenker, H., Psychas, P., Lynch, M., … Harvey, S. a. (2015). Outdoor-sleeping and other night-time activities in northern Ghana: implications for residual transmission and malaria prevention. *Malaria Journal*, *14*(1), 35. http://doi.org/10.1186/s12936-015-0543-4

Moody, A. (2002). Rapid Diagnostic Tests for Malaria Parasites Rapid Diagnostic Tests for Malaria Parasites. *Clinical Microbiology Reviews*, *15*(1), 66–78. http://doi.org/10.1128/CMR.15.1.66

Moormann, A. M., Chelimo, K., Sumba, O. P., Lutzke, M. L., Ploutz-Snyder, R., Newton, D., … Rochford, R. (2005). Exposure to holoendemic malaria results in elevated Epstein-Barr virus loads in children. *The Journal of Infectious Diseases*, *191*(8), 1233–1238. http://doi.org/10.1086/428910

Mori, A., Ngalesoni, F., Norheim, O. F., & Robberstad, B. (2014). Cost-effectiveness of dihydroartemisinin-piperaquine compared with artemether-lumefantrine for treating uncomplicated malaria in children at a district hospital in Tanzania. *Malaria Journal*, *13*(1), 363. http://doi.org/10.1186/1475-2875-13-363

Mori, Y., & Notomi, T. (2009). Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. *Journal of Infection and Chemotherapy : Official Journal of the Japan Society of Chemotherapy*, *15*(2), 62–9. http://doi.org/10.1007/s10156-009-0669-9

Morris, U., Khamis, M., Aydin-Schmidt, B., Abass, A. K., Msellem, M. I., Nassor, M. H., … Cook, J. (2015). Field deployment of loop-mediated isothermal amplification for centralized mass-screening of asymptomatic malaria in Zanzibar: a pre-elimination setting. *Malaria Journal*, *14*(1), 205. http://doi.org/10.1186/s12936-015-0731-2

Mosha, J. F., Sturrock, H. J. W., Greenhouse, B., Greenwood, B., Sutherland, C. J., Gadalla, N., … Gosling, R. (2013). Epidemiology of subpatent Plasmodium falciparum infection: implications for detection of hotspots with imperfect diagnostics. *Malaria Journal*, *12*(1), 221. http://doi.org/10.1186/1475-2875-12-221

Mouatcho, J. ., Munhenga, G., Hargreaves, K., Brooke, B. ., Coetzee, M., & Koekemoer, L. . (2009). Pyrethroid resistance in a major African malaria vector Anopheles arabiensis from Mamfene, northern KwaZulu-Natal, South Africa. *South African Journal Of Science*, *105*(April), 127–131.

Mubi, M., Kakoko, D., Ngasala, B., Premji, Z., Peterson, S., Björkman, A., & Mårtensson, A. (2013). Malaria diagnosis and treatment practices following introduction of rapid diagnostic tests in Kibaha District, Coast Region, Tanzania. *Malaria Journal*, *12*(1), 293. http://doi.org/10.1186/1475-2875-12-293

Murray, C. K., Gasser, R. a., Magill, A. J., & Miller, R. S. (2008). Update on rapid diagnostic testing for malaria. *Clinical Microbiology Reviews*, *21*(1), 97–110. http://doi.org/10.1128/CMR.00035-07

Najera, J. a, & Zaim, M. (2001). Malaria Vector Control: Insecticides for indoor residual spraying. WHO/CDS/WHOPES/2001.3.

Namibia Statistics Agency. (2011). Namibia 2011 Population and Housing Census Indicators.

National Statistics Agency. (2012). 2011 Namibia Population and Housing Census.

Ndjinga, J. K., & Minakawa, N. (2010). The importance of education to increase the use of bed nets in villages outside of Kinshasa, Democratic Republic of the Congo. *Malaria Journal*, *9*, 279. http://doi.org/10.1186/1475-2875-9-279

Ndoen, E., Wild, C., Dale, P., Sipe, N., & Dale, M. (2012). Mosquito Longevity, Vector Capacity, and Malaria Incidence in West Timor and Central Java, Indonesia. *ISRN Public Health*, *2012*, 1–5. http://doi.org/10.5402/2012/143863

Ngondi, J. M., Graves, P. M., Gebre, T., Mosher, A. W., Shargie, E. B., Emerson, P. M., & Richards, F. O. (2011). Which nets are being used: factors associated with mosquito net use in Amhara, Oromia and Southern Nations, Nationalities and Peoples' Regions of Ethiopia. *Malaria Journal*, *10*(1), 92. http://doi.org/10.1186/1475-2875-10-92

Noden, B. H., & van der Colf, B. E. (2013). Neglected tropical diseases of Namibia: Unsolved mysteries. *Acta Tropica*, *125*(1), 1–17. http://doi.org/http://dx.doi.org/10.1016/j.actatropica.2012.09.007

Nonaka, D., Maazou, a, Yamagata, S., Oumarou, I., Uchida, T., Jg Yacouba, H., … Mizoue, T. (2014). Can Long-lasting Insecticide-treated Bednets with Holes Protect Children from Malaria? *Tropical Medicine & Health*, *42*(3), 99–105. http://doi.org/http://dx.doi.org/10.2149/tmh.2013-21

Nyunt, M. H., Aye, K. M., Kyaw, M. P., Kyaw, T. T., Hlaing, T., Oo, K., … San, N. a. (2014). Challenges in universal coverage and utilization of insecticide-treated bed nets in migrant plantation workers in Myanmar. *Malaria Journal*, *13*(1), 211. http://doi.org/10.1186/1475-2875-13-211

Obaldia, N. (2015). Determinants of low socio-economic status and risk of Plasmodium vivax malaria infection in Panama (2009-2012): a case-control study. *Malaria Journal*, *14*, 14. http://doi.org/10.1186/s12936-014-0529-7

Ojulong, J., Kh, M., & Sn, I. (2011). Knowledge and attitudes of infection prevention and control among health sciences students at University of Namibia, 1071–1078.

Omusati Regional Council. (2010). *Omusati Regional Profile*. Outapi. Retrieved from http://www.omusatirc.gov.na/pdf images/Omusati_Regionall_Profile_2010.pdf

Onwujekwe, O., Uzochukwu, B., Dike, N., Okoli, C., Eze, S., & Chukwuogo, O. (2009). Are there geographic and socio-economic differences in incidence, burden and prevention of malaria? A study in southeast Nigeria. *International Journal for Equity in Health*, *8*(1), 45. http://doi.org/10.1186/1475-9276-8-45

Oriero, E. C., Okebe, J., Jacobs, J., Van, J. P., Nwakanma, D., & Alessandro, U. D. (2015). Diagnostic performance of a novel loop-mediated isothermal amplification (LAMP) assay targeting the apicoplast genome for malaria diagnosis in a field setting in sub-Saharan Africa. *Malaria Journal*, 1–6. http://doi.org/10.1186/s12936-015-0926-6

Papa Mze, N., Ndiaye, Y. D., Diedhiou, C. K., Rahamatou, S., Dieye, B., Daniels, R. F., … Ndiaye, D. (2015). RDTs as a source of DNA to study Plasmodium falciparum drug resistance in isolates from Senegal and the Comoros Islands. *Malar J*, *14*, 373. http://doi.org/10.1186/s12936-015-0861-6

Paris, D. H., Imwong, M., Faiz, A. M., Hasan, M., Yunus, E. Bin, Silamut, K., … Dondorp, A. M. (2007). Loop-mediated isothermal PCR (LAMP) for the diagnosis of falciparum malaria. *American Journal of Tropical Medicine and Hygiene*, *77*(5), 972–976.

Pemberton-Ross, P., Smith, T. a, Hodel, E. M., Kay, K., & Penny, M. a. (2015). Age-shifting in malaria incidence as a result of induced immunological deficit: a simulation study. *Malaria Journal*, *14*(1), 287. http://doi.org/10.1186/s12936-015-0805-1

Pindolia, D. K., Garcia, A. J., Wesolowski, A., Smith, D. L., Buckee, C. O., Noor, A. M., … Tatem, A. J. (2012). Human movement data for malaria control and elimination strategic planning. *Malaria Journal*, *11*(1), 205. http://doi.org/10.1186/1475-2875-11-205

Plowe, C. V. (2007). Combination therapy for malaria: mission accomplished? *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *44*(8), 1075–7. http://doi.org/10.1086/512743

Polley, S. D., González, I. J., Mohamed, D., Daly, R., Bowers, K., Mewse, E., … Programme, M. C. (2013). Highly sensitive detection of malaria parasitemia in an endemic setting:

Performance of a new LAMP kit in a remote clinic in Uganda. *Journal of Infectious Diseases*, *44*(0).

Polley, S. D., Gonzalez, I. J., Mohamed, D., Daly, R., Bowers, K., Watson, J., … Sutherland, C. J. (2013). Clinical Evaluation of a Loop-Mediated Amplification Kit for Diagnosis of Imported Malaria. *Journal of Infectious Diseases*, *208*(4), 637–644. http://doi.org/10.1093/infdis/jit183

Portero, J.-L., Rubio-Yuste, M., Descalzo, M. A., Raso, J., Lwanga, M., Obono, J., … Cano, J. (2010). Accuracy of an Immunochromatographic Diagnostic Test (ICT Malaria Combo Cassette Test) Compared to Microscopy among under Five-Year-Old Children when Diagnosing Malaria in Equatorial Guinea. *Malaria Research and Treatment*, *2010*, 858427. http://doi.org/10.4061/2010/858427

Pulford, J., Hetzel, M. W., Bryant, M., Siba, P. M., & Mueller, I. (2011). Reported reasons for not using a mosquito net when one is available: a review of the published literature. *Malaria Journal*, *10*(1), 83. http://doi.org/10.1186/1475-2875-10-83

Ratovonjato, J., Randrianarivelojosia, M., Rakotondrainibe, M. E., Raharimanga, V., Andrianaivolambo, L., Le Goff, G., … Robert, V. (2014). Entomological and parasitological impacts of indoor residual spraying with DDT, alphacypermethrin and deltamethrin in the western foothill area of Madagascar. *Malaria Journal*, *13*, 21. http://doi.org/10.1186/1475-2875-13-21

Reddy, M. R., Overgaard, H. J., Abaga, S., Reddy, V. P., Caccone, A., Kiszewski, A. E., & Slotman, M. a. (2011). Outdoor host seeking behaviour of Anopheles gambiae mosquitoes following initiation of malaria vector control on Bioko Island, Equatorial Guinea. *Malaria Journal*, *10*(1), 184. http://doi.org/10.1186/1475-2875-10-184

Roayaei, M., & Galehdari, H. (2008). Cloning and Expression of Thermus aquaticus DNA polymerase in Escherichia coli. *Jundishapur Journal of Microbiology*, *1*(1), 1–5.

Roca-Feltrer, A., Schellenberg, J. R. M. A., Smith, L., & Carneiro, I. (2009). A simple method for defining malaria seasonality. *Malaria Journal*, *8*, 276. http://doi.org/10.1186/1475-2875-8-276

Roll Back Malaria. (2010). *Namibia Country Profile*.

Roll Back Malaria Partnership (WHO). (2008). *THE GLOBAL GLOBAL MALARIA MALARIA ACTION PLAN For a malaria free world*. http://doi.org/http://www.rollbackmalaria.org/gmap/gmap.pdf.

Romi, R., Boccolini, D., D’Amato, S., Cenci, C., Peragallo, M., D’Ancona, F., … Majori, G. (2015). Incidence of malaria and risk factors in Italian travelers to malaria endemic

countries. *Travel Medicine and Infectious Disease*, *8*(3), 144–154. http://doi.org/10.1016/j.tmaid.2010.02.001

Rosas-Aguirre, A., Llanos-Cuentas, A., Speybroeck, N., Cook, J., Contreras-Mancilla, J., Soto, V., … Erhart, A. (2013). Assessing malaria transmission in a low endemicity area of north-western Peru. *Malaria Journal*, *12*(1), 339. http://doi.org/10.1186/1475-2875-12-339

Rosas-Aguirre, A., Ponce, O. J., Carrasco-Escobar, G., Speybroeck, N., Contreras-Mancilla, J., Gamboa, D., … Llanos-Cuentas, A. (2015). Plasmodium vivax malaria at households: spatial clustering and risk factors in a low endemicity urban area of the northwestern Peruvian coast. *Malaria Journal*, *14*(1), 1–11. http://doi.org/10.1186/s12936-015-0670-y

Sadasivaiah, S., Tozan, Y., & Breman, J. G. (2007). Dichlorodiphenyltrichloroethane (DDT) for indoor residual spraying in Africa: How can it be used for malaria control? *American Journal of Tropical Medicine and Hygiene*, *77*(SUPPL. 6), 249–263. http://doi.org/77/6_Suppl/249 [pii]

Saiwichai, T., Maneepak, M., Songprakhon, P., Harnyuttanakorn, P., & Nithiuthai, S. (2009). Species-specific Nested PCR for Detecting, *32*(2), 75–81.

Sanders, K., Gueye, C. S., Phillips, A. A., & Gosling, R. (2012). Active Case Detection for Malaria Elimination : a Confusion of Acronyms and Definitions. *Ashdin Publishing*, *1*, 1–5.

Schwartz, E., Sadetzki, S., Murad, H., & Raveh, D. (2001). Age as a risk factor for severe Plasmodium falciparum malaria in nonimmune patients. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *33*(10), 1774–1777. http://doi.org/10.1086/322522

Sema, M., Alemu, A., Bayih, A. G., Getie, S., Getnet, G., Guelig, D., … Pillai, D. R. (2015). Evaluation of non-instrumented nucleic acid amplification by loop-mediated isothermal amplification (NINA-LAMP) for the diagnosis of malaria in Northwest Ethiopia, 1–9. http://doi.org/10.1186/s12936-015-0559-9

Sharp, B. L., Ridl, F. C., Govender, D., Kuklinski, J., & Kleinschmidt, I. (2007). Malaria vector control by indoor residual insecticide spraying on the tropical island of Bioko, Equatorial Guinea. *Malaria Journal*, *6*, 52. http://doi.org/10.1186/1475-2875-6-52

Simon, C., Moakofhi, K., Mosweunyane, T., Jibril, H. B., Nkomo, B., Motlaleng, M., … Haque, U. (2013). Malaria control in Botswana, 2008-2012: the path towards elimination. *Malaria Journal*, *12*, 458. http://doi.org/10.1186/1475-2875-12-458

Singh, B., Bobogare, A., Cox-Singh, J., Snounou, G., Abdullah, M. S., & Rahman, H. A. (1999). A genus- and species-specific nested polymerase chain reaction malaria detection assay for

epidemiologic studies. *American Journal of Tropical Medicine and Hygiene*, *60*(4), 687–692.

Slutsker, L. (2012). Challenges in surveillance and response. *Malaria Journal*, *Conference*(Suppl 1), S1–S2. http://doi.org/10.1186/1475-2875-11-S1-O3

Sluydts, V., Heng, S., Durnez, L., Van Roey, K., Gryseels, C., Lydie, C., … Coosemans, M. (2014). Spatial clustering and risk factors of different plasmodium species in Ratanakiri province Cambodia. *Malaria Journal*, *13*(1), 1–12. http://doi.org/10.1186/1475-2875-13-387

Smith Gueye, C., Gerigk, M., Newby, G., Lourenco, C., Uusiku, P., & Liu, J. (2014). Namibia's path toward malaria elimination: a case study of malaria strategies and costs along the northern border. *BMC Public Health*, *14*(1), 1190. http://doi.org/10.1186/1471-2458-14-1190

Smith Gueye, C., Sanders, K. C., Galappaththy, G. N. L., Rundi, C., Tobgay, T., Sovannaroth, S., … Gosling, R. D. (2013). Active case detection for malaria elimination: a survey among Asia Pacific countries. *Malaria Journal*, *12*(1), 358. http://doi.org/10.1186/1475-2875-12-358

Snounou, G., & Singh, B. (2002). Nested PCR analysis of Plasmodium parasites. *Methods in Molecular Medicine*, *72*, 189–203. http://doi.org/10.1385/1-59259-271-6:189

Somi, M. F., Butler, J. R., Vahid, F., Njau, J. D., Kachur, S. P., & Abdulla, S. (2008). Use of proxy measures in estimating socioeconomic inequalities in malaria prevalence. *Tropical Medicine and International Health*, *13*(3), 354–364. http://doi.org/10.1111/j.1365-3156.2008.02009.x

Sonko, S. T., Jaiteh, M., Jafali, J., Jarju, L. B., D'Alessandro, U., Camara, A., … Saho, A. (2014). Does socio-economic status explain the differentials in malaria parasite prevalence? Evidence from The Gambia. *Malaria Journal*, *13*(1), 449. http://doi.org/10.1186/1475-2875-13-449

Southern Africa Roll Back Malaria Network. (2012). *A situational analysis of Malaria Control in SADC*.

Staedke, S. G., Nottingham, E. W., Cox, J., Kamya, M. R., Rosenthal, P. J., & Dorsey, G. (2003). Short report: proximity to mosquito breeding sites as a risk factor for clinical malaria episodes in an urban cohort of Ugandan children. *The American Journal of Tropical Medicine and Hygiene*, *69*(3), 244–246.

Steinhardt, L. C., Yeka, A., Nasr, S., Wiegand, R. E., Rubahika, D., Sserwanga, A., … Filler, S. (2013). The effect of indoor residual spraying on malaria and anemia in a high-transmission

area of Northern Uganda. *American Journal of Tropical Medicine and Hygiene*, *88*(5), 855–861. http://doi.org/10.4269/ajtmh.12-0747

Strøm, G. E. a, Moyo, S., Fataki, M., Langeland, N., & Blomberg, B. (2014). PCR targeting Plasmodium mitochondrial genome of DNA extracted from dried blood on filter paper compared to whole blood. *Malaria*, *13*(1), 137. http://doi.org/10.1093/trstmh/tru084

Strøm, G. E. a, Tellevik, M. G., Hanevik, K., Langeland, N., & Blomberg, B. (2014). Comparison of four methods for extracting DNA from dried blood on filter paper for PCR targeting the mitochondrial Plasmodium genome. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *108*(8), 488–494. http://doi.org/10.1093/trstmh/tru084

Sturrock, Bousema, T., Mosha, J., & Gosling, R. D. (2012). Finding hotspots: The role of active surveillance methods in malaria control and elimination. *Malaria Journal*, *Conference*(Suppl 1), S2. http://doi.org/10.1186/1475-2875-11-S1-O6

Sturrock, H. J. W., Roberts, K. W., Wegbreit, J., Ohrt, C., & Gosling, R. D. (2015). Tackling Imported Malaria: An Elimination Endgame. *American Journal of Tropical Medicine and Hygiene*, *93*(1), ajtmh.14–0256–. http://doi.org/10.4269/ajtmh.14-0256

Sutcliffe, J. F., & Yin, S. (2014). Behavioural responses of females of two anopheline mosquito species to human-occupied , insecticide-treated and untreated bed nets. *Malaria Journal*, *13*(1), 294.

Tangena, J. A. a, Adiamoh, M., D'Alessandro, U., Jarju, L., Jawara, M., Jeffries, D., … Pinder, M. (2013). Alternative Treatments for Indoor Residual Spraying for Malaria Control in a Village with Pyrethroid- and DDT-Resistant Vectors in The Gambia. *PLoS ONE*, *8*(9), 1–8. http://doi.org/10.1371/journal.pone.0074351

Tanser, F. C., Pluess, B., Lengeler, C., & Sharp, B. L. (2007). Indoor residual spraying for preventing malaria. *Cochrane Database of Systematic Reviews*, (3). http://doi.org/10.1002/14651858.CD006657

Tiemi Shio, M., Eisenbarth, S. C., Savaria, M., Vinet, A. F., Bellemare, M. J., Harder, K. W., … Olivier, M. (2009). Malarial hemozoin activates the NLRP3 inflammasome through Lyn and Syk kinases. *PLoS Pathogens*, *5*(8). http://doi.org/10.1371/journal.ppat.1000559

Tumwiine, J., Mugisha, J. Y. T., & Luboobi, L. S. (2007). On Oscillatory Pattern of Malaria Dynamics in a Population with Temporary Immunity. *Computational and Mathematical Methods in Medicine*, *8*(3), 191–203. http://doi.org/10.1080/17486700701529002

Tusting, L. S., Willey, B., Lucas, H., Thompson, J., Kafy, H. T., Smith, R., & Lindsay, S. W. (2015). Socioeconomic development as an intervention against malaria: a systematic review

and meta-analysis. *The Lancet*, *382*(9896), 963–972. http://doi.org/10.1016/S0140-6736(13)60851-X

UNHCR. (2010). *Distribution of Long-lasting Insecticide Treated Nets in Refugee Situations*.

Vale, N., Aguiar, L., & Gomes, P. (2014). Antimicrobial peptides: a new class of antimalarial drugs? *Frontiers in Pharmacology*, *5*(December), 1–13. http://doi.org/10.3389/fphar.2014.00275

Vallejo, A. F., Martínez, N. L., González, I. J., Arévalo-Herrera, M., & Herrera, S. (2015). Evaluation of the Loop Mediated Isothermal DNA Amplification (LAMP) Kit for Malaria Diagnosis in P. vivax Endemic Settings of Colombia. *PLoS Neglected Tropical Diseases*, *9*(1), e3453. http://doi.org/10.1371/journal.pntd.0003453

Veron, V., & Carme, B. (2006). Short report: Recovery and use of Plasmodium DNA from malaria rapid diagnostic tests. *American Journal of Tropical Medicine and Hygiene*, *74*(6), 941–943.

Walker, N. F., Nadjm, B., & Whitty, C. J. (2014). Malaria. *Medicine*, *42*(2), 100–106.

White, N. (2011). The parasite clearance curve. *Malaria Journal*, *10*(1), 278. http://doi.org/10.1186/1475-2875-10-278

WHO. (2011). *Progress report on the implementation of regional committee resolution AFR/RC59/R3 on accelerated malaria control*.

WHO. (2012a). *Handbook for integrated vector management* (Vol. 24). http://doi.org/10.1564/v24_jun_14

WHO. Moving towards sustainable elimination in Cape Verde (2012). http://doi.org/10.1126/science.1240539

WHO. (2012c). *World Malaria Report 2012*.

WHO. (2014). WHO recommendations for achieving universal coverage with long-lasting insecticidal nets in malaria control. *WHO*, (September 2013).

Wickramage, K., Premaratne, R. G., Peiris, S. L., & Mosca, D. (2013). High attack rate for malaria through irregular migration routes to a country on verge of elimination. *Malaria Journal*, *12*(1), 276. http://doi.org/10.1186/1475-2875-12-276

Wilhelm, M. (2012). *The Impact of Climate Change in Namibia- A Case Study of Omusati Region*.

Williams, G. S., Mweya, C., Stewart, L., Mtove, G., Reyburn, H., Cook, J., … Drakeley, C. J. (2009). Immunophoretic rapid diagnostic tests as a source of immunoglobulins for

estimating malaria sero-prevalence and transmission intensity. *Malaria Journal*, *8*, 168. http://doi.org/10.1186/1475-2875-8-168

Winskill, P., Rowland, M., Mtove, G., Malima, R. C., & Kirby, M. J. (2011). Malaria risk factors in north-east Tanzania. *Malaria Journal*, *10*(1), 98. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21507217

World Health Organization. (2005). *The Roll Back Malaria strategy for improving access to treatment through home management of malaria*. *World Health Organization*.

World Health Organization. (2009). Elimination of malaria. *World Malarial Report 2009*, 45–56. Retrieved from http://www.who.int/malaria/world_malaria_report_2009/mal2009_rep_chap5_0040.pd

World Health Organization. (2011). World Malaria Report 2011 Briefing on Revised Estimates of Cases and Deaths, 25–26.

World Health Organization. (2014). *World Malaria Report 2014*. Geneva.

Worrall, E., Basu, S., & Hanson, K. (2003). The relationship between socio-economic status and malaria : a review of the literature. *World*, (January), 1–46. http://doi.org/10.1080/0013191500020305

Xiaodong, S., Tambo, E., Chun, W., Zhibin, C., Yan, D., Jian, W., … Xiaonong, Z. (2013). Diagnostic performance of CareStart[TM] malaria HRP2/pLDH (Pf/pan) combo test versus standard microscopy on falciparum and vivax malaria between China-Myanmar endemic borders. *Malaria Journal*, *12*(1), 6. http://doi.org/10.1186/1475-2875-12-6

Yadav, K., Dhiman, S., Rabha, B., Saikia, P., & Veer, V. (2014). Socio-economic determinants for malaria transmission risk in an endemic primary health centre in Assam, India. *Infectious Diseases of Poverty*, *3*(1), 19. http://doi.org/10.1186/2049-9957-3-19

Yan, J., Li, N., Wei, X., Li, P., Zhao, Z., Wang, L., … Fan, Q. (2013). Performance of two rapid diagnostic tests for malaria diagnosis at the China-Myanmar border area. *Malaria Journal*, *12*(1), 73. http://doi.org/10.1186/1475-2875-12-73

Youssef, R. M., Alegana, V. a, Amran, J., Noor, a M., & Snow, R. W. (2010). Fever prevalence and management among three rural communities in the North West Zone, Somalia. *Eastern Mediterranean Health Journal*, *16*(6), 595–601. Retrieved from http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=med5&AN=20799585 http://digitaal.uba.uva.nl:9003/uva-linker?sid=OVID:medline&id=pmid:20799585&id=doi:&issn=1020-3397&isbn=&volume=16&issue=6&spage=595&pages=595-601&date=2010&title=Easte

8. APPENDICES

Appendix 1: English Consent form

UNIVERSITY OF NAMIBIA / NATIONAL MALARIA CONTROL PROGRAMME
CONSENT TO BE IN RESEARCH

Study Title: Epidemiology of border malaria in Namibia

This is a medical research study, and you do not have to take part. The study coordinator from the University of Namibia/National Malaria Control Programme will explain this study to you. If you have any questions, you may ask the research coordinator.

You are being asked to take part in this study either because you have been diagnosed with malaria or you are a healthy individual with whom we can compare results. In this study, the researchers are collecting blood samples to learn more about the causes of malaria in this area. By looking at who does and does not have malaria, it is possible to find out why the disease is only occurring in some people. This information can then be used to better target control measures in those areas most at risk. About 7020 people will give blood samples for this research. The Bill and Melinda Gates Foundation is paying for this research. The sponsors of this study cannot influence the results of this study.

What will happen if I take part in this study?

If you agree to be in this study, we will prick your finger to take a small amount of blood. This will take about one minute. The blood will be stored in laboratories in Windhoek and will be analyzed in Namibia and the USA for malaria parasites. We will also ask you a series of questions about your recent travel history, your household structure and other factors that might influence whether you are at risk of malaria.

How long will I be in the study?

Participation in the study will take a total of about 30 minutes.

Are there risks?

The finger prick may hurt a little. There is a small risk of bruising, and a rare risk of infection.

Are there benefits?

If you have a fever we can test you for malaria and refer you to a hospital if necessary. The results of this study may help the National Malaria Control Programme to develop better strategies to prevent malaria in this area. There is no other direct benefit to you. The blood will be used only for research into malaria.

Can I say "No"?

Yes, you do not have to donate a blood sample for this study.

Will my information be kept confidential?

We will do our best to protect the information we collect from you and your medical record. Information which identifies you will be kept secure and restricted. However, your personal information may be given out if required by law. If information from this research is published or presented at scientific meetings, your name and other identifiers will not be used. Information

which identifies you will be destroyed when this research is complete. The following organizations may look at information about you in your medical and research records: University of Namibia, University of California San Francisco, London School of Hygiene and Tropical Medicine.

Are there any costs or payments?
No. You will neither be paid nor charged to donate a blood sample.

What if I get injured?
Tell the study coordinator if you feel that you have been injured because of being in this research.

Treatment and Compensation for Injury: If you are injured as a result of being in this study, treatment will be available. The costs of the treatment may be covered by the University of California depending on a number of factors. The University does not normally provide any other form of compensation for injury. For further information about this, you may call the office of the Committee on Human Research at +1 415- 476-1814.

Who can answer my questions about the study?
You can talk to the study coordinator(s) about any questions, concerns, or complaints you have about this study. Contact the study coordinator Joyce Auala at 081 442 5739/ 081 207 3473.
If you wish to ask questions about the study or your rights as a research participant to someone other than the researchers or if you wish to voice any problems or concerns you may have about the study, please call the Office of the Committee on Human Research at +1 415-476-1814 or Chris Lourenco (Tel: 081 692 9940), the malaria elimination analyst at the Southern Africa Malaria Elimination Support Team or Stark Katokele (Tel: 081 292 8754), the National Malaria Control Program deputy manager in Namibia. You have been given copies of this consent form and the Experimental Subject's Bill of Rights to keep.

If you wish to be in this study, please sign or provide a thumb print below.

____________ ________________________________
Date Participant's Signature/Thumb Print for Consent

____________ ________________________________
Date Person Obtaining Consent

____________ ________________________________
Date Witness signature (if participant does not speak/read English)

Appendix 2: Oshiwambo Consent form

(Oshikwanyama)

OUNIVEESITI YANAMIBIA/OPROGRAMA YOPASHIWANA YEKONDOLOLO LOMALARIA
UNIVERSITY OF NAMIBIA/NATIONAL MALARIA CONTROL PROGRAMME

EPITIKILO LOKUKALA MOMAPEKAPEKO
CONSENT TO BE IN RESEARCH

Oshipalanyole shElihongo: **Eshiivo leengaba domukifi woMalaria muNamibia**
Study Title: *Epidemiology of border malaria in Namibia*

Eshi osho elihongo lomapekapeko opaunamiti, na ito pumbwa okukufa ombinga musho. Omuunganeki welihongo wokoUniveesiti yaNamibia/Oprograma yoPashiwana yEkondololo loMalaria ote ku fatulule elihongo eli. Ngeenge ou na epulo lasha, oto dulu yoo okupula omuunganeki womapekapeko.

Oto pulwa u kufe ombinga melihongo eli ngeenge pamwe owa monika omalaria ile u na oukolele, opo tu dule okuyelekanifa naye oidjemo. Melihongo eli, ovakonakoni otava ongele omalolelo eehonde, opo ve li honge shihapu kombinga yoshietifi shoMalaria moshitukulwa osho. Okutala ou a kwatwa naao i na kwatwa komalaria, oshipu okumona kutya omolwashike omukifi ou ha u monika ashike movanhu vamwe. Omauyelele aa otaa dulu okulongifwa, opo ku ningwe omakondololo meukililo moitukulwa oyo i li monghalo yefyo. Ovanhu konyala 7020, otava ka yandja omalolelo ohonde komapekapeko aa. Ehangano *loBill and Melinda Gates Foundation,* olo tali futu omapekapeko aa. Ovafutili velihongo eli itava dulu okunwefa mo oidjemo yelihongo eli.

Oshike tashi holoka po ngeenge nda kufa ombinga melihongo eli?
Ngeenge owa dimina okukala melihongo eli, ohatu ku tu komunwe woye tu kufe mo eta lohonde. Eshi otashi pula konyala omunute umwe. Ohonde otai ka longifwa opo u konakonwe omalaria. Ohonde otai ka tuvikilwa molabora yomOvenduka notai ka dongokununwa mokukonga mo oupuka womalaria omu moNamibia noko-USA. Ohatu ke ku pula yo oupulo vamwe kombinga yomalweendo oye mefimbo lapita, omutungilo weumbo loye naikwao ya wedwa po oyo tai holola kutya onghalo yoye oi li moshiponga komalaria.

Oule u fike peni handi ka kala melihongo?
Omukufimbinga melihongo ota kufa konyala oule wominute 30.

Omu na omalixupulo eemwenyo?

Ekufo lohonde komunwe ohali yehameke kanini. Opu na okalixupulomwenyo kanini ketunhilo nokalixupulomwenyo kanafangwa kekwato lombuto.

Omu na omalikolo?

Ngeenge owa monika omalaria, oto pangwa diva komupangi. Oidjemo yelihongo eli otai ka kwafela Oprograma yoPashiwana yEkondololo lyoMalaria okulimonena eemhito diwa mekondjifo lomalaria moshitukulwa eshi. Ovakonakoni otava ka yandja wo omauyelelehongo nhumbi mu na okuliamena komalaria. Kamu na omalikolo amwe e ku yukilila. Ohonde otai ka longifwa ashike momapekapeko oMalaria.

Ohai dulu okutya "Ahowe"?

Heeno, oto dulu okukala inoo yandja omalolelo ohonde kelihongo eli.

Omauyelele ange otaa dulu tuu okukalekwa meameno?

Ohatu ka fya noshisho okwaamena omauyelele, oo twa kufa kwoove nomavalulohokololo oye opaunamiti. Omauyelele taa ku hollola, otaa tulwa meameno nomengambeko. Nande ongaho, omauyelele opaumwene otaa dulu yoo okuyandjwa ngeenge okwa pumbiwa paveta. Omauyelele aa omapekapeko ngeenge okwa nyanyangidwa ile a yelifwa moyoongalele yopaunongononi, Edina loye namadina aavo ve ku holola itaa ka longifwa. Omauyelele oo ta ku holola otaa ka pombolwa po konima eshi epekapeko eli la pwa. Omalutu taa shikula otaa dulu okukonga omauyelele momavalulohokololo oye opaunamiti nomomapekapeko: *University of Namibia, University of California San Francisco, London School of Hygiene and Tropical Medicine.*

Otapa futwa sha ile tapa yandjwa eefuto?

Ahowe. Ove ito dulu okufutwa ile u futifwe eshi to yandje omalolelo ohonde.

Ongahelipi ngeenge onda yehamekwa?

Shiivifila omuunganeki welihongo ngeenge ou udite wa yehamekwa momapekapeko aa.

Ouhaku neefuto kOmayehameko:

Ngeenge owa yehamekwa eshi u li melihongo eli, ouhaku otau monika. Eefuto douhaku otadi dulu okufutwa kOuniveesiti yaCalifornia she likolelela komivalu doiningifi. Ouniveesiti ihai yandje naanaa omikalo dimwe domafutilo komayehameko.

Omauyelele a wedwa po kombinga ei, dengela kombelewa yOkomiti i na sha *noHuman Research,* konomola: + 1 415-476-1814.

Olye ta dulu okunyamukula omapulo ange kombinga yelihongo?

Oto dulu okupopya nomuunganeki (ovaunganeki) welihongo kombinga yepulo keshe, omalimbililo ile omanyenyeto oo u na kombinga yelihongo eli. Monafana nomuunganeki welihongo, Joyce Auala, konomola: 081 442 5739/081 207 3473. Ngeenge ou na omapulo kombinga yelihongo ile wa hala okulongifa oufemba woye ongomukufimbinga momapekapeko okupula umwe e lili ehe shi ovaunganeki, ile ngeenge owa hala okuholola udjuu keshe ile omaliudo omalimbililo oo u na kombinga yelihongo, alikana dengela kongodi yomombelewa yOkomiti *yoHuman Research,* konomola: + 1 415-476-1814 ile kuChris Lourenco (Tel: 081 692 9940), omudongokononi nomuxulifipo womalaria mokangudu ko*Southern Africa Malaria Elimination Support Team*, ile kuStark Katokele (Tel: 081 292 8754), omuwiliki wopedu muNamibia wOprograma yoPashiwana yEkondololo loMalaria. Owa pewa eekopi dofooloma yepitikilo nosho yo okukala nofooloma yedina: *Experimental Subject's Bill of Rights*.

Ngeenge owa hala okukala melihongo eli, alikana shaina ile tula po oshihako shomunwe wakula wokomake okuyuka pedu.

___________ ____

Efiku Eshainokasha lomukufimbinga / Oshihako shOmunwe wakula onga Epitikilo

___________ ____

Efiku Omunhu oo e na oufemba wEpitikilo

___________ ____

Efiku Eshainokasha lOmbangi (ngeenge omukufimbinga iha popi / lesha Oshiingilisa)

Appendix 3: Sample Questionnaire

1. Sleeping structures

Main material of the floor
- Natural floor:
  - Earth/sand
  - Dung
- Rudimentary floor
  - Wood planks
  - Palm/bamboo
- Finished floor
  - Parquet/Polished wood
  - Vinyl or Asphal strips
  - Ceremic tiles
  - Cement
  - Carpet
  - Other

Main Material of exterior floor
- Natural wall
  - Grass
  - Cane/trucks/bamboo/reed
- Rudimentary wall
  - Bamboo/wood with mud
  - Stone with mud
  - Plywood
  - Carton
  - Reused wood
- Finished wall
  - Cement
  - Stone with lime/cement
  - Bricks
  - Mud blocks
  - Wood planks/shingles
  - Other

When was the last time this structure was replastered or painted?
- Less than 1 year ago
- More than a year ago
- Not sure
- Never painted

Type of Windows
- With Glass
- No Windows
- Without any cover
- With cloth/screen
- With wooden
- With metal
- Other covers

Type of door
- Doorway without any cover
- Doorway with cloth/screen
- Doorway with metal door
- Other types of doors

Is there open space between the roof and the walls?
- Yes
- No
- Don't know

Indoor residual spraying
- Yes
  - *(if Yes)* Hurt Card present
    - Yes/No
  - *(if yes)* Who sprayed the house?
    - Government worker/program
    - Private company
    - Don't know
    - Others
- No
  - *(if No)* What are some of the reasons why spray operators visited the house but did not spray all or part of your household?
    - Did not know the reason for spraying (Y/N)
    - Did not know they were coming (Y/N)
    - Do not like fumes (Y/N)
    - Do not like the effect on appearance o f the walls (Y/N)
    - Afraid of health effect on those living in house (Y/N)
    - Malaria no longer an issue in this area (Y/N)
    - All of my household was sprayed (Y/N)
    - No one came to spray my household (Y/N)
    - Others (*specify others*) (Y/N)
- Don't know

1. Add Individual

Name
Code
Age
Sex
- [Male/Female]

Guest
- [Yes/No]

Did (name) sleep here last night?
- [Yes/No]

Did (name) sleep under a mosquito net last night?
- [Yes/No]

Relationship to the household head:
- Head
- Wife/Husband
- Son/daughter
- Son-in-law/daughter-in-law
- Grandchild
- Parent
- Parent-in-law
- Brother/sister
- Other relatives
- Adopted/foster/stepchild
- Not related
- Don't know

Consent granted?
- Yes
  - Dry Blood Spot (DBS) [done/not present/refused]
  - RDT results [Positive/Negative/Not valid/Not done]

Reported fever in last 48 hours? [Yes/No]
Did you sleep outside last night (10pm-6am)? [Yes/No/Don't know]
Where you outside for any other reason last night (10pm-6am)? [Yes/No/Don't know]
The last time you had a fever, did you seek treatment for it? [Yes/No/Never had a fever]
- *If yes,* where did you go for treatement?
  - Government hospital/clinic
  - Private clinic
  - Private pharmacist
  - Traditional healer
  - Other
- Why did you use this place for treatment?
  - Cheapest
  - Closest
  - Best service
  - Other
- *If No,* why did you not seek for treatment?
  - Treatment is too expensive

Travel is too expensive

Health facility too far away

Fever not thought to be serious

Have medicines at home

Other

Have you been diagnosed with malaria in the past 2 weeks?

[Yes/No/Don't know]

Have you taken any drugs in the last two weeks?

Coartem/ACT

Chloroquine

SP/Fansidar

Quinine

Other

None

Have you spent any night outside this district in the last 6 weeks?

[Yes/No/Don't know]

No

Not present

1. Household

Index Household? [Yes/No]

Urban or rural area? [Urban/Rural]

2. New Neighbourhood

Case or Control [Case/Control]

Any breeding sites found nearby? [Yes/No]

3. Neighbourhood sites

4. Mosquito net ownership & usage

Net Condition?

Good condition

Average (a few small holes)

Ask respondent to show you the nets in the structure that they sleep in?

Net hanging

Net used or still in bag

Net taken during the day

Not observed

How long ago did you obtain the mosquito net?

Less than 6 months ago

6-12 months ago

1-3 years ago

More than 3 years ago

Don't know

Where did you obtain the net?

Government clinic/hospital

Distribution at home?

Community health worker

Retail shop/market

Pharmacy

Work place

Don't know

Others

Was the net purchased?

[Yes/No/Don't know]

Brand of the mosquito net?

Olyset

Lifenet

Permanet

More than 3 years ago

Don't know

When you go the net, was it already factory-treated with an insecticide to kill or repel mosquitoes?

[Yes/No/Not sure]

Since you got the mosquito net, was it ever soaked or dipped in a liquid to repel mosquito bugs?

[Yes/No/Not sure}

How long was the net last soaked or dipped?

- Less than a month
- More than 2 years ago
- Never soaked
- Not sure

1. Socio-economic indicators

What is the main source of drinking water for members of your household?

- If piped water
  - Piped into dwelling
  - Piped into yard/standpipe
  - Public tap/standpipe
  - Borehole
- If dug well
  - Protected well
  - Unprotected well
- If water from spring
  - Protected spring
  - Unprotected spring
  - Rain water
  - Tanker truck
- If surface water
  - Dam
  - River
  - Lake
  - Pond
  - Stream
  - Canal/irrigation channel
  - Bottle water

What kind of toilet facilities does your household use?

- Flush/pour flush toilet
- Ordinal toilet
- Ventilated improved pit latrine
- Pit latrine with slab
- Composite toilet
- No facility/bush/field
- Others

Does your household have?

- Electricity [Yes/No]
- Radio [Yes/No]
- Television [Yes/No]
- Mobile phone [Yes/No]
- Non-mobile phone [Yes/No]
- Refrigerator [Yes/No]
- Stove [Yes/No]

What type of fuel does your household mainly use for cooking?

- Electricity
- Charcoal
- Wood
- Gas
- Paraffin
- Coal
- Straw/shrub/grass
- No food cooked
- Others

Does any member of your household own?

- Bicycle [Yes/No]
- Motorcycle/motor scooter [Yes/No]
- Car/truck [Yes/No]
- Donkey [Yes/No]
- Tractor [Yes/No]

1. Mosquito nets

Does your household have any mosquito nets used while sleeping?[Yes/No/Don’t know]

Has the community health worker in your village ever helped hang a mosquito net in the house?

[Yes/No/Don’t Know]

Are there instances when children do not sleep under a mosquito net?

[Yes/No or N/A]

Why do children sometimes not sleep under a mosquito net?

- Always sleep under a net
- Too hot
- Too cold
- Child cries
- Child afraid
- Not enough nets
- Net not hung up
- Used by adults
- Net dirty
- Net bad for children’s health
- Not needed in dry season
- Don’t know
- Others

Are there instances when adults in the household do not sleep under a mosquito net?

[Yes/No]

If No, why do the adults sometimes not sleep under a mosquito net?

- Always sleep under a net
- Too hot
- Too cold
- Net not hung up
- Used by children

Appendix 4: Carestart RDT Manufactures guideline

REF G0131

CareStart™ Malaria HRP2/pLDH (Pf/PAN) Combo

A rapid test for the detection of malaria HRP2 and plasmodium LDH in human blood.

Intended Use

For the rapid qualitative detection of malaria histidine-rich protein 2 (HRP2) (*P. falciparum*) and lactate dehydrogenase (pLDH) (*P. falciparum, P. vivax, P. ovale* and *P. malariae*) in human blood as an aid in the diagnosis of malaria infection.

Summary

Malaria is a serious parasitic disease characterized by fever, chills, and anemia and is caused by a protozoan parasite that is transmitted to humans through biting of infected *Anopheles* mosquitoes. There are four types of human malaria: *Plasmodium falciparum, P. vivax, P. ovale,* and *P. malariae.* In humans after an infected mosquito biting, the parasites (called sporozoites) migrate to the liver where they mature and are released into blood stream, infecting red blood cells. Malaria infection occurs in more than 90 countries worldwide, but is mostly prevalent in sub-Saharan Africa. It is estimated that there are over 500 million clinical cases and 2.7 million malaria-caused deaths per year.

The *CareStart*™ Malaria HRP2/pLDH (Pf/PAN) Combo contains a membrane strip, which is pre-coated with two monoclonal antibodies as two separate lines across a test strip. One monoclonal antibody (test line 2) is PAN specific to lactate dehydrogenase (pLDH) of the *Plasmodium* species (*P. falciparum, P.vivax, P.malariae, P.ovale*) and the other line (test line 1) consists of a monoclonal antibody specific to histidine-rich protein 2 (HRP2) of the *P. falciparum.* The conjugate pad is dispensed with antibodies absorbed on gold particles, which are specific to pLDH of PAN and specific to HRP2 of *P. falciparum.*

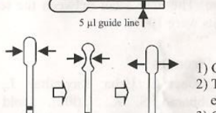
The *CareStart*™ Malaria HRP2/pLDH (Pf/PAN) Combo is designed for the differential diagnosis of *P. falciparum, P.vivax, P.malariae,* and *P.ovale* infection.

Materials Provided

CareStart™ Malaria HRP2/pLDH (Pf/PAN) Combo contains the following items:

Test Device (Test device sealed in aluminum pouch with desiccant)
Instructions for Use
Assay Buffer (Borax buffered SDS and saponin solution)
Option: sample pipette / lancet / alcohol pad

- Sample pipette description and instruction -



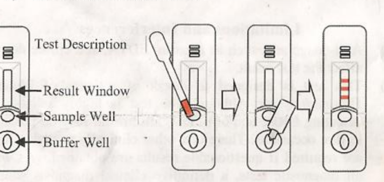
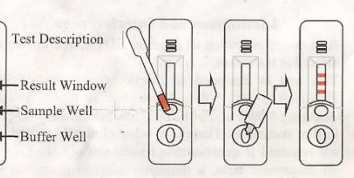
Warnings and Precautions

The following requirements must be observed:

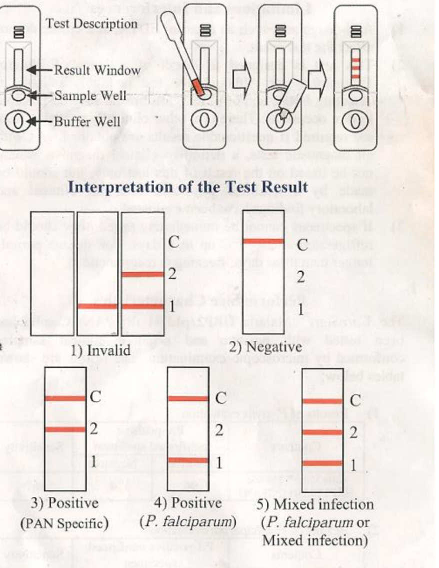
1) For in vitro diagnostic and professional use only.
2) Read provided instructions for use before using the test kit.
3) Observe storage condition indicated on the aluminum pouch and outer package.
4) Do not use components from other lots.
5) Use the test device and optional components (lancet / alcohol pad) immediately after opening its package.
6) Use disposable protective gloves while handling potentially infectious materials and performing the assay. Wash hands thoroughly afterwards.
7) Do not swallow the Assay Buffer solution.
8) Do not use the kit beyond the expiration date that is indicated on the outer package.
9) All provided materials are for single-use, do not re-use any of contents.
10) Do not eat or smoke while handling specimens.
11) Clean up spills thoroughly using an appropriate disinfectant.
12) The test procedure, precautions and interpretation of results for this test must be closely followed when testing.
13) If the test kit is stored in a refrigerator (Do not freeze the test kit), the devices need to equilibrate to room temperature prior to use.
14) Do not use, if lancet cap is loose and/or if package of alcohol pad or device pouch is damaged.

Test Procedure

1) Clean the area to be pierced with an alcohol pad.
2) Squeeze the end of a fingertip and pierce it using venipuncture or provided lancet.
3) Wipe out the first drop of blood with sterile gauze or cotton. (Not provided.)
4) Collect the blood sample (5 µl) using pipette provided or micro-pipette.
5) Add whole blood (5 µl) to the sample well.
6) Add assay buffer 2 drops (60 µl) to the buffer well.
7) Read result in 20 minutes.

Interpretation of the Test Result



Appendix 5: Eiken PAN/Pf LAMP guidelines

380201-A

For performance evaluation | REF LMP561

Loopamp™ MALARIA Pan Detection Kit

INTENDED USE

The Loopamp™ MALARIA Pan Detection Kit is for qualitative detection of *Plasmodium* DNA extracted from human blood samples.

TEST PRINCIPLES

This product is based on the nucleic acid amplification method, LAMP (Loop-mediated Isothermal Amplification), developed by Eiken Chemical Co., Ltd.

The LAMP method has the following characteristics: (1) Only one polymerase enzyme is required and the amplification reaction proceeds under isothermal conditions;[1),2)] (2) it has extremely high specificity because of the use of four primers recognizing six distinct regions on the target; (3) it has high amplification efficiency and can produce a high concentration of amplified product in a short time, which makes visual or automated detection possible.[3),4)]

The Malaria Pan (genus)-specific primers provided with this product have been designed to detect the mitochondrial DNA of the four most widespread *Plasmodium* species causing malaria (*P. falciparum, P. vivax, P. ovale* and *P. malariae*). The targeted DNA sequences have been confirmed by alignment analysis to have well-conserved base sequence in all these *Plasmodium* species.

The test DNA solution extracted from blood samples is dispensed into a reaction tube. The strand displacement DNA polymerase, deoxynucleotide triphosphates (dATP, dCTP, dGTP and dTTP), calcein, reaction buffers and Malaria Pan (genus)-specific primers are stored in dried form in the cap of the reaction tube. These dried LAMP reagents (Malaria Pan detection reagents (dMAL Pan)) dissolve when the DNA solution is added. The reaction tube is then incubated at 65.0°C and the DNA is amplified by the strand displacement DNA polymerase in accordance with LAMP reaction.

The detection of amplified products is based on turbidimetric measurement of magnesium pyrophosphate (a white precipitate produced as a by-product of DNA amplification).[3)] Alternatively, visual detection under ultraviolet light may be used. Before DNA amplification, calcein contained in the reagent is in the quenched state as it is bound to manganese ions. At the start of DNA amplification, the pyrophosphate ions that are generated out-compete the manganese ions for binding sites, and thus the calcein becomes fluorescent.[4)]

CONTENTS OF THE KIT

Reagents are stable until the date on the label assuming the container remains unopened within a storage temperature of 1 – 30°C.

Malaria Pan detection reagent (dMAL Pan) ················ 10 X 48 tubes

The following reagents in dried form are contained in each reaction tube:

- *Bst* DNA polymerase*1
- Deoxynucleotide triphosphates
- Magnesium sulfate
- Calcein
- Manganese chloride
- Primers*2

Positive control Mal (PC Mal)*3 ···························· 5 X 1.0 mL

Negative control Mal (NC Mal) ·························· 20 X 0.5 mL

*1: *Bst* DNA polymerase derived from *Bacillus stearothermophilus* is a strand displacement DNA polymerase that lacks 5'→3' exonuclease activity.

*2: Primers designed for the mitochondria DNA of *Plasmodium* parasites, purified from synthesized oligonucleotides by HPLC.

*3: PC Mal contains a product resulting from *in vitro* amplification of an artificial gene designed from the mitochondrial DNA of *Plasmodium falciparum* (GenBank No.M76611).

The abbreviations of names of the following reagents, their Lot No. and the manufacturer (EKN), are printed on the containers.

Reagents	Labelling on the tube	Code on the cap
Positive control Mal	PC Mal Lot No., EKN	PC Mal
Negative control Mal	NC Mal Lot No., EKN	NC Mal

WARNINGS AND PRECAUTIONS

(1) For performance evaluation only. Do not use for other purposes including for any medical purpose such as patient diagnosis or patient care decisions.
(2) This product is designed only for detection of DNA of *P. falciparum, P. vivax, P. malariae and P. ovale* parasites in blood samples of human origin. Do not use for other purposes.
(3) When using this product, always follow this package insert.
(4) Do not freeze the reagents.
(5) Do not use any expired reagent.
(6) Do not mix reagents from different lots.
(7) Do not replenish any reagent.
(8) Performance of the Loopamp™ MALARIA Pan Detection Kit is dependent on operator proficiency and adherence to procedural directions. Testing should be performed by properly trained personnel strictly according to the instructions provided.
(9) Exposure to heat, humidity and light may cause deterioration of the dMAL Pan. Remove only the required number of reaction tubes (number of samples + number of controls) and re-seal the aluminium pouch immediately.
(10) Do not remove the desiccant from the aluminium pouch. High humidity can cause deterioration of the dried LAMP reagent in the reaction tubes.
(11) Read the instruction manual and ensure required equipment (turbidimeter or incubator) is available before commencing the procedure.
(12) Blood samples pose a potential risk for infection. Use universal precautions to minimize biohazard.[5)]
(13) PC Mal and NC Mal both contain a small amount of sodium azide as preservative. Sodium azide is classified as toxic. Avoid any contact with eyes, mouth, or skin.
(14) In case of accidental contact of any reagent with eyes, mouth, or skin, immediately rinse the affected site with running water and seek medical advice.
(15) Do not dilute or add the PC Mal to the samples. Use the PC Mal only as described in this package insert in order to avoid DNA contamination.
(16) Store the PC Mal and any positive blood samples separately from the other kit reagents.
(17) The cap of each reaction tube contains dMAL Pan in dried form. Do not touch the inside of the cap.
(18) Before using the reaction tubes, check carefully to see if they have any cracks or scratches. Damaged tubes might give false results and lead to DNA contamination of the incubator and work area.
(19) Do not expose reaction tubes to UV light before the end of the LAMP reaction. Prolonged exposure to UV light might damage the tubes and lead to false results.
(20) When a UV lamp is used for visual fluorescence judgment, do not stare directly at UV light. Since UV light is harmful to the eyes, even watching for a short period may irritate eyes and cause symptoms similar to conjunctivitis. Use a glass screen or wear protective goggles/glasses/face shield whenever looking directly at the UV lamp.
(21) Refer to the manual of the incubator. When the LF-160 or the real-time turbidimeter is used, be careful when removing the reaction tubes from the incubator to avoid burns.

WASTE DISPOSAL

(1) Do not open the tubes after DNA amplification. Leave the cap closed and dispose of the used tubes as medical waste by incineration or after double bagging with sealable plastic bags.
(2) Never autoclave or re-use the reaction tubes. Amplified products will disperse and cause contamination.

(3) The main material for the reaction tubes and reagent tubes is polypropylene (PP); for the reaction tube tray, polyethylene (PET); for the aluminium pouch, aluminium; and for the kit case, paper.
(4) Dispose of any other reagent, container, or lab ware in accordance with regulations.

SPECIMEN COLLECTION

(1) Blood samples should be used immediately after collection.
(2) Collect blood in a separate room from the LAMP amplification room. Aerosols containing *Plasmodium* DNA can be generated during blood collection and may cause contamination.
(3) DO NOT USE EDTA and Citrate as anticoagulant for blood collection if the result is to be read by fluorescence. The use of heparin as anticoagulant is recommended.

MATERIALS REQUIRED BUT NOT PROVIDED

- Loopamp™ PURE DNA Extraction Kit (REF LMC802) (optional)

For Visual fluorescence detection

(For LF-160 incubator)

- LF-160 (REF MVKM17)

(For other incubator using UV lamp)

- Incubator (temperature accuracy: ±0.5°C; with hot bonnet)
- Heating block
- UV lamp (wavelength: 240 to 260 nm, and 350 to 370 nm)
- Goggles/glasses or other UV-blocking eye mask

For real-time turbidity detection

- Real-time turbidimeter LA-500 (REF MVL300) (only for use in the LAMP method; wavelength: 600 to 700 nm; amplification temperature: 65.0°C)

For reagent and sample mixing

- Micropipettes (10 to 100 µL, and 20 to 200 µL) and pipette tips with filter
- Centrifuge for micro-tubes (optional)
- Centrifuge for eight connected tubes (optional)

PREPARATION OF SAMPLE DNA SOLUTION

The following DNA extraction methods are recommended.

(For boil and spin)

To extract the DNA from blood sample, follow the standard operating procedures published at http://www. finddiagnostics.org/programs/malaria-afs/lamp/standard_procedures/index.html.

(For PURE)

To extract the DNA from blood sample, follow the instruction for the Loopamp™ PURE DNA Extraction Kit[6] (sold separately). Use the DNA solution obtained for LAMP amplification.

PREPARATION OF REAGENTS

(1) Malaria Pan detection reagent

Remove the required number of tubes from the aluminium pouch and put them in the rack provided. (number of samples + number of controls).

Note: After removing the required tubes, seal the aluminium pouch with any unused tubes immediately.

(2) Negative control Mal (NC Mal)

Flick (or spin) down the tube before using, in order to collect the content at the bottom of the tube.

Note: NC Mal should be used with every run.

(3) Positive control Mal (PC Mal)

Flick (or spin) down the tube before using, in order to collect the content at the bottom of the tube.

Note: PC Mal should be used with every run.

MEASUREMENT PROCEDURE

Reagent and sample mixing

(1) Turn on the incubator or the real-time turbidimeter.
(2) Dispense 30µL of extracted DNA solution into a reaction tube using the pipette, and close the cap.
(3) Dispense 30 µL of NC Mal into a reaction tube using the pipette, and close the cap.
(4) Dispense 30 µL of PC Mal into a reaction tube using the pipette, and close the cap.
(5) Flick (or spin) down all tubes to collect the solution at the bottom of the tubes.

Note: When using the PURE device make sure the liquid level is closer to the upper line of the two lines on a reaction tube to ensure that the correct volume has been dispensed.

(6) Reconstitute the dried reagent in the cap by inverting the reaction tubes and collecting the DNA solution in the cap. Leave the tubes standing upside down for 2 minutes to reconstitute the dried reagent.
(7) Invert the reaction tubes five times to mix the contents. Make sure that the dried reagent in the cap is fully dissolved (the solution should have a slight orange colour).
(8) Flick (or spin) down all tubes to collect the solution at the bottom of the tubes.

Amplification

For visual fluorescence detection

(for LF-160)

(1) Check that the temperature showing on the incubator is 65.0°C.
(2) Load the reaction tubes into the LF-160 incubator and press the green button to start the LAMP reaction (40 minutes at 65.0°C). See the LF-160 instruction manual for details on how to operate the incubator.
(3) Confirm the completion of polymerase inactivation (automatically completed by the LF-160). Take all reaction tubes from the LF-160.

(for other incubator using UV lamp)

(1) Set the temperature of the incubator at 65.0°C (with hot bonnet temperature set to 10 degrees above the reaction temperature or as near to this figure as possible – temperature accuracy: ±0.5°C). Wait until the temperature displayed reaches the set value.
(2) Load the reaction tubes, and then start amplification reaction (for 40 minutes at 65.0°C).
(3) Forty minutes later, inactivate the polymerase using the heating block (for 5 minutes at 80°C, or for 2 minutes at 95°C) to terminate the reaction.

For real-time turbidity detection (see Flow chart of the procedure)

(1) If not already correctly configured, configure the real-time turbidimeter for detection with this product.
(2) Check if the temperature displayed reaches 65.0°C (allow the turbidimeter to warm up for 20 minutes before use).
(3) Load the reaction tubes, and start measurement.
(4) Watch the display of the turbidimeter to check the positive and negative controls for any increase in turbidity. If the turbidity increases in the positive but not in the negative control solution, amplification reaction is proceeding properly (Fig1). If this is not the case, amplification reaction may be proceeding in a wrong way. In such a case, restart testing from reagent preparation.
(5) Confirm the completion of polymerase inactivation (automatically completed by the turbidimeter). Take all reaction tubes from the turbidimeter and discard them without opening.

Amplification plots by Malaria Pan detection reagent

(Analyzer: real-time turbidimeter LA-500 (two different reaction blocks are shown here))

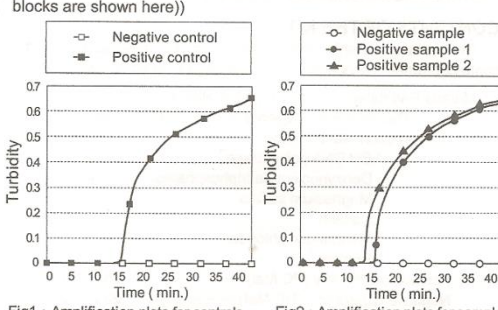


Fig1 : Amplification plots for controls   Fig2 : Amplification plots for samples

PROCEDURAL NOTES

(1) The LAMP reaction is very sensitive, and contamination with small amounts of amplified product might lead to false positive results.
(2) Separate the sample preparation and the amplification areas.
(3) Clean benches with over 0.5% sodium hypochlorite before and after performing the test.

(4) Change gloves after transferring the blood or if the gloves come into contact with the DNA solution.
(5) When handling this product, avoid microbial contamination and nuclease contamination. Even a small amount of contamination of the reaction tube from sweat or saliva may decompose DNA and cause a false result.
(6) Furthermore, read the instruction manual of DNA extraction kit when performing DNA extraction.
(7) The DNA solution should ideally be used immediately after preparation.
(8) **(For LF-160 or other incubator using UV lamp)**
If bubbles are present, flick the tubes to release them.
(For real-time turbidimeter)
Since bubbles in reaction solution may interfere with turbidity measurement and cause a false result, avoid forming any bubble when mixing reagent and sample solution. If bubbles occur, spin or flick the tube to release them.
(9) dMAL Pan should be fully dissolved. Any undissolved portion may influence performance, such as causing a decrease in sensitivity.
(10) The PC Mal contains a high copy number of control DNA. Avoid any contamination of other samples with the PC Mal. Dispense the samples and the NC Mal and close all reaction tubes before dispensing the PC Mal.
(11) Flick (or spin) down the PC Mal tube before opening it, in order to collect the content at the bottom of the tube. Close the tube immediately after dispensing the PC Mal.
(12) Never open the reaction tubes once the LAMP reaction has started or after completion. Be particularly careful when unloading the reaction tubes from the incubator to avoid opening the tubes accidentally.
(13) When the LF-160 incubator or the real-time turbidimeter is used, polymerase inactivation is automatically performed.
(14) For other incubators, when visual fluorescence judgment is chosen, inactivate the polymerase (for 5 minutes at 80°C, or for 2 minutes at 95°C) before reading, or false results will be caused.
(15) Do not reuse any amplified product in the tubes for electrophoresis or other applications.

INTERPRETATION OF RESULTS

For visual fluorescence detection

(For LF-160)

Set each reaction tube into the Fluorescence Visual Check Unit, irradiate and observe the tube from the side.

(For other incubator using UV lamp)

Irradiate the bottom of each reaction tube and observe from the side through goggles or other UV-protective eye shielding.

For a valid run, the following results must be obtained when read at the specified time:

- Positive Control: Green fluorescent light is emitted.
- Negative Control: No fluorescent light is emitted.

If any control is invalid, all samples in the run should be reported as invalid and the test should be repeated.

After confirming that the run is valid, evaluate samples as follows:

- Positive Sample: Green fluorescent light is emitted.
- Negative Sample: No fluorescent light is emitted.

For real-time turbidity detection

After confirming that the turbidity increases in the positive control but doesn't in the negative control solution, evaluate samples in accordance with the following criteria (Fig 1 and 2).

- Positive: Some increase is observed in turbidity.
- Negative: No increase is observed in turbidity.

Notes:

(1) The minimum detectable sensitivity of the MALARIA Pan detection kit is 5.0 copies per test.
(2) Although the primers have been designed to target a region containing a relatively small number of variations, it is conceivable that *Plasmodium* infections may occur with variations in this region that are not well detected by this product.
(3) Test results may be affected by specimen collection, transport, specimen preparation, inhibitors and other laboratory procedural errors. A negative test does not exclude the presence of *Plasmodium* DNA from the specimen.
(4) This product is a kit for qualitative detection; it is not designed for quantitative measurement. The intensity of fluorescent light observed or the rise time of turbidity measured by the real-time turbidimeter does not correlate with the concentration of template DNA.

INTERFERING SUBSTANCES

Our in-house studies have revealed that turbidimetry measurement was not affected by the presence of EDTA (6.0 nmol/test), free bilirubin (1.2 µg/test), conjugated bilirubin (1.2 µg/test), chyle (formazine turbidity: 14,400), and hemolytic hemoglobin (3.4 µg/test), and human genomic DNA (1.2 µg/test). EDTA may cause false positive results when the result is read by fluorescence.

With regard to drugs, our in-house studies have revealed that measurement was not affected by the presence of isoniazid (840 ng/test), ethambutol (204 ng/test), rifampicin (958 ng/test), pyrazinamide (4.2 µg/test), acetaminophen (322 ng/test), loxoprofen sodium (637 ng/test), cefotaxime sodium (12 µg/test), levofloxacin (270 ng/test), atovaquone (1.59 µg/test), proguanil (20 ng/test), chloroquine (40 ng/test), quinine (960 ng/test), doxycycline hydrochloride (360 ng/test), mefloquine (168 ng/test), primaquine (18 ng /test), and artemisinin (93 ng/test).

PERFORMANCE CHARACTERISTICS

(1) Sensitivity and accuracy

In testing the following samples:

- negative sample (concentration: 0 copy/test)
- positive sample 1 (100 copies/test)
- positive sample 2 (1000 copies/test);

The negative sample shall test negative, and the positive samples 1 and 2 shall test positive.

(2) Within-run reproducibility

In testing five negative and five positive samples simultaneously, the negative samples shall test negative throughout, and the positive samples shall test positive throughout.

(3) Limit of detection

5.0 copies/test

(4) Cross-reactivity

The measurement system tested positive for *Plasmodium* species and negative for other pathogens, as detailed in the table below:

Plasmodium genus	
Plasmodium falciparum	Positive
Plasmodium vivax	Positive
Plasmodium ovale	Positive
Plasmodium malariae	Positive
Other pathogens	
Trypanosoma brucei	Negative
Trypanosoma cruzi	Negative
Leishmania donovani	Negative
Schistosoma mansoni	Negative
Theileria parva	Negative
Mycobacterium tuberculosis	Negative
Influenza A virus	Negative
Human immunodeficiency virus	Negative

(5) Information about a Calibrator

The performance test for this product used plasmid DNA containing the mitochondrial DNA of *Plasmodium falciparum* as a calibrator.

ORDERING INFORMATION

Product Code	Product Name	Contents
LMP561	***Loopamp™ MALARIA Pan Detection Kit***	***480 tests***
MVKM17	LF-160	1 Main unit 1 Fluorescence Visual Check Unit
LMC802	Loopamp™ PURE DNA Extraction Kit	90 tests
MVL300	LA-500	1 control unit 1 amplification unit

Note: For detection of DNA of *P. falciparum* parasites in blood, use Malaria Pf detection reagent (dMAL Pf) in the Loopamp™ MALARIA Pan/Pf Detection Kit (REF LMC562) (sold separately).

Appendix 6: MoHSS Case Investigation Form

MALARIA CASE INVESTIGATION FORM

(*TO BE COMPLETED AT HEALTH FACILITY, FOR ALL MALARIA POSITIVE PATIENTS*)

Namibia National Vector-Borne Disease Control Programme, MoHSS, Directorate of Special Programmes, Windhoek

Investigation Date ..../............/...............

Region_______________________ Health District _________________________ Health Facility _______________________

Investigation was conducted by:

Name ________________________Job Title ______________________Contact details_________________________________

PATIENT DETAILS

Patient Name _____________________________________
Patient contact number _____________________________________
Head of household name _____________________________________
Home village/town name _____________________________________
Village headman name _____________________________________

Age in completed years ________
Gender ☐ Male ☐Female
Pregnant ☐ Yes (Trimester________) ☐ No

Nationality ☐ Namibian
☐ Other, enter country name ______________________________

Current occupation

☐Unemployed ☐Farming/Agriculture ☐Other Manual Labour ☐Small-market sales or trade
☐Nurse / Teacher / Professional ☐Fisherman ☐Other (specify ________________________________)

Location/ Place of Occupation _____________________________________

DIAGNOSIS

Presented with fever ☐ Yes (Temperature: _______°C) ☐ No
History of fever ☐ Yes ☐ No

Original diagnosis confirmed by: ☐ RDT ☐ Microscopy ☐ Both

RDT result: Positive ☐ Negative ☐
Result type: *P. falciparum* ☐ *Other species* ☐ Mixed ☐

Microscopic result: positive ☐ negative ☐ not tested ☐
Specify species...
Microscopy (Specify, how many _____parasites per microlitre (μl))

Diagnosis Type
☐ Uncomplicated ☐ Severe ☐ Unknown

PLEASE ASK THE FOLLOWING FEW QUESTIONS:
For how long were you experiencing signs & symptoms before you sought treatment at the health facility? _____days
Has a family member been sick, with fever, in the past week? ☐ Yes ☐ No ☐ Don't know
Have you previously been diagnosed with malaria in the past 12 months? ☐ Yes ☐ No

TREATMENT

Treatment Prescribed: ☐ Artemether Lumefantrine ☐ Oral Quinine ☐ IV Quinine ☐ IM Quinine ☐ Other (specify ______________________________

What malaria signs and symptoms were present?

☐ Fever ☐ Abdominal Pain ☐ Abdominal Pain ☐ Chills ☐ Anaemia
☐ Nausea, Vomiting ☐ Sweating ☐ Motor weakness ☐ Diarrhoea ☐ Headache
☐ Joint pain ☐ Other (Specify_____________________)

TRAVEL HISTORY

Have you spend a night anywhere else besides your home village/town in the past 4 weeks? ☐ Yes ☐ No
If no, skip to Vector Control section

1. Within Namibia			2. Outside of Namibia		
Village / town/ District where you have spend the night(s)	Duration of stay		Country/Province/Town where you have spend the night(s)	Duration of stay	
	First Night YY/MM/DD	Last Night YY/MM/DD		First Night YY/MM/DD	Last Night YY/MM/DD

What was your reason for travel?
☐ Business/trade ☐ Visiting family or friends ☐ Shopping ☐ Holiday/tourism ☐ Other (specify ____________________)

What was your means of travel?
☐ Bus ☐ Mini-bus ☐ Car/Taxi ☐ Boat ☐ Walked ☐ Airplane ☐ Other (specify______________________)

Did you use any of the following personal protection measures to prevent contracting malaria (during travel)?
☐ Chemoprophylaxis (specify _________________) ☐ Bed net ☐ Mosquito repellent or coil ☐ None

---

VECTOR CONTROL & PERSONAL PROTECTION MEASURES

What are the exterior walls of the house primarily (main material) made of?
☐ Sticks/Grass ☐ Mud/Cow dung ☐ Wood ☐ Cement block or brick ☐ Corrugated iron sheets
☐ Other (specify _________________)

What is the roof primarily comprised of?
☐ Grass / Sticks ☐ Corrugated iron sheets ☐ Asbestos ☐ Other (specify _________________)

Has this home been sprayed in the past year? ☐ Yes ☐ No ☐ Don't Know
Do you own a bed net? ☐ Yes ☐ No

If no, DO NOT ASK THE FOLLOWING QUESTIONS
If yes,
How many bed nets do you have in the house? ___________
Do you use ALL the nets in the house? ☐ Yes ☐ No ***if no, ask why all nets are not used***
____

How many people live in the house? (Include all usual members) ___________
Is your bed net treated with insecticide? ☐ Yes ☐ No
Did you sleep under your bed net anytime within the past week? ☐ Yes ☐ No

IF NO, Why didn't you sleep under a mosquito bed net within the past week?
☐ Too hot ☐ used by someone else ☐ net not hung up ☐ net worn out/poor condition ☐ net is/was dirty
☐ Don't like shape or colour ☐ other reason __________

Appendix 7: Proportions and Odds ratio calculations for the type of material used in the floor, wall, window, construct of sleeping structures in case and control households

	Case n (%)	Control n (%)	Odds Ratio (O.R)	95% C.I	p-value
Type of Floor Material					
Other	4 (1.6)	0	1		
Earth/sand	83 (33.2)	72 (29.9)	0.21	0.046-6.00	0.5
Palm/bamboo	2 (0.8)	0	0.1	0.018-21.14	0.773
Vinly or Asphal strips	9 (3.6)	7 (2.9)	0.177	0.035-9.27	0.59
Ceramic tiles	5 (2)	7 (2.9)	0.09	0.01-6.28	0.39
Cement	138 (55.2)	137 (56.85)	0.18	0.04-5.15	0.44
Carpet	9 (3.6)	18 (7.47)	0.08	0.01-3.511	0.24
Type of Wall Material					
Ply wood	1 (0.40)	0	1		
Cane/trucks/bamboo/reed	7 (2.82)	1 (0.42)	0.71	0.10-156.82	0.38
Bamboo/wood with mud	15 (6.05)	8 (3.33)	0.5	0.08-42.79	0.71
Cement	94 (37.9)	129 (53.75)	0.25	0.044-13.98	0.95
Bricks	26 (10.48)	21 (8.75)	0.38	0.067-25.94	0.85
Mud blocks	31 (12.5)	24 (10)	0.41	0.07-26.59	0.84
Wood planks/shingles	7 (2.82)	7 (2.92)	0.26	0.04-27.08	0.93
Corrugated metal	63 (25.4)	50 (20.83)	0.41	0.07-24.63	0.84
Cloth	2 (0.81)	0	0.19	0.03-68.95	0.79
Other	2 (2.82)	0	0.67	0.099-137.81	0.42
Type of Window					
Other covers	0	2 (0.82)	1		
With Glass	99 (39.29)	149 (61.57)	0.34	0.06-11.72	0.97
No windows	123 (48.81)	69 (28.51)	0.9	0.16-31.40	0.55
Without any cover	21 (8.33)	15 (6.2)	0.63	0.107-28.20	0.67
With cloth/screen	9 (3.57)	4 (1.65)	0.73	0.11-54.33	0.52
With metal	0	3 (1.24)	0.122	0.02-27.16	0.87
Type of Doorway					
Other types of doors	6 (2.38)	2 (0.83)	1		
Doorway without any cover	34 (13.49)	21 (8.68)	0.34	0.077-6.46	0.65
Doorway with cloth/screen	23 (9.13)	22 (9.09)	0.22	0.04-4.36	0.43

Doorway with wooden door	143 (56.75)	155 (64.05)	0.21	0.051-3.3	0.34
Doorway with metal door	46 (18.25)	42 (17.36)	0.24	0.056-4.18	0.43
Eaves					
Yes	166 (65.87)	166 (68.60)	0.85	0.49-1.59	
No	86 (34.13)	76 (31.40)			
Last painting or replastering of structure					
Not sure	0	4 (1.66)	1		
Less than 1 year ago	24 (9.52)	14 (5.79)	1.55	0.262-45.03	0.277
More than 1 year ago	164 (65.08)	174 (71.9)	0.98	0.17-21.44	0.477
Never painted	64 (25.4)	50 (20.66)	1.29	0.23-30.31	0.357