

**ASSESSING THE BIO-EFFICACY, PHYSICAL  
INTEGRITY, AND CARE PRACTICES OF YORKKOL®  
PYRETHROID-BASED LONG-LASTING  
INSECTICIDAL NETS AFTER ONE YEAR OF USE IN  
SEME, KISUMU COUNTY**

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**Assessing the Bio-Efficacy, Physical Integrity, and Care Practices of  
Yorkool® Pyrethroid-Based Long-Lasting Insecticidal Nets after  
one Year of use in Seme, Kisumu County**

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**A Thesis Submitted in Partial Fulfillment of the Requirements for  
the Degree of Master of Science in Parasitology and Entomology of  
the Jomo Kenyatta University of Agriculture and Technology**

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## DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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## **DEDICATION**

I dedicate this work to my family for their continued support and encouragement throughout the duration of my studies.

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## **ABBREVIATIONS AND ACRONYMS**

<b>Bfc</b>	Blood Fed in Control
<b>CBRD</b>	Centre for Biotechnology Research and Development
<b>CHWs</b>	Community Health Workers
<b>DNMCP</b>	Division of National Malaria Control Programme
<b>ESACIPAC</b>	Eastern and Southern Africa Centre for International Parasite Control
<b>GTS</b>	Global Technical Strategy
<b>IRS</b>	Indoor Residual Spraying
<b>KD</b>	Knock Down
<b>KEMRI</b>	Kenya Medical Research Institute
<b>KMIs</b>	Kenya Malaria Indicator Survey
<b>KMS</b>	Kenya Malaria Strategy
<b>LLINs</b>	Long Lasting Insecticidal Treated Nets
<b>NMCP</b>	National Malaria Control Program
<b>PMI</b>	The President Malaria Initiative
<b>SERU</b>	Scientific and Ethics Review Unit
<b>WHO</b>	World Health Organisation
<b>WHOPES</b>	WHO Pesticide Evaluation Scheme

## ABSTRACT

The physical integrity and insecticide efficacy of long-lasting insecticide-treated nets (LLINs) are influenced by handling practices during use. This was an evaluation of Yorkool<sup>®</sup> pyrethroid-based LLINs after 12 months of use to determine the washing and drying practices, physical conditions, and bio-efficacy. This was a cross-sectional household survey in an area where rectangular Yorkool<sup>®</sup> pyrethroid LLINs were distributed during the national mass net distribution campaign in 2021. Questionnaires were used for data collection, and bioassay tests were conducted on 30 randomly selected nets. A total of 262 household heads were interviewed, with their mean age being 46.9 years (SD 17.4 years), and the level of education was mainly primary (70.2%). Most households (71.8%) had 1 to 2 sleeping spaces. The households had 468 LLINs, with 95.1% having been washed at least once. About three-quarters (74.4%) of the LLINs had been washed using local bar soap, with 99.6% having not been scrubbed on a hard surface during washing, and 72.1% were dried under a shade. Those evaluated for physical integrity and had at least one hole made up 77.8% of the nets and 57.2% were too torn and required replacement. Bio-efficacy test recorded a mean KD of 92% after 1 hour, and a mean mortality of 86% after 24 hours. The nets that passed the WHO efficacy cut-off for mortality ( $\geq 80\%$ ) after 24 hours were 83.3%, and 70% passed the KD ( $\geq 95\%$ ) cut-off after 1 hour. In conclusion, while more than half of the Yorkool<sup>®</sup> LLINs needed replacement due to poor physical integrity after one year of use in the households, the bio-efficacy of the LLINs was relatively high with the mortality on vectors meeting the WHO threshold.

**Key words:** Integrity, Efficacy, Bioassay, Washing, Drying, Knockdown

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background of the Study

Malaria is a major global public health problem, with an estimated 249 million malaria cases and more than 608,000 malaria-related deaths being recorded in 2022. Out of these, the World Health Organization (WHO) African Region accounted for more than 90% of the cases and deaths (WHO, 2024). Use of long-lasting insecticide-treated nets (LLINs) is currently one of the key malaria prevention measures (WHO, 2024). The LLINs have insecticide that has been incorporated into or coated onto the fibre and thus prevents human-mosquito contact by creating both a physical and an insecticidal barrier, which remains effective even after repeated washing and extended use (Randriamaherijsaona *et al.*, 2017). The extra benefits of LLINs include their dirt repellent capacity, high durability to washing, and a wide mesh size to provide good airflow (WHO, 2023). Physical integrity of LLINs and their insecticidal efficacy determine their prevention effectiveness in preventing mosquito bites. Presence and the number of holes on the nets as well as deterioration of the impregnated insecticide compromises their efficacy. Several studies have reported varying results on physical integrity of LLINs depending on the conditions to which the nets are exposed. A study in Guatemala found a high functional survivorship of up to 88-94% after 18 to 32 months of use (Castellanos *et al.*, 2021). Another study in Zambia recorded that 94% of nets had holes after a median of 31 months of use (Craig *et al.*, 2015) The common causes of holes on the nets include direct contact with the bed frame or mattress during tucking-in of the net, fires in single room houses, tin lamps and tearing caused by animals like cats or rodents (Mutuku *et al.*, 2013). Other causes listed in studies include tearing after the net is caught on an object during use and also children (Castellanos *et al.*, 2021).

Most LLINs are currently treated with pyrethroids due to the relative safety of this class of insecticide for humans at low dosage, repellent, rapid knocking down rates and killing effects (WHO, 2023). The physical and insecticidal durability of LLNs is influenced by many factors including the frequency of washing, type of laundry

soap, washing and drying techniques, and other wear-and-tear parameters (Castellanos *et al.*, 2021). LLINs are expected to retain effectiveness against susceptible *Anopheles* species vectors for up to 20 standard WHO laboratory washes and 3 years of recommended field usage. Nets washed gently with water and a bar soap under controlled conditions retain all formulations showing good resistance with 100% anopheles' mortality observed in the cone bioassay after three washes (WHO, 2022). A study in Kenya reported that nets retained efficacy when hand-rubbed instead of beating on rocks while washing (Syme *et al.*, 2025). Washing frequency also has an influence on the durability and efficacy of nets demonstrated by increased feeding success and survival rates of mosquitoes with increasing number of washes up to 100% in certain brands of nets (Kilian *et al.*, 2021). Evidence has been published from laboratory-controlled studies showing a decline in the bio-efficacy of several brands of LLINs after repeated washings. For instance, in Iran, there was a significant increase in the mean values of the median knockdown time (MMKDT) and reduced mortality as a result of more washing of a brand of deltamethrin-impregnated nets which was more evident after 15 and 21 washings (Kayedi *et al.*, 2017). Similarly, another brand impregnated with deltamethrin was also studied in Kenya and was found to have a significant drop of mortality from 95% with unwashed nets to 72% after 15 washings (Etang *et al.*, 2016). The net drying practices in the households have also been a concern in relation to maintaining efficacy of the LLINs. However, bioassay tests on deltamethrin-impregnated nets showed no significant difference between the sun-dried and shade-dried bed nets (Kayedi *et al.*, 2017). The current study sought to determine the physical integrity and bio-efficacy of Yorkool<sup>®</sup> pyrethroid-based LLINs and to assess the washing and drying practices at 12 months of use in Seme community in Western Kenya where the LLINs were distributed under the National Malaria Control Programme mass net distribution.

## **1.2 Problem Statement**

Malaria is a major global public health problem, with Africa carrying most of the burden. Use of long-lasting insecticide-treated nets is currently one of the key malaria prevention measures and it's highly recommended by World Health

Organization. According to WHO standards, LLINs should retain their insecticidal activity after at least 20 standardized laboratory washes and maintain protective effectiveness for up to three years under field conditions. Manufacturer claims of 36-month LLINs effectiveness in terms of bio-efficacy and physical integrity but studies have reported that LLINs are losing their insecticidal activity within 12 months of household usage (Randriamaherijsaona *et al.*, 2017). In addition to decreased bio-efficacy, deterioration of the net fabric caused by the formation of holes and tears compromises the physical integrity of LLINs, limiting their ability to prevent human-mosquito interaction. The continuous loss of insecticidal activity and physical integrity poses a danger to LLINs' overall protective effectiveness. Household factors such as washing frequency, detergent type, washing procedure, drying methods, and net handling practices can all contribute to increased insecticidal degradation and physical damage. The WHO Pesticide Evaluation Scheme (WHOPES) laboratory washing processes may not accurately represent abrasive field conditions, thus leading to overestimation of LLIN durability. Despite the widespread distribution of Yorkool® pyrethroid-based LLINs in Kisumu County, there is no study that has been done to evaluate these parameters in Seme Sub-county, nor has it examined household practices that may influence the Yorkool® pyrethroid-based LLINs performance. Effective malaria prevention and evidence-based decisions on net replacement and community education are constrained by limited localized data on the performance of Yorkool® LLINs under real-world conditions. This study evaluated the bio-efficacy and physical integrity of Yorkool® pyrethroid LLINs over 12 months of household use in Seme Sub-county and determined washing and drying practices that may compromise net effectiveness.

### **1.3 Justification**

Malaria remains a significant public health burden in several countries especially Kenya. Use of long-lasting insecticide-treated nets (LLINs) is currently one of the major key malaria prevention measures (WHO, 2024). According to the WHO, LLINs should retain effectiveness against susceptible *Anopheles* spp. vectors for up to 20 standards WHO laboratory washes and 3 years of recommended usage under field conditions (WHO & WHOPES, 2013). However, recent studies suggest that the

lifespan of LLINs may be less than three years and that there is reduction of insecticidal efficacy of LLINs as early as from 12 months (Randriamaherijaona *et al.*, 2017) despite the fact the manufacturers claim the LLINs efficacy is 36 months. A study by Randriamaherijaona *et al.* reported that Yorkool® LLINs bio-efficacy significantly decreased within one year of household use (Randriamaherijaona *et al.*, 2017), contradicting the manufacturer's three-year durability claim. Such findings raise concerns regarding the sustained protective value of LLINs distributed in malaria-endemic settings. Despite this evidence, Yorkool® pyrethroid-based LLINs continue to be widely distributed during mass net campaigns in Kenya. The continuous dependence on these nets, without locally created durability data, provides a possible gap in evidence-based malaria control programs. There have been no durability studies on Yorkool® LLINs in Kenya to evaluate their bio-efficacy and physical integrity under local household conditions. Variations in washing frequency, detergent type, drying methods, and general handling practices may significantly influence insecticide retention and fabric strength. Without actual data from Kenyan communities, policymakers and malaria control programs may be making procurement and replacement decisions based on assumptions that do not accurately reflect field performance. There is therefore a critical need to evaluate the bio-efficacy, physical integrity of Yorkool® LLINs as well as household washing and drying practices that may affect their effectiveness. Generating continuous, context-specific durability data on nets distributed in Kenya will support informed decision-making regarding procurement, replacement intervals, and community education strategies. Such evidence is essential to ensure that LLIN interventions achieve their intended long-term impact in reducing malaria transmission and maintaining advances in malaria control.

#### **1.4 Research Questions**

- i. What is the physical integrity status of Yorkool® LLINs after one year of household use in Seme, Kisumu County?
- ii. What are the household washing and drying practices of Yorkool® LLINs in Seme, Kisumu County?

- iii. What is the bio-efficacy of the Yorkool® LLINs after one year of household use in Seme, Kisumu County?

## **1.5 Main Objective**

To determine the insecticidal activity and physical integrity of Yorkool®, a pyrethroid-based long-lasting insecticidal net, and to assess household factors that may influence its bio-efficacy in Seme, Kisumu County after one year of use.

### **1.5.1 Specific Objectives**

1. To determine the physical integrity of Yorkool® LLINs in Seme, Kisumu County, after one year of use in households.
2. To investigate households' washing and drying practices of Yorkool® LLINs in Seme, Kisumu County.
3. To assess the bio-efficacy of Yorkool® LLINs in Seme, Kisumu County, after one year of use in households.

## **1.6 Scope of the Study**

This study focused on evaluating the insecticidal activity and physical integrity of Yorkool®, a pyrethroid-based Long-Lasting Insecticidal Net (LLIN), after one year of use in households within Seme Sub-County, Kisumu County. The study assessed the structural condition of the nets, including the presence, number, and size of holes, to determine their serviceability and continued protective capacity. In addition, it examined household factors that may influence the bio-efficacy of these nets, particularly washing and drying practices.

The scope of the study was limited to Yorkool® LLINs distributed during the 2021 mass net distribution program by the Ministry of Health under the National Malaria Control Programme. The study primarily targeted households that received these nets, with a sample, size of 262 households determined through Yamane sampling technique.

Bio-efficacy assessment was conducted using standard WHO cone bioassay tests with the Kisumu strain of *Anopheles gambiae*, a principal malaria vector. The bioassays measured knockdown rates after one hour and mortality rates after 24 hours to determine whether the nets met the WHO efficacy criteria ( $\geq 95\%$  knockdown after one hour and/or  $\geq 80\%$  mortality after 24 hours).

Physical integrity assessment involved examination of the nets for holes and tears, and classification of their condition based on established durability indicators. The research employed a cross-sectional survey design, collecting data at a single point in time. Systematic random sampling was used to select households for participation to ensure unbiased representation. Structured questionnaires were administered to household heads to assess washing frequency, detergent type, washing techniques, drying methods, and other handling practices. Selected nets were retrieved for laboratory bioassay testing and replaced with new ones to ensure continued malaria protection for participants.

The study provided insights into the real-world durability and performance of Yorkool® LLINs under field conditions, generating context-specific evidence to inform malaria vector control strategies and future LLIN distribution and replacement planning.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Introduction

Malaria continues to be one of the most significant public health challenges worldwide, with heavy burden felt in sub-Saharan Africa. The disease is caused by Plasmodium parasites transmitted through the bite of infected female Anopheles mosquitoes. Despite global efforts to eliminate malaria, the disease continues to cause high morbidity and mortality, especially among under five years children and expectant mothers in endemic regions (WHO,2024). Long-lasting insecticidal nets (LLINs) have been a cornerstone of malaria prevention strategies, since they provide both a physical and chemical barrier against mosquito bites. However, the insecticidal effectiveness and physical integrity of these nets is influenced by various factors, including insecticide resistance, washing and drying practices of the households, and environmental conditions. The efficacy of LLINs is largely dependent not only on the retention of insecticides such as pyrethroid over time, but also on the maintenance of their physical integrity. Pyrethroid-based LLINs, including Yorkool®, are widely distributed in malaria-endemic regions due to their affordability and long-lasting insecticidal properties. However, their bio-efficacy may degrade with continuous use, especially due to frequent washing, exposure to sunlight, and improper handling. In addition to insecticide degradation, the development of tears, holes, and seam failures significantly compromises the protective barrier function of the nets. Physical damage of the nets allows mosquito entry even when insecticide is still present, thereby reducing overall effectiveness. Factors such as daily wear and tear, sleeping arrangements, washing practices, drying practices, and general household conditions can accelerate LLINs' deterioration. Therefore, assessing both insecticide retention and physical integrity is crucial for a comprehensive evaluation of LLIN performance and durability. Understanding the extent of insecticidal loss alongside the physical condition of LLINs, and the impact of household practices on both parameters, is crucial for improving malaria control

strategies. This literature review will explore existing research on malaria burden, LLIN efficacy, insecticide retention, and physical durability, providing a foundation for understanding the study's findings (WHO,2023).

### **2.1.1 The Burden of Malaria Disease**

#### **2.1.2 Global Malaria Epidemiology**

Malaria is a major global health problem. Globally, there were an estimated 263 million malaria cases in 2023 in 83 malaria-endemic countries, an increase of 11 million cases compared with 2022. Most of this increase was from countries in the WHO African Region(WHO,2024). At the Global Technical Strategy for malaria 2016–2030 (GTS) baseline survey of 2015, there were 231 million estimated malaria cases (World Malaria Report, 2024). The World Health Organization also reported an estimated more than 597 000 deaths from malaria in 2023 globally, with a mortality rate of 13.7 per 100000. Children under 5 years were affected the most (World Malaria Report, 2024). The WHO African region bears a disproportionately large share of the global malaria burden, with a mortality of 95%. In the WHO African Region, there were 569 000 fatalities and 246 million cases in 2023. Estimated malaria cases and deaths rose by 23 million and 24,000, respectively, over the previous five years, from 2019 to 2023. Children under the age of five accounted for about 76% of all malaria deaths in the region (WHO,2023). Africa is the most affected region, and thus malaria poses a significant socioeconomic burden to African countries.

#### **2.1.3 Epidemiology of Malaria in Kenya**

Malaria is still a major source of morbidity and mortality in Kenya, with more than 70% of the population at risk (Kenya Malaria Indicator Survey,2020). In Kenya, nearly four million cases of malaria are reported yearly, with a 5.1 percent fatality rate among patients admitted with severe malaria(Kenya Malaria Indicator Survey,2020). Malaria risk varies according to altitude and rainfall. Around 70% of Kenyans are at risk, with 14 million living in low-lying areas where malaria is endemic and 17 million living in higher land where malaria is periodically episodic

and occasionally epidemic (Kenya Malaria Indicator Survey, 2020). The Kenya Malaria Indicator Survey 2019-2023 classifies four epidemiological zones in Kenya endemic (lake and coast), epidemic (highland), seasonal (semi-arid), and low risk with variation in risk of malaria infection. These zones have been determined based on several factors, including malaria prevalence, climate factors (i.e., temperature, rainfall, altitude), and topography (Kenya Malaria Indicator Survey, 2020). Prevalence-based models incorporating data from Malaria Indicator Survey, school surveys, malaria vaccine, and climate data from 2000 to 2020 indicate that counties in the lake endemic zone have transitioned from high to moderate malaria transmission (U.S. President's Malaria Initiative Kenya Malaria Operational Plan, 2022). Malaria transmission in Kenya's western highlands is linked to seasonal changes and population movements, and it occurs in epidemics with high morbidity and mortality rates (PMI Kenya MOP, 2022). Kisumu county the area of study, is a lake endemic malaria region and it's located in southwest Kenya on the eastern coasts of Lake Victoria's Winam Gulf, stretching from Kisumu town, on the Kano plains (1100 m altitude), to Kericho 2100 m (KNBS, 2019), 80 km further east and it is regarded as a high-transmission-risk location (Macharia *et al.*, 2018). With altitudes ranging from 0 to 1300 meters, these are places with stable malaria transmission. Around Lake Victoria, eight counties account for 19.4 percent of Kenya's population (Macharia *et al.*, 2018). The determinants of malaria transmission are rainfall, temperature, and humidity. Due to the favorable climatic conditions, the vector life cycle is usually brief, with a high survival rate. Malaria transmission is intense throughout the year, with community-based *Plasmodium falciparum* prevalence among children exceeding 30% in 2009 and with the highest rates of malaria transmission in Kenya in 2015 (Macharia *et al.*, 2018). Malaria is one of the most causes of morbidity and mortality in Kisumu, accounting for 40 percent of all outpatient hospital visits in the county. Mosquitoes abound in Kisumu, where the humid climate makes the area an ideal breeding ground for the female anopheles' mosquito, the vector for the disease (Achieng *et al.*, 2020).

#### 2.1.4 Malaria Parasites

Malaria is a life-threatening disease caused by plasmodium parasites that are transmitted to people through the bites of infected female Anopheles mosquitoes thus referred to as malaria vectors. In low-income nations, it is the eighth largest cause of death (WHO, 2023). Malaria is characterized by symptoms such as fever, exhaustion, vomiting, and headaches. There are five species that causes malaria; *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium falciparum*, and *Plasmodium knowlesi*. The first four species are specific for humans while *P.knowlesi* is naturally maintained in macaque monkeys and causes zoonotic malaria widely in South East Asia (WHO, 2023). Malaria is transmitted through the bite of an infected female *Anopheles* mosquito (WHO, 2023). Among the five human malaria parasite species, *Plasmodium falciparum* accounts for the majority of malaria infections globally and is responsible for most malaria-related morbidity and mortality, particularly in Sub-Saharan Africa (Weiss *et al.*, 2019). Although *Plasmodium vivax* has been considered less prevalent in Sub-Saharan Africa due to the overwhelming burden of *P. falciparum*, recent evidence indicates that *P. vivax* is more widespread across Africa than previously recognized (Twohig *et al.*, 2019). Globally, *P. vivax* has the widest geographic distribution of all human malaria parasites and remains a major public health concern, particularly in Asia and the Americas (Battle *et al.*, 2019). *Plasmodium ovale* is less common and occurs predominantly in West Africa and parts of Asia, while *Plasmodium malariae* is distributed worldwide but at a low prevalence (Kotepui *et al.*, 2020). *Plasmodium knowlesi*, a zoonotic malaria parasite of Old World monkeys, naturally infects macaque species such as *Macaca fascicularis* and *Macaca nemestrina*. Once considered rare in humans, *P. knowlesi* is now recognized as a significant cause of human malaria in Southeast Asia, particularly in Malaysia (Cooper *et al.*, 2020). The mosquito is often the insect vector that allows plasmodium species to spread across vertebrate hosts. Not only is the vector a carrier, but it also serves as the final host, facilitating sexual reproduction of Plasmodium species. The parasite's growth in the vector insect is necessary for its transfer to the subsequent vertebrate host. Anopheles mosquitoes are the only vector by which the five Plasmodium species that cause malaria in humans are transmitted. The spectrum of insect species that may sustain

the crucial development of Plasmodium varies depending on the specific parasite type (Sato *et al.*, 2021).

## **2.1.5 Malaria Parasite Life Cycle**

### **2.1.5.1 Life Cycle in Human Body**

Mosquitoes acquire the malaria parasite by feeding on an infected human carrier. The Plasmodium life cycle starts when parasites called Sporozoites generated in the insect vector enter the blood of the vertebrate host after a bite (Frischknecht & Matuschewski, 2017). Sporozoites are deposited in the dermis and rapidly migrate to the liver where they invade the hepatocytes and multiply into thousands of parasites through a process called schizogony (Prudêncio *et al.*, 2021). The resultant parasites, known as merozoites, are released back into the circulation and infect erythrocytes. In an erythrocyte, one merozoite multiplies asexually by schizogony to generate between 8 and 64 new merozoites this number depends on the type species (Rudlaff *et al.*, 2020). These new merozoites are released back into the bloodstream, and the parasites restart the intraerythrocytic propagation cycle every 48 hours for *P. falciparum*, *P. ovale*, *P. vivax*, 72 hours for *P. malariae* and 24 hours for *P. knowlesi*. Some merozoites differentiate into the subsequent developmental stage known as the gametocyte for sexual reproduction (Subramaniam *et al.*, 2025). The onset of gametocytogenesis, or gametocyte differentiation, varies depending on the species. For instance, *P. vivax* continuously produces gametocytes even in its early intraerythrocytic propagation cycles; whereas *P. falciparum* requires several cycles of intraerythrocytic propagation before it begins differentiation into gametocytes. Even though all gametocytes look alike in their early stages of development, they are preprogrammed to differentiate into either male or female gametes (a process known as "gametogenesis," or sexual commitment) (Subramaniam *et al.*, 2025).

### **2.1.5.2 Life Cycle in the Mosquito**

After a blood meal, development beyond the gametocyte stage typically occurs in the mosquito midgut lumen, where the male and female gametes fuse (Sparkes *et al.*, 2024). Nonetheless, there are infrequent accounts of *P. falciparum*'s exflagellated forms male gametes being seen in human tissue (Sable *et al.*, 2020). When the insect

consumes blood-containing gametocytes from an infected vertebrate host, the second phase of the life cycle in the insect vector starts. After being exposed to the specific environment of the mosquito midgut lumen, the gametocytes become activated. The male and female gametocytes then differentiate to produce macrogametes and microgametes, respectively (Sparkes *et al.*, 2024). The microgamete fertilises the macrogamete to produce a zygote, the only developmental stage of the parasite that has a diploid genome (Ouologuem *et al.*, 2023). Recombination can occur in zygotes, as shown by genetic crossing tests using gametocytes from two clones of *P. falciparum* with distinct allelic variants. Soon the zygote undergoes meiosis and differentiates into a motile form, the ookinete, that now contains four haploid genomes in its nucleus (Jeninga *et al.*, 2023). The ookinete penetrates the wall of the mosquito midgut and forms an oocyst on the outer side (Siciliano *et al.*, 2020). Multiple rounds of mitosis occur in the oocyst, and sporogony produces a large number of sporozoites (Araki *et al.*, 2020). When the oocyst matures, it ruptures, and sporozoites released into the haemolymph migrate to the salivary glands, where they acquire the ability to infect human cells during a blood meal, when released into the body of a vertebrate host stage of their lifecycle, which converts gametocytes into Sporozoites that are ready to infect another person (Ouologuem *et al.*, 2023)

### **2.1.6 Malaria Vector**

The knowledge of major malaria vectors and their bionomics in Africa remains a problem, since various malaria vectors have different behaviours and capacities for parasite transmission. Malaria, being a localized illness, will vary in features from location to location (Msugupakulya *et al.*, 2023)

Over fifty members of species complexes remain nameless, whereas 465 officially recognized species have been identified. Out of these species, about 70 have the ability to spread human malaria parasites, and 41 are dominant vector species complexes that can spread malaria at a level that poses a serious threat to public health (Msugupakulya *et al.*, 2023). Different *Anopheles* mosquito species, with varying behaviors and vectorial capacities, aid in the spread of malaria throughout Africa. The world's most anthropophilic *Anopheles* species, *Anopheles gambiae*,

*Anopheles funestus*, *Anopheles coluzzii*, and *Anopheles arabiensis*, are generally the four main vector species (WHO,2023). The characteristics of vector populations specific to a given site have a close relationship with the distribution of malaria within human populations. Less than five vector species are typically found in any given area, despite the fact that each species' biology is distinct in many ways, such as the locations of larval development, adult mosquito behavior (particularly the behavior of biting humans), susceptibility to Plasmodium parasites, and capacity to spread these parasites (Takken *et al.*, 2024). The major anopheline malaria vectors across sub-Saharan Africa are *Anopheles funestus* s.s. and three members of the *Anopheles gambiae* complex: *An. gambiae* s.s., *Anopheles coluzzii* and *Anopheles arabiensis*. In coastal East Africa, mosquitoes belonging to the *Anopheles gambiae* complex are the main vectors of malaria (Antonio-Nkondjio *et al.*, 2021). For instance, the species in the *An. gambiae* complex range from the non-vectors *Anopheles quadriannulatus* and *Anopheles amharicus* to the major vectors *An. gambiae*, *An. coluzzii*, and *An. arabiensis*, as well as the minor vectors *Anopheles melas*, *Anopheles merus*, and *Anopheles bwambae* (Belay *et al.*, 2024). Nonetheless, several other species, such as the *Anopheles mouchti* and *Anopheles nili* families, are also involved in the transmission of malaria within their own geographic ranges (Zanga *et al.*, 2024). There are plenty of incidental vectors The *Anopheles* genus has about 500 species worldwide, although only a few are known to transmit malaria (Antonio-Nkondjio *et al.*, 2021). Effective transmission of mosquito-borne disease requires successful contact between female mosquitoes and their hosts. The *Anopheles* genus is well-known among Anophelinae for its role in the global transmission of filariasis and malaria (WHO,2023). The malaria caused by Plasmodium parasite is one of the greatest killer diseases in the world. *Anopheles* mosquito abundance, feeding habits, resting habits, and Plasmodium infectivity are among the factors that determine the endemicity of malaria in any given region (Takken *et al.*, 2024). Identification and monitoring of malarial vectors have long been found to benefit from entomological studies that concentrate on the diversity, density, behavioral patterns, and temporal variations of *Anopheles* species. The ability of a vector to spread malaria is determined by a number of factors, including

female longevity, infection rates, anthropophily, zoophily, abundance, and susceptibility to infection by the malaria parasite (Takken *et al.*, 2024).

### **2.1.7 Malaria Control**

Malaria control is one of the biggest public health concerns facing the globe today, particularly in sub-Saharan Africa, where over 80% of the illness cases occur (WHO,2023). Malaria prevention started back in 1895 by Ronald Ross when he discovered the presence of the malarial parasites within the anopheles mosquito. This finding started a new era in the prevention of malaria, particularly in vector control. Malaria vector control is an important component in malaria prevention and elimination because it can be extremely effective in providing personal protection and reducing disease transmission. Prevention of mosquito bites is one of the interventions recommended by the World Health Organization (WHO) to reduce malaria transmission in high-risk communities. Earlier in the days, major vector control strategies included larviciding with oil, biological control with the use of pond-dwelling larval fish, and environmental cleaning with drainage to remove the larval mosquito habitat (CDC, 2024). All of them proved to be quite successful, particularly in many countries. The employment of biological techniques is still regarded as a crucial aspect of the recently started malaria eradication programme and has so far demonstrated encouraging results, albeit this strategy is still in its infancy (WHO,2023). For a period of two decades, the use of insecticides in controlling vector borne diseases, including malaria, was among the most reliable methods. Many compounds like mercuric chloride, Paris Green, phenols and cresols, naphthalene, Bordeaux mixture, rosin-fish oil soap, calcium arsenate, and nicotine sulfate, were used as conventional pesticides (Moyes *et al.*, 2020). One of the most significant initiatives that has been successful in recent years is the eradication of malaria by targeting the Anopheles vector with insecticide-treated nets (ITNs), long-lasting insecticidal material (LMs), indoor residual spraying (IRS), and space spraying, together with appropriate preventive measures (WHO,2023). Long lasting Insecticide-treated nets (LLINs) and indoor residual spraying (IRS) are effective malaria prevention methods that have been widely used, and their use has contributed significantly to the decline in malaria in the twenty-first century (Furnival-Adams *et*

*al.*, 2020). Since 2013, the president malaria initiative (PMI) has prioritized malaria-endemic areas in Kenya in order to achieve the greatest reduction in malaria morbidity and mortality. With an estimated population of 9.8 million in 2019, the eight counties of Bungoma, Busia, HomaBay, Kakamega, Kisumu, Migori, Siaya, and Vihiga form the lake endemic zone and have the highest malaria burden. President's Malaria Initiative (PMI) has focused its support on vector control. Although there has been an extensive scale-up of malaria interventions in Kenya, malaria infections persist at unacceptably high levels in some of the regions. Even with renewed calls to eradicate the disease through increased international donor assistance and country-specific government involvement, malaria is still a cause of worry in endemic regions. These epidemiological zones are used to guide the implementation of malaria control interventions in endemic areas, such as intermittent preventive treatment in pregnancy (IPTp) and community case management (PMI Kenya MOP,2022). Kenya tailors its malaria control efforts and prioritizes malaria endemic areas in order to achieve the highest reduction rate. There has been significant progress in reporting malaria prevention and treatment measures with the help of other partners such as the Global Fund and the National Malaria Control Program. Malaria remains a source of concern in endemic areas, despite renewed pledges to eradicate the illness through increasing international donor assistance and country-specific government involvement (Achieng *et al.*, 2020). In light of this discourse, the Kenyan government and other developing partners in the health sector have remained committed to improving health service delivery in various parts of the country, with a focus on malaria prevention and control measures in endemic areas, with the goal of eventually eliminating the disease. This was one of the objectives that were to be met by 2018, with the goal of reducing malaria burden by two-thirds (PMI Kenya MOP,2022). Long-lasting insecticide-treated nets (LLINs) have proven to be an effective tool, and their use has increased. Bed nets have been shown to reduce the incidence of uncomplicated malaria cases by 50%, severe malaria cases by 45%, and malaria mortality by 55% (WHO,2019). The distribution of long-lasting insecticidal nets (LLINs) by governments, non-governmental organizations (NGOs), and donors has resulted in a dramatic increase in their ownership and contributed to the decline in malaria burden since 2000 (World

Malaria Report,2024). Tremendous efforts have been made in the last five years to combat malaria through prevention and treatment interventions such as mass and routine mosquito net distribution programs to achieve universal coverage, intermittent preventive treatment for malaria during pregnancy, and parasitological diagnosis and management of malaria cases (WHO,2024).

## **2.2 Long-Lasting Insecticide Nets (LLINs)**

Long-lasting insecticide nets (LLINs) create a chemical and physical barrier against mosquitoes, thus preventing malaria. Not only does the insecticidal coating kill mosquitoes that attempt to bite a sleeping person beneath an LLIN, but the netting also blocks their path. Research indicates that the implementation of LLINs decreased the prevalence of malaria by 50% in sub-Saharan Africa, a region that has over 90% of the world's malaria cases (WHO, 2023). Long-lasting insecticide nets (LLINs) are nets in which the insecticide is either incorporated into the fiber during extrusion or coated on the fiber after extrusion, and they typically retain effectiveness against susceptible *Anopheles* spp. vectors for up to 20 standards World Health Organization laboratory washes and three years of field usage are recommended (WHO, 2023). Long-lasting insecticide nets (LLINs), came about in reaction to the poor rates of ITN retreatment, particularly in Africa, WHO encouraged manufacturers to create durable insecticidal nets (LLIN). These are ready-to-use, factory-pre-treated nets, which are supposed to last for at least 3years before severely tearing and not need any additional treatment. The LLINs are regarded as a significant advancement in the prevention of malaria and as a potential remedy for the poor net re-treatment rate. They are significantly more durable than regular ITNs, according to evaluations conducted in comparison with the ITNs (WHO, 2023).

Pyrethroids are currently the only class of insecticides recommended for treating netting fabric because of their rapid effects on mosquitoes at minimal dosages requirements combined with their relatively low at health risks (World Malaria Report, 2022).Pyrethroid are a class of chemicals that have a neurotoxic mode of action. To have an effect, pyrethroids can enter an organism's body in a number of ways. The first mode is non-stereospecific and penetrates the epidermis quickly. It is

then taken up by the haemolymph or blood carrier proteins and distributed throughout the body. The primary pathway for pyrethroid distribution to the central nervous system (CNS) following penetration is by diffusion along the cells of the epidermis. Pyrethroids can also directly enter the central nervous system (CNS) by coming into contact with peripheral nervous system sensory organs. Both vertebrates and invertebrates have sensory organs that are susceptible to pyrethroids. Although they can potentially enter the body through the airway in the vapor phase, pyrethroids only make a little contribution in this method (Dong *et al.*, 2025).

The insecticides recommended by the WHO Pesticide Evaluation Scheme (WHOPES) are alpha-cypermethrin (10%), cyfluthrin(5%), deltamethrin (1-25%), etofenprox (10%), lambda-cyhalothrin (2.5%), and permethrin (10%). Three LLINs which have been fully recommended by the World Health Organisation and are now commercially available are Olyset®, PermaNet®2.0 and Yorkool®LLIN (WHO,2023).Yorkool® net is among the LLIN brands that have been given full approval in December 2009 by the WHO Pesticide Evaluation Scheme(WHOPES), manufactured by Yorkool International Limited, China. Its 100% polyester with deltamethrin content of 55mg/m<sup>2</sup>, which is a non-systemic synthetic pyrethroid. The insecticide in it is incorporated within the 75-100 denier high-density polyethylene monofilaments, which slowly diffuses to the surface (controlled release of insecticide) and a small amount of insecticide available on the surface (bio-availability) is sufficient to kill the mosquitoes (WHO,2023).The active component of many long-lasting insecticidal nets (LLINs), deltamethrin, disrupts the insect's nervous system by interacting with sodium channels in the neuron membrane. This causes the channels to open for a prolonged period of time, which causes excessive nerve firing. The insecticide is exposed to the mosquito's body when it lands on a net infused with deltamethrin. This allows the insecticide to enter the mosquito's body through its exoskeleton and enter its nervous system thus leading to a rapid "knockdown" due to the rapid interruption of nerve signals, which renders it paralyzed and incapable of flying. Eventually leading to death, this mechanism is known as a neurotoxic effect (Dong *et al.*, 2025). Yorkool®LLINs dimensional stability is not more than 5% shrinkage/expansion in both dimension, its bursting

strength is not less than 350kpa and the available shapes are circular, rectangular and hammock (WHO, 2023).



**Figure 2.1: Long-Lasting Insecticidal Net (Yorkool®) Net** (Source: Wikimedia.Org)

### **2.3 Bio-Efficacy of Long-Lasting Insecticide Nets (LLINs)**

Bio-efficacy is one of the components measured when assessing LLINs durability. The standard WHO methods for evaluating net bio efficacy comprise the cone test, tunnel test, and experimental hut trials (World Malaria Report,2019). Cone test, a contact assay, mosquitoes are kept close to the LLINs, and after 60 minutes and 24 hours, respectively, mosquito knockdown (KD) and 24-hour mortality are reported. In tunnel test a live animal is used as a bait (guinea pig, rabbit or mice), allowing mosquitoes to engage in host-seeking behaviour, and LLINs efficacy is assessed by measuring mosquito mortality and blood feeding inhibition. In experimental hut trial

LLINs that fulfil laboratory (phase I) testing requirements are assessed using small-scale field (phase II) testing tests. The huts are built to resemble little local homes; huts are placed in places where target mosquito populations are dense. Features like window traps and baffles are added to the houses to keep mosquitoes inside. In the same way that they would in nearby homes, wild mosquitoes try to feed on and interact with the LLINs while volunteers sleep beneath them. Key outcome measures that translate to individual and group protection against malaria are both feeding inhibition and mortality (WHO, 2023).

Long-lasting insecticide treated nets (LLINs), when used it leads to a reduction of human-vector contact by providing a physical barrier and through insecticidal and/or repellent effects. Long-lasting insecticidal nets (LLINs) were developed to eliminate the need for retreatment every 6 months (Gunasekaran *et al.*, 2016). Nets must retain their effective biological activity without re-treatment for at least 20 WHO standard washes under laboratory conditions and three years of recommended use in the field to be classified as an LLIN (WHO, 2023). To maintain biological activity, two techniques have been developed: incorporating the insecticide into the textile polymer via extrusion (as with polyethylene and polypropylene), and mixing the insecticide with a wash-resistant resin that is bound around the polymer fibers (polyester). Pyrethroids are the only insecticide class currently recommended for use on mosquito nets. The insecticides of choice for net treatment have been pyrethroid-based because of their safety profiles on non-target arthropods, low mammalian toxicity and rapid knockdown of targeted vectors. The use of pyrethroid-impregnated bed nets is one of the main malaria vector control strategies worldwide (WHO, 2023). The WHO Pesticide Evaluation Scheme (WHOPES) currently recommends twelve net types that use permethrin, deltamethrin, or alpha-cypermethrin, with one combination net using deltamethrin combined with the synergist piperonylbutoxide (PBO) in the product's roof. However, there have been increasing reports of malaria vectors developing resistance to the pyrethroids commonly used in LLINs and thus threatens to undermine the efficacy of the current generation of pyrethroid-treated LNs (WHO, 2023). Pyrethroid resistance is now well established across Africa (Ranson & Lissenden, 2016). In Kenya ,DNMP, with global fund resources, continues to procure pyrethroid-only ITNS for the rest of the country due to concerns

about the higher cost of PBO ITNS and the reduced coverage that would result if a switch were made without additional resources (PMI Kenya MOP, 2022). Some studies have shown that bio-efficacy analysis shows that average mortality rates of Yorkool were below World Health Organization's optimal effectiveness of  $\geq 80\%$  compared to other netting brands (Ghimire *et al.*, 2020). The lifespan of LLINs should be at least 3 years after which they will be in bad condition and need replacement (WHO, 2013). The protection offered by LLINs does not last long due to physical and chemical factors. The physical factors include wear and tear, resulting in the formation of holes in the fabric over time. Chemical factors are attributed to factors leading to the reduced or loss of insecticides. It is assumed that there are variations in the physical and chemical decay among LLINs, which seem to be significant, and the effective LLINs life could be less than three years. This assumption was later considered to be overly optimistic. Studies in Benin (Ahogni *et al.*, 2019), Madagascar (Randriamaherijsaona *et al.*, 2017), Benin (Gnanguenon *et al.*, 2014), Eritrea (Yenus *et al.*, 2024) and Zambia (Kilian *et al.*, 2021) have shown that LLINs lifespan is less than 3 years, which contradicts the manufacturer's claims of 3 years lifespan. Some studies have also shown that household factors like LLINs washing and drying practices under field conditions affect the insecticidal activity of the LLINs. The standard WHOPES washing protocol underestimates the amount of insecticide washed from LLINs compared to the abrasive washing procedures that are used in the field (Syme *et al.*, 2025).

#### **2.4 Long Lasting Insecticidal Nets (LLINs) Washing and Drying Practices**

The effectiveness of Long lasting insecticidal nets (LLINs) for malaria control is reduced by washing them (Briet *et al.*, 2020). The LLINs washing practices conditions set by WHOPES stipulates that LLINs should be washed at every 3 months. Frequent washing can degrade both the physical and chemical integrity of the LLINs (WHO, 2023). Washing of the LLINs impact on the insecticidal properties and there is some evidence that over washing of LLINs may also deteriorate the fabric integrity (Briet *et al.*, 2020). The standard WHOPES washing protocol underestimates the amount of insecticide washed from LLINs compared to the abrasive washing procedures that are used in the field. Washing of the LLINs removes insecticide from the surface of

the LN, but it is replenished over time by migration from within the fibers (WHO, 2019). Studies have shown that washing of the nets reduces the effectiveness of the insecticide-treated materials (ITMs) for malaria control. When the nets are washed under controlled conditions, gently with water and a bar soap, all formulations show good resistance, with 100% anopheles mortality after three washes (WHO, 2023). Long lasting insecticide nets are usually recommended to be washed at most every 3 months (PMI Kenya MOP, 2022). Although this frequency depends on local cultural practices, the type of soap available in that area, and water availability. Some studies in Western Kenya (Ochomo *et al.*, 2017) have shown that the LLINs, which were washed using detergent powder instead of bar soap there insecticidal activity deteriorated very fast. Studies have also shown that when the impregnated nets are soaked for 30-60 minutes and washed with soap powder and tap water in the usual way, the mortality drops significantly after 4 washes (Ochomo *et al.*, 2017). Some studies have shown that the majority of LLINs tend to be washed using detergents, which was a common general practice probably because of ease accessibility of the detergent powder (Sahu *et al.*, 2020). However, WHO advises using regular bar soap in place of detergent powder for washing treated nets, as it is not a recommended practice (WHO, 2023).

Drying of nets in shade would be appropriate to preserve the effectiveness of the insecticide in the net after washing. According to some published studies, suggest that net survival are caused by living conditions and household behaviors rather than the LLIN material (Koenker *et al.*, 2023).

Previous studies have reported that the insecticidal content in LLINs decay over time (Briet *et al.*, 2020) ,while permethrin and PBO contents may be lost due to natural decay and evaporation, drying of the nets under direct sunlight are known to contribute to such losses and reduces the biological efficacy of the nets (Staedke *et al.*, 2019). Drying LLINs in the sun is not recommended because direct sunlight degrades insecticides this is consistent with a study by Protopopof et al. (Protopopoff *et al.*, 2023) and in Nicaragua (Villalta *et al.*, 2021) reported that LLINs were exposed to sunlight while drying led to unexpectedly low bio-efficacy. Previously, it had been demonstrated that direct sunlight was harmful to pyrethroid-based

insecticides because UV rays break down pyrethrin molecules, rendering the insecticide inactive (Ochomo *et al.*, 2017). Some studies suggest that nets that were dried hanging on line under the shade generally retained more insecticide compared to those that were air-dried hanging in direct sunlight, and spreading on the ground under the shade and spreading on the ground in direct sunlight (Ochomo *et al.*, 2017).

## 2.5 Physical Integrity of Long-Lasting Insecticidal Nets (LLINs)

Physical durability of long-lasting-insecticidal nets (LLIN) is an important aspect of the effectiveness of LLIN as a malaria prevention tool. It is the structural state of the LLIN fabric that acts as a physical barrier against mosquito bites. This includes the quantity, size, and distribution of holes and tears that develop over time during use (Hiruy *et al.*, 2021). The World Health Organization (WHO) guidelines on durable LLINs advocate standard evaluation of physical integrity, since diminishing physical integrity impairs net effectiveness even when insecticidal properties remain unchanged. (Tan *et al.*, 2016). Physical integrity is mainly quantified using the Proportionate Hole Index (pHI), which is an indicator that weights holes by sizes to approximate total damaged surface area. The ‘proportionate hole index’ (pHI) for LLINs are calculated using WHO guidelines. These holes are categorized into four: Size 1 (0.5–<2.0 cm), Size 2 (2–<10 cm), Size 3 (10–25 cm), and Size 4 (>25 cm), with weights of 1, 23, 196, and 576, respectively, applied during pHI calculation (WHO & WHOPEs, 2013). Depending on total weighted scores, nets can be categorized as **good** (pHI < 64), **serviceable** (pHI 64–642), or **torn** (pHI > 642), reflecting progressively degraded physical protection capacity. Studies have shown that LLINs are deteriorating faster physically, in that they accumulate holes rapidly after distribution. Study in a village in Benin that received Yorkool® LLINs reported 23% having holes after 12 months of use (Ahogni *et al.*, 2019). Another prospective cohort study in Benin designed to compare seven brands of nets reported an even lower proportion at 6.4% of holes existence in Yorkool® LLINs brand after 12 months of use (Ahogni *et al.*, 2020a). Study in Madagascar reported higher proportion of holes present in Yorkool® brand at 68.5% with 31.5% found to be intact after 12 months of use (Randriamaherijaona *et al.*, 2017). Net physical damage

is majorly caused by mechanical damage which are physical forces and interactions that weaken fibers and lead to hole formation during routine household use. The main types of mechanical damage are snagging, abrasion, tearing, seam failure, cutting or puncturing and laddering. These are identified as the principal mechanisms of hole formation in field conditions (Wheldrake *et al.*, 2021). Studies have reported that snagging is the single most frequent mechanical damage mechanism, whilst the largest sized holes in LLINs result from tearing and seam failure. Hole enlargement and abrasion are also responsible for a progressive loss in the physical integrity of nets (Wheldrake *et al.*, 2021). In addition to mechanical damage, animal damage particularly from rodent contributes greatly to the number of holes, typically producing small but numerous perforations that compromise net integrity (Lorenz *et al.*, 2020). Fire damage from candles, cooking fires, open flames can burn holes in LLIN fabrics. Fire damage on nets occurs less frequently than mechanical damage or animal damage but notable cause of localized burns and melting, especially in settings that depend on open flames for lighting or cooking. Exposure to fire still contributes noticeably to hole formation in some communities (Wheldrake *et al.*, 2021). Other contributing factors that influence deterioration of LLINs net condition are household usage practices like frequent handling and folding, improper hanging and nets tied to sharp nails or rough hooks which are prone to tearing (Feio-dos-Santos *et al.*, 2024). Frequent washing and rough cleaning may contribute to physical deterioration of the nets overtime in some settings. Frequent washing and abrasiveness weakens the fibre with time (Gnanguenon *et al.*, 2014). Lastly net fabric influence susceptibility to physical deterioration, polyester multifilament nets are mostly prone to snagging and tearing than polyethylene monofilament nets, and fiber denier and mesh construction affecting resistance to abrasion and rupture (Villalta *et al.*, 2021).

## 2.6 Conceptual Framework

### INDEPENDENT VARIABLES (Community Household Net Handling Practices)

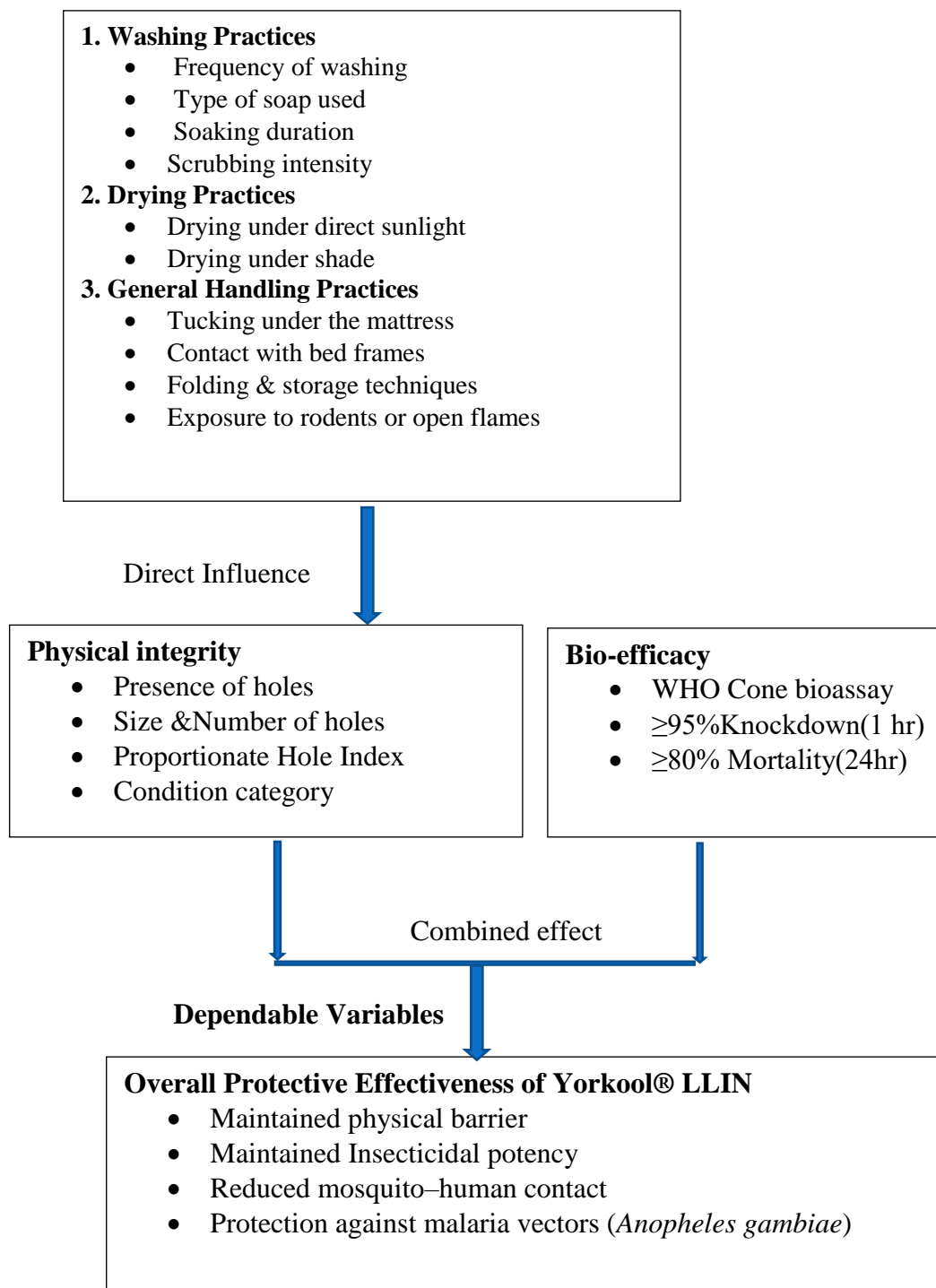


Figure 2.2: Conceptual Framework

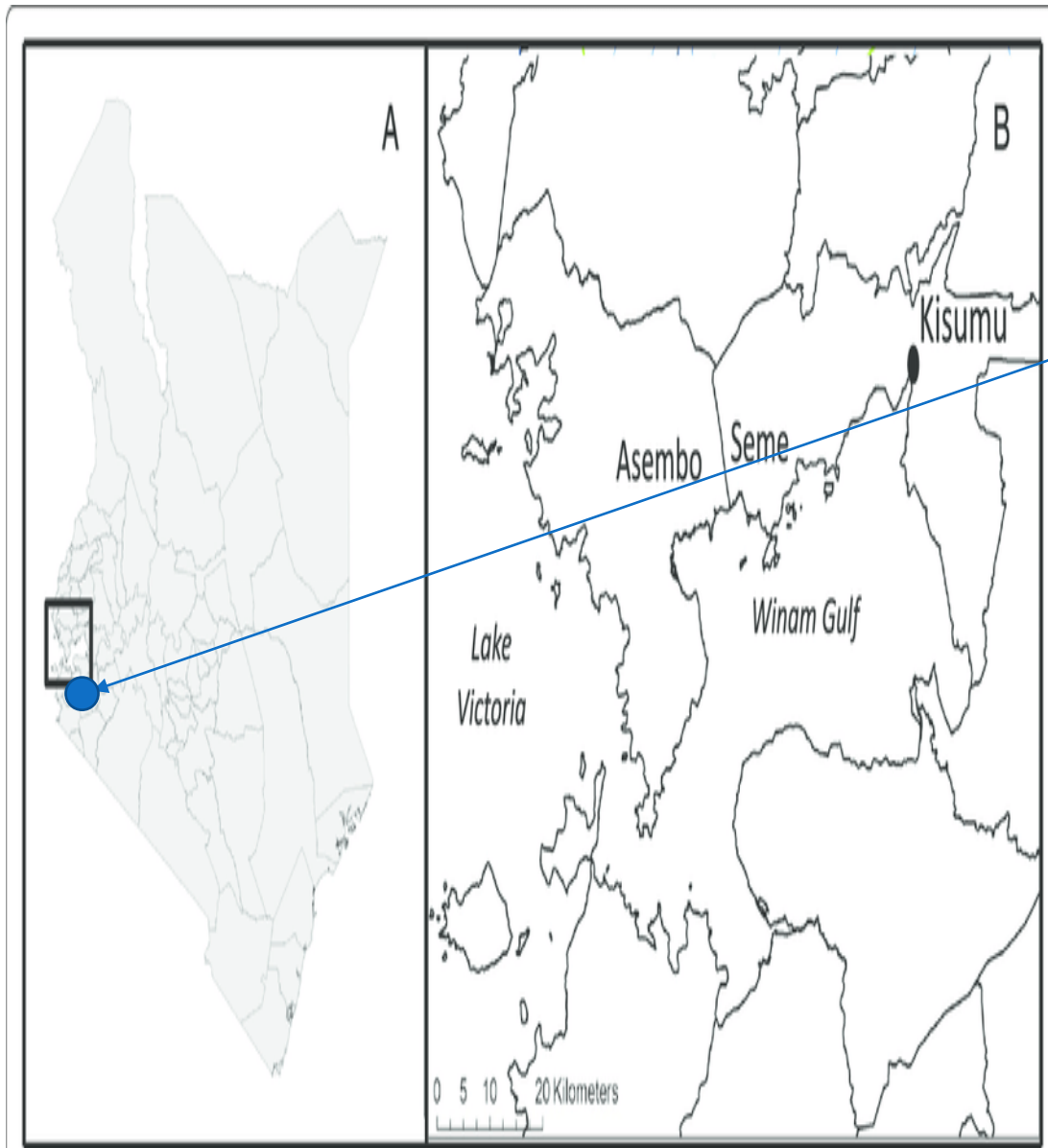
The conceptual framework proposes that household washing, drying, and general handling practices (independent variables) directly influence the physical integrity and bio-efficacy (mediating variables) of Yorkool® LLINs. These two mediators collectively determine the overall protective effectiveness (dependent variable) of the nets after one year of field use.

## **CHAPTER THREE**

### **METHODOLOGY**

#### **3.1 Study Site**

This study was conducted in Seme Sub-County, which is situated in the southwestern part of Kisumu County within the Nyanza region of Kenya. It lies between latitudes 00 20' and 00 50' South and longitudes 300 20' and 350 20'E. The Sub-County's low ridges, seasonal rivers, Lake Victoria, and scalps are some of its most notable geographical characteristics. The mean annual rainfall varies with altitude and proximity to the highlands along the Nandi Escarpment. There are two rainy seasons in the Sub-County, with prolonged showers in April and May. The average yearly rainfall during the August and September brief rains is between 450 and 600 mm. This is a malaria-endemic lake region and is classified as a malaria endemic zone (PMI Kenya MOP, 2022). Malaria transmission occurs all year round and peaks following the rainy season in the area. Because of its vulnerability as a malaria endemic zone, the area has received mosquito nets as malaria control interventions over the years. The economic activities engaged by the people of this region include fishing, which is the major preoccupation because of Lake Victoria, small-scale farming of maize, millet, sorghum and beans. Lastly, a section of the population also engages in small-scale businesses.



**Figure 3.1: Study Site, (Source: gadm.org)**

### **3.2 Study Design**

The study was a cross-sectional household survey and it evaluated rectangular Yorkool® pyrethroid LLINs distributed by the Ministry of Health during the 2021 mass net distribution campaign. The choice of the study design was informed by the fact that this study had the sole objective of evaluating the nets at the household level at a single point in time, which was 12 months after distribution.

### **3.3 Study Population**

The targeted population were household heads in the area, which received rectangular Yorkool®LLINs during mass net distribution in 2021 from the Ministry of Health.

#### **3.3.1 Inclusion Criteria**

Households with Yorkool® pyrethroid LLINs obtained from 2021 mass net distribution.

Households where the head of the household consented to the study.

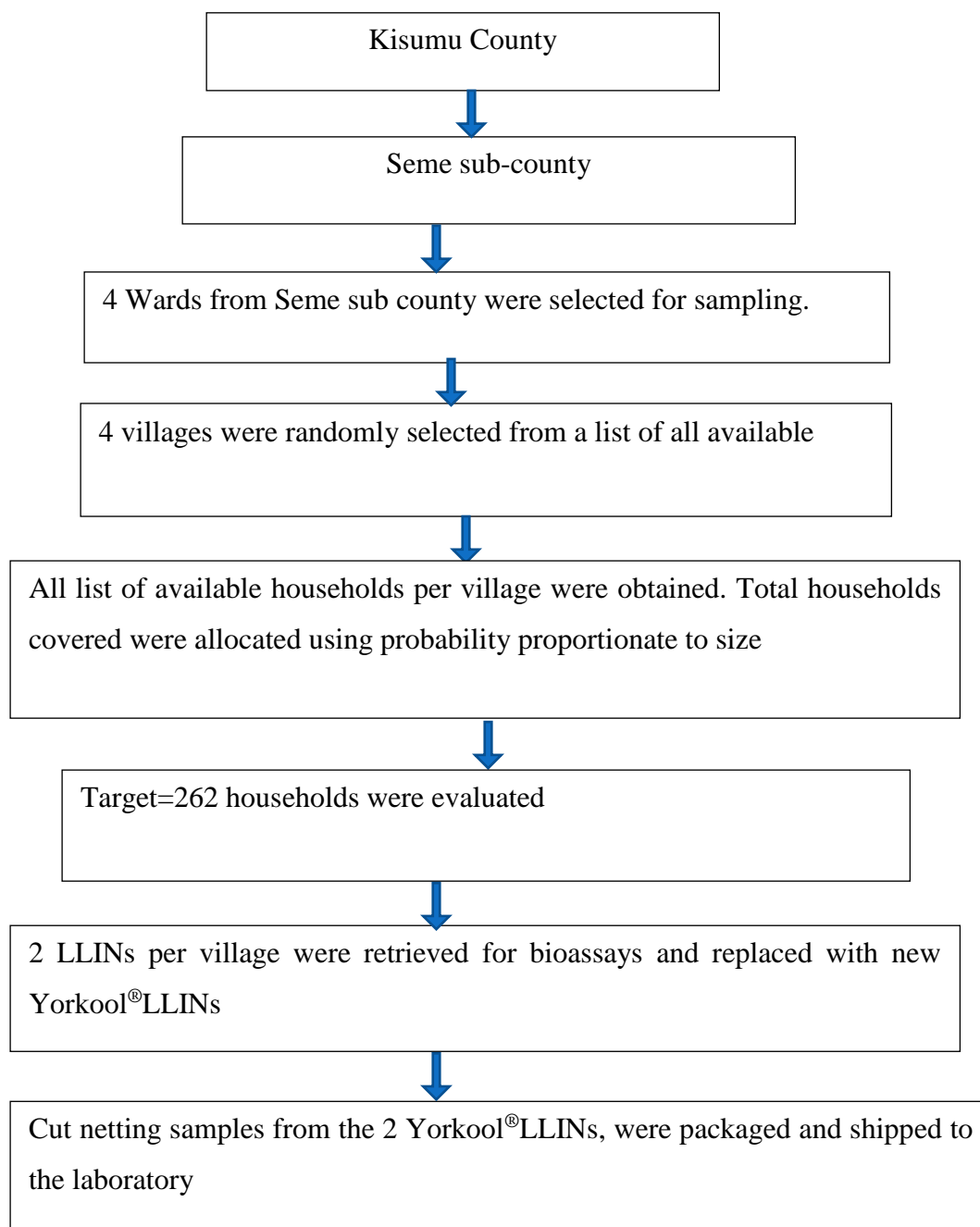
#### **3.3.2 Exclusion Criteria**

Households with other types of nets other than Yorkool®pyrethroid LLINs and households with Yorkool®pyrethroid LLINs but not obtained from the mass distribution of 2021.

Households where the head of the household did not consent to the study.

### 3.4 Sampling Methods

#### 3.4.1 Sampling Frame



**Figure 3.2: Sampling Frame**

### 3.4.2 Sample Size Determination for Bioassay Testing

For the efficacy testing, the study adopted WHO recommendations for minimum sample size of 30 LLINs (WHO,2022).

#### 3.4.2.1 Sample Size Determination for Household Survey

The Yamane Sampling technique was used to determine the sample size for the study as follows:

$$n = \frac{N}{1+N(e)^2}$$

Where  $n$  =the required Sample size

$N$  = the total number of households that received the Yorkool® nets in the study

$e$  =level of precision=0.05

$$n = \frac{760}{1+760(0.05)^2}$$

$$n= 262$$

Sample size of 262 households were recruited.

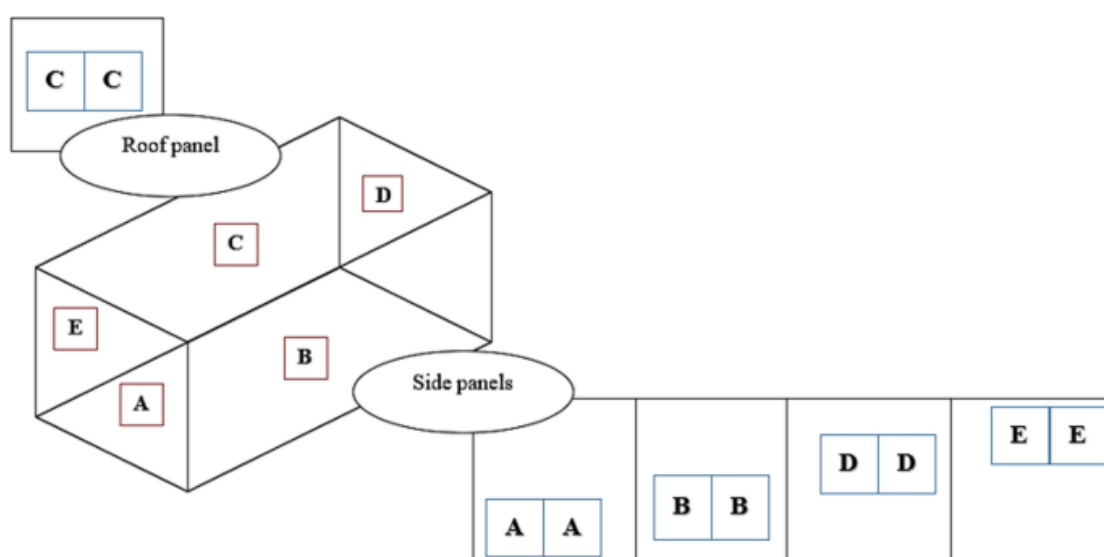
### 3.4.3 Sampling Procedure

Seme sub-county was purposively selected owing to the fact that Yorkool® nets had been distributed earlier. Multistage cluster sampling technique was used to sample households for inclusion in the study. All the four wards in the sub county were selected (Central, North, East and West Seme). A list of all the available villages in the wards, and households that received LLINs in the 2021 mass net distribution was obtained from the sub county malaria coordinator. Four villages from each ward were randomly selected to participate in the study. A list of all households in the selected villages was obtained from the village head and the community health worker. Depending on the number of households across each village, the targeted

sample size was allocated to the 16 villages using probability proportionate to size. At the village level, the first household was selected from the middle of the village and thereafter, every fourth household was systematically sampled until the allocated households in that village were exhausted. For purposes of evaluating the physical integrity of the nets, a sample 180 nets were randomly selected which translated to approximately 10 nets per village. In addition, 30 nets were destructively retrieved from selected households and taken to laboratory for bioassay tests. Households where the nets were retrieved from were given new nets for replacement.

### 3.4.4 LLIN Cuttings for Bioassay

The netting pieces were cut from the sampled nets from positions A–E for bioassays (WHO&WHOPES, 2013). All the nets that were sampled and retrieved were replaced with new ones of similar product. For each LLIN, 30" × 30" sub-samples were cut from all five sides of the net i.e., the roof panel and each of the four sides of the LLINs. These net cuttings were covered in an aluminum foil envelope, labelled, kept individually at 4 °C and transported to the Kenya Medical Research Institute (KEMRI). The samples for bioassay were taken to the Centre for Biotechnology Research and Development (CBRD) and analyzed.



**Figure 3.3: A Rectangular Net and its Individual Panels Showing Positions for Cut Netting Pieces for Bioassay Cone Testing Positions (WHO, 2022)**

### **3.5 Data Collection Procedures**

#### **3.5.1 Household Questionnaires**

Interviews were conducted at the household level to collect data about utilization of the nets. A structured questionnaire was administered to the household head or an adult available in the household after obtaining informed consent. In each sampled village, households were sampled using systematic random sampling and only those households which received Yorkool<sup>®</sup>LLINs in the 2021 mass net distribution and the head of the household consented to the study was interviewed inside the house or outside. The questionnaire captured household demographic information, and the information about the LLINs and handling practices. This data collection technique was the most appropriate as the study was quantitative. The interviews were conducted by the research team field personnel in the presence of the CHW (Appendix 3).

#### **3.5.2 Physical Integrity Assessment**

Physical integrity of Yorkool<sup>®</sup> nets was measured through assessment of holes on the nets. The nets were draped on a frame and inspected for presence of any holes on the sides and roof panels. The number of holes including tears and seam openings were counted and categorized into 4 groups: Hole size T1, were holes smaller than one inch (0.5–2.0 cm), Hole size T2 were holes larger than one inch but smaller than a fist (2–10 cm), Hole size T3 were holes between a closed fist but smaller than a head (10–25 cm) and Hole size T4 were holes bigger than a head (> 25 cm). A proportionate hole index (pHI), which characterized the midpoint diameter to an estimated hole size was calculated by making the sum of the holes weighted by size for each net. The weights were as per the WHO recommendations i.e. 1, 23, 196 and 576. To better translate the hole index to an integrity status (net condition) for each sampled net, the pHI was then categorized into “good” (0–64), “damaged but still useful” (65–642), and “too torn” that protection from mosquitoes is judged to be

compromised ( $\geq 643$ ) (WHO, 2022). Serviceable nets constituted all LLINs in the category of good condition and damaged.

### **3.5.3 Laboratory Analysis**

#### **3.5.3.1 Mosquito Rearing**

The mosquito colonies used in this study were from the insectary. The eggs were obtained from an existing colony in Kisumu KEMRI because the mosquitoes have already adapted to artificial rearing and insectary environment. Kisumu strain *Anopheles gambiae* have been colonized since 1954 and do not exhibit any discernible mechanism of insecticide resistance (WHO, 2018). Fresh batch of Kisumu strain *Anopheles gambiae* eggs were collected and were shipped through an express courier. The eggs were covered with moist filter paper and cotton wool and it was then sealed to avoid desiccation. The *An. gambiae* mosquitoes used in this study were bred in the insectary at KEMRI/CBRD under conventional circumstances, which included a 12-hour light/dark cycle, 80% relative humidity, and controlled temperature of  $25 \pm 2$  °.

Adults were kept on a 10% sugar solution, while larvae were fed sera baby fish food every day.

The rearing of the *Anopheles gambiae* were as per the protocol of mosquito rearing in the insectary (WHO, 2018). The eggs obtained from KEMRI Kisumu insectary were on Whatman filter paper. The filter paper was cautiously discharged into water in a plastic tray flooded with water to prevent them from adhering to the rearing tray's edge. The distilled water where the eggs were submerged contained a pinch of sera baby fish food. The eggs then hatched to larvae. The larvae were fed everyday with sera fish food and monitored for density and population. On the fifth day (5-day old larvae) the larvae population was diluted from one tray to the other trays with a pinch of sera baby fish food. On the 9<sup>th</sup> day the water was changed with fresh water and sera baby fish food was added. The pupae started developing at this stage. The trays were then covered with nets to prevent an escape of the adult mosquitoes as they emerge. The pupae were allowed to emerge to adults for the next 2-3 days. Baby

fish food was given every day to the larvae and pupae by carefully removing the nets to avoid escaping of the adults. The adults were collected into a cage through an aspirator connected to vacuum. The cage consisted of a small 100 ml bottle with a cotton wick that was soaked with 10% glucose solution (autoclaved) and a paper towel lining on the bottom that was used to soak any potential sugar spills which occurred during cage handling.

The adults (both males and females) were then kept in the insectary room for 3-5 days, fed on 10% glucose solution. The 3-5 days old female anopheles' mosquitoes were taken for bioassay testing. Some of 3-5 days old mosquitoes that were not used for bioassay testing were blood-fed to begin the next cycle for the continuity of the colonies that were to be used for bioassay testing. The mosquitoes were used to lay eggs more than once. As regards the blood meal, the mice were laid on top of the cage, mosquitoes were thus being permitted to feed on it through the netting. The mosquitoes were allowed to feed for 30 minutes. The females laid eggs two days after they were blood fed. A filter paper was wrapped in a conical shape and was put in a beaker containing distilled water to make sure the filter paper was getting moist. The beaker was kept inside the cage overnight for the mosquitoes to lay eggs. Lastly, the mosquitoes that were not needed for experiments or rearing were killed by placing the cage in a freezer.

### **3.5.3.2 WHO Cone Bioassay Test**

The insecticidal effect of the Yorkool® LLINs was evaluated using cone bioassays as per the WHO guidelines (WHO, 2022). The bioassays testing was done at 12 months of net use. On each of the netting pieces cut for bioassays, standard WHO plastic cones were held in place using a plastic manifold and a total of 50 laboratory bred, susceptible Kisumu strain *Anopheles gambiae* that were known to be susceptible to pyrethroid insecticides (non-blood fed, 3–5 day old) were exposed for 3 min (5 pieces per net × 5 mosquitoes per test × 2 replicates). After the exposure, the mosquitoes were removed gently from the cones and were transferred into holding plastic cups provided with cotton-wool moistened with 10% glucose solution. Knockdown (KD) was recorded at 60 min post exposure. Mortality was recorded at

24 h after exposure. Mosquitoes were considered knocked down or dead if they could not fly or could not stand upright on either the side or the bottom of the plastic cups. The mean percentage knock down (KD) at 3 minutes and mortality at 24 hours post-exposure were estimated for each of the evaluated bed nets. The bioassays were carried out at  $27 \pm 2$  °C temperature and  $80\% \pm 10\%$  relative humidity (WHO, 2022).

#### **3.5.4 Statistical Analysis**

Data entry was entered in Microsoft Excel spreadsheets and cross-checked for accuracy. Data was exported for analysis in STATA version 15.1 (Stata corporation, college station, TX, USA). Households were described by summarizing the demographic characteristics and net usage information into frequencies and percentages. The presence, location and type of holes on the nets were analyzed and presented as percentages. The holes were categorized into sizes and presented as counts with 95% confidence interval by part of the net affected. In addition, fabric integrity was represented using pHI which was categorized into “good”, “damaged” and “needs replacement” then presented as percentages. Net washing practices were described based on whether washing was done at least once in past year, duration between washes, detergents used, soaking and scrubbing practices. Drying of nets after washing focused on exposure to the sun. The practices on net washing and drying were analyzed and presented as percentages of the of the households where interviews were conducted. The bio-efficacy of Yorkool® LLINs was analysed using mean knock-down and mortality of the mosquitoes and presented as percentages with 95% confidence interval (CI). Long-Lasting Insecticide Nets passed the WHO efficacy criteria if the mosquito knockdown rate was  $\geq 95\%$  and/or mortality rate  $\geq 80\%$  in cone bioassays.

#### **3.6 Ethical Considerations**

Ethical approval was sought from KEMRI Scientific and Ethics Review Unit and NACOSTI.

KEMRI/SERU/CBRD/479/4587 (Appendix V) and NACOSTI/P/23/23968 (Appendix IV).

Participation was voluntary from respondents and they had a right to refuse or drop from the study anytime. There was no prejudicial treatment of those who declined to participate or withdrew from the study after agreeing to participate. The participants who were not able to read or write were assisted by an independent witness and ensured the study was clearly explained to them either in English or Dholuo and guided them to give a thumb print on the consent form (Appendix I and II) after they agreed to participate in the study. There was respect to the autonomy of all participants and participants with diminished autonomy were entitled to additional protection. Sufficient information about the purpose and objectives of the study was provided to the participants, the participants understood and were sure about taking part in the study and willingly decided whether or not to do so without any pressure or persuasion. The participants were assured that the study had no risk or discomfort and it tried to decrease chances of uncomfortable events from occurring during the visits. They were asked to allow the research team to get into their houses to see the nets. The study did not give any monetary compensation to the participants while visiting their houses. However, for the households where a mosquito net was withdrawn to be used in the study, a new Yorkool<sup>®</sup> net was given for replacement. The participants were assured that all the information related to their participation was confidential. The study team were the only ones who knew their names because of the direct contact they had with the respondents. Their identity was not revealed in any reports or publications resulting from the study. Unique identifier was used to ensure anonymity. All original documents were kept in a secure location at ESACIPAC KEMRI to which only members of the research team had access.

As for the mosquitoes that were used, the KEMRI Animal Care and Use Committee (ACUC) provided ethical oversight for the use of *Anopheles gambiae* Kisumu strain mosquitoes, ensuring that all animal handling and experimental procedures met globally established criteria for the care and use of laboratory animals. Mosquito rearing and blood-feeding methods were carried out compassionately and in accordance with established ACUC protocols, reducing suffering and ensuring that mosquitoes were only employed for bioassay testing and colony maintenance. The ACUC certification emphasizes that all experimental manipulations of mosquitos, including feeding and final killing of surplus individuals, were carried out in

accordance with strict ethical norms intended to encourage responsible and humane scientific conduct.

## CHAPTER FOUR

### RESULTS

#### 4.1 Demographic Characteristics of the Study Population

A total of 262 households participated in the study, where the household heads were interviewed. The mean age of the household heads was 46.9 years (SD 17.4 years), ranging from 18 to 92 years. Most of the participants were in the 31 to 50 years (36.6%) age bracket. A majority of them had attained primary level education (70.2%), and 42.3% of the households used electricity as the main source of lighting. A majority of the households (71.8%) had 1-2 sleeping spaces. (Table 4.1).

**Table 4.1: Household Demographic Characteristics**

<b>Variable</b>	<b>Category</b>	<b>Percentage (%)</b>
Age	18-30	63 (24.1)
	31-50	96 (36.6)
	51-70	82 (31.3)
	71-95	21 (8.0)
Education level	No formal education	11 (4.2)
	Primary	184 (70.2)
	Secondary	53 (20.2)
	Tertiary	14 (5.3)
Electricity	Yes	116 (42.3)
	No	146 (55.7)
Sleeping space	1-2	188 (71.8)
	3-4	67 (25.6)
	5-6	5 (1.9)

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7-8	2 (0.7)
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## 4.2 Net Location

A total of 180 LLINs were physically examined for physical integrity in the households that participated in the study. A majority of these LLINs (98.9%) were located inside the house. The floor type in the houses where the nets were found was mainly soil or sand (49.4%) and cement (48.3%). The walls were mud brick with wood frames (57.9%) or concrete (37.6%) and 99.4% of the houses had corrugated iron roofs (Table 4.2).

**Table 4.2: Net Location and Socio-Economic Parameters**

Variable	Category	Total nets=180 n (%)
Location of the net	Inside	178 (98.9)
	Outside	2 (1.1)
Type of floor	Soil or sand	88 (49.4)
	Cement	86 (48.3)
	Cement (incl. vinyl)	2 (1.1)
	Other	2 (1.1)
Walls of the room	Mud brick with wood frames	103 (57.9)
	Concrete	67 (37.6)
	Mud brick	4 (2.3)
	Lime plaster	4 (2.3)
Roof or ceiling of the room	Corrugated iron	177 (99.4)
	Concrete	1 (0.6)

**% percentages**

### 4.3 Net Conditions

Most of the nets examined (77.8%) had at least one hole, with a majority of them (93.6%) having holes in the lower part. Horizontal tears (76.7%) were the most common types of holes observed. The highest number holes were size 1 (n=2015) while size 2 were 1216, size 3 were 995 and size 4 were 314. The lower part of the nets had the highest number of holes of all sizes (Table 4.3).

**Table 4.3: Net Conditions**

Variable	Category	Total nets = 180 n (%)
Nets with holes	Yes	140 (77.8)
	No	40 (22.2)
Specific area of holes	Roof	65 (46.4)
	Upper part	30 (21.4)
	Lower part	131 (93.6)
	Seams	17 (12.1)
Type of holes observed	Horizontal tears at bottom	138 (76.7)
	Holes at hanging points	4 (2.2)
	Open seams	21 (11.7)
	Burn holes	4 (2.2)
	Holes from rodents	0
	Whole section missing	1 (5.6)
Holes observed by size and specific area, # of holes (95% CI)		
<b>Size 1</b>	Roof	212 (89.3-334.7)
	Lower part	1580 (1141.4-2018.6)
	Upper part	220 (79.2-376.7)
	Seams	3 (0-7.3)
	Total	2015 (1517.7-2512.3)
<b>Size 2</b>	Roof	136 (80.3-191.7)
	Lower part	946 (659.2-1232.8)
	Upper part	118 (59.5-176.5)
	Seams	16 (4.3-27.7)
	Total	1216 (909.4-1522.6)
<b>Size 3</b>	Roof	272 (173-371)
	Lower part	565 (440.2-689.8)
	Upper part	144 (79.1-208.9)
	Seams	14 (2.3-25.7)
	Total	995 (821.8-1168.2)

<b>Size 4</b>	Roof	35 (19.6-50.4)
	Lower part	249 (178.0-320)
	Upper part	18 (2.9-33.2)
	Seams	12 (1.7-22.3)
	Total	314 (236.8-391.2)

# Number, CI confidence interval, % percentage

#### 4.4 Proportionate Hole Index (pHI)

From the pHI classification, 48 (26.7%) nets were found to be in good condition, 29 (16.1%) needed repairs and 103 (57.2%) needed to be replaced. Overall, 42.8% of all the LLINs examined after one year of study were of serviceable condition and thus were offering protection to the users. (Table 4.4).

**Table 4.4: Proportionate Hole Index (pHI)**

<b>Total number of LLINs inspected</b>	<b>180</b>
Proportion of nets with any hole n (% , CI)	140 (77.8, 71.7-83.9)
Mean pHI	2254.8
Median pHI	1041.0
IQR	3109.0
Proportion of nets in pHI<64 (good category)	48 (26.7; 20.2-33.1)
Proportion of nets pHI ≤642 (Serviceable category)	29 (16.1; 10.7-21.5)
Proportion of nets pHI >642 (needed replacement category)	103 (57.2; 49.9-64.2)

#### 4.5 Factors Associated with LLIN Loss of Physical Integrity

Of the 180 nets inspected to assess the loss of physical integrity, 103 (57.2%) had lost their physical integrity and needed to be replaced. Univariable analysis results revealed several factors were associated with net replacement. These factors included the type of fire used in the household where the LLIN was found. Nets found near wood fires were significantly more likely to be completely worn out compared to those found in areas without wood fires (OR=4.1, 95% CI: 1.2-13.8, P=0.023). Furthermore, horizontal tears at the bottom were much more likely to require replacement compared to intact nets (OR=54.6, 95% CI: 12.6-237.3, P<0.001). Similarly, nets with open seams were also more likely to be replaced compared to those without (OR=3.6, 95% CI: 1.2-11.2, P=0.026). (Table 4.5).

The multivariable analysis confirmed that some factors remained significantly associated with net replacement after accounting for other variables. Nets found in areas with wood fires were more likely to be replaced compared to nets found in areas without wood fires (adjusted OR=7.5, 95% CI: 1.4-40.7, P=0.019). In addition, nets with horizontal tears at the bottom are more likely to be replaced compared to nets without the horizontal tears (adjusted OR=96.2, 95% CI: 9.6-966.4, P=0.026). (Table 4.6)

**Table 4.5: Univariable Analysis of Factors Associated with Loss of Physical Integrity**

Factors	Number of replaceable nets (%), n=103	Odds ratio (95% CI)	p-value
<b>Where net is found</b>			
Inside	101 (98.1)	Reference	-
Outside	2 (1.9)	Insufficient observation	-
<b>Type of floor where net is found</b>			
Soil or sand	56 (55.5)	Reference	-
Cement (incl. vinyl)	0	Insufficient observations	-
Cement	44 (43.5)	0.6 (0.3-1.1)	0.097
Other	1 (1.0)	0.6 (0.0-9.5)	0.696
<b>Walls of the room where net is found</b>			
Concrete	41 (40.6)	Reference	-
Mud brick	2 (2.0)	0.6 (0.1-4.8)	0.659
Mud brick with wood frames	56 (55.5)	0.8 (0.4-1.4)	0.380
Lime plaster	2 (2.0)	0.6 (0.1-4.8)	0.659
<b>Use of open flame where net is found</b>			
Yes	27 (26.2)	Reference	-
No	76 (73.8)	1.7 (0.9-3.2)	0.102
<b>Type of fire (n=27)</b>			
Wood fire			
Yes	22 (81.5)	4.1 (1.2-13.8)	<b>0.023*</b>
No	5 (18.5)	Reference	-
Charcoal fire			
Yes	19 (70.4)	Reference	-
No	8 (29.6)	2.6 (0.7-10.1)	0.157
<b>Type of hole</b>			
Horizontal tears at the bottom			
Yes	101 (98.1)	54.6 (12.6-237.3)	<b>&lt;0.001*</b>
No	2 (1.9)	Reference	-
Holes at hanging points			
Yes	3 (2.9)	Reference	-
No	100 (97.1)	0.4 (0.0-4.3)	0.479

Open seams				
Yes	17 (16.5)	3.6 (1.2-11.2)		<b>0.026*</b>
No	86 (83.5)	Reference		
Burn holes				
Yes	3 (2.9)	Reference		
No	100 (97.1)	0.4 (0.0-4.3)		0.479

\*Significant p-value

**Table 4.6: Multivariable analysis of Factors Associated with Loss of Physical Integrity**

<b>Factor</b>	<b>Number of Replaceable nets (%), n=103</b>	<b>Adjusted Odds ratio (95% CI)</b>	<b>P value</b>
Type of fire (n=27)			
Wood fire			
<b>Yes</b>	22 (81.5)	7.5 (1.4-40.7)	<b>0.019*</b>
<b>No</b>	5 (18.5)	Reference	
Type of hole			
Horizontal tears at the bottom			
<b>Yes</b>	101 (98.1)	96.2 (9.6-966.4)	<b>&lt;0.001*</b>
<b>No</b>	2 (1.9)	Reference	

\*Significant p-values

#### 4.6 Yorkkool® LLINs Washing and Drying Practices

A total 468 nets were recorded from all the 262 households which participated in the study. Of these, 95.1% (445 nets) was reported to have been washed at least once. At the time of the survey, 82% of the nets were reported to have been washed in the last one month prior to survey, with 74.4% of the nets reported to have been washed using local bar soap. Majority of the participants (90.8%) reported not soaking the nets before washing for. The practice of scrubbing the nets while washing was uncommon, having being reported in only 2 nets. Drying practice was mainly outside in the shade (72.1%) while those that were dried outside in the sun were 26.5% (Table 4.7).

**Table 4.7: Yorkool® LLINs Washing and Drying Practices in the Households**

<b>Variable</b>	<b>Category</b>	<b>Frequency (%)</b>
Ever been washed	Yes	445 (95.1)
	No	23 (4.9)
Duration since last washing	<1 month	365 (82.0)
	1-3 months	69 (15.5)
	3+ months	11 (2.5)
Type of soap used	Local bar soap	331 (74.4)
	Detergent powder	85 (19.1)
	Mix (bar and detergent)	29 (6.5)
Net soaking duration	Did not soak	404 (90.8)
	<1 hour	39 (8.8)
	>1 hour	2 (0.5)
Net scrubbed on hard surface	Yes	2 (0.5)
	No	443 (99.6)
Net dried	Outside in the sun	118 (26.5)
	Outside in the shade	321 (72.1)
	Inside	6 (1.4)

#### **4.7 Bio-Efficacy of Yorkool® LLINs at 12 Months**

After 12 months of use, the nets tested recorded a mean KD of 92% after 1 hour, and mean mortality of 86% after 24 hours. Overall, 83.3% of the nets passed the WHO efficacy cut off for mortality ( $\geq 80\%$ ) after 24 hours, with another 70% passing the cut off for KD ( $\geq 95\%$  knockdown) after 1 hour (Table 4.8).

**Table 4.8: Knock-Down and Mean Mortality Observed with WHO Cone Tests at 12 Months**

<b>No of nets tested</b>	<b>30</b>
<b>Mean 1h % KD (95% CI)</b>	92 (90.7-93.5)
<b>Mean 24h % mortality (95% CI)</b>	86 (83.2-89.7)
<b>Nets that passed WHO cut off by KD criteria alone</b>	21 (70%)
<b>Nets that passed WHO cut off by Mortality criteria alone</b>	25 (83.3%)

## CHAPTER FIVE

### DISCUSSION, CONCLUSION AND RECOMMENDATIONS

#### 5.1 Discussions

Long lasting insecticidal nets have played a central role in preventing mosquito bites in the malaria-endemic zones including the East Africa's Lake Victoria region. In countries where malaria is endemic, the distribution of LLINs to communities living in endemic areas has become a priority (WHO,2023). However, several factors including condition, use and maintenance of the net by the user and the type of the net tends to affect the durability, and thus determining its ability to offer protection to the end user. This study therefore determined the physical integrity and bio-efficacy of Yorkool® net after one-year of use in a malaria endemic zone in Kenya. The washing and drying practices were also assessed among the household users of the nets. The study found out that a majority of the LLINs needed replacement because of poor physical integrity. The communities reported good net handling practices, with washing of the LLINs been done mainly using local bar soap and without soaking and scrubbing on hard surface, and drying of the nets under the shade. Handling of LLINs at the household level needs to follow the set guidelines given during the distribution period to ensure sustained physical integrity of the nets.

##### **5.1.1 Physical Integrity of Yorkool® LLINs in Seme, Kisumu County after One Year of Use in Households**

The fabric integrity is a design parameter that measures physical damage sustained by the net and coverage (Randriamaherijaona *et al.*, 2017). Net handling practices influence the physical sustainability of the nets for the duration of use. Over-washing has been identified as one among other reasons compromising physical integrity of bed nets (Villalta *et al.*, 2021).Nets that are handled using rough methods may develop holes which influences the ability of the net to protect the user from mosquito bites.

A majority of the LLINs inspected in this study had at least one hole located more frequently at the lower part of the net. Previous studies on this type of LLIN have reported lower proportions of holes during the same duration of use. A study in a village in Benin that routinely received Yorkool® nets reported 23% having holes after the 12 months of use (Ahogni *et al.*, 2020a). Another prospective cohort study in Benin designed to compare seven brands of nets reported an even lower proportion at 6.4% of holes existence in Yorkool® brand after 12 months of use (Ahogni *et al.*, 2020b). A study in Madagascar reported higher proportion of holes present in Yorkool® brand at 68.5% with 31.5% found to be intact after 12 months of use (Randriamaherijaona *et al.*, 2017). The variation in prevalence of holes in bed nets are attributable to the differences in handling practices and the brand of the net may also have an influence.

The number and sizes of holes on the nets determine the usability of the nets in barring the mosquitoes which is evaluated using the proportionate hole index (pHI) (WHO, 2011). Proportionate hole index is a standardized approach that describes changes in LLIN fabric integrity, and gives a threshold of good, damaged but still useful and compromised nets. In the present study, slightly higher than a quarter (26.7%) of the nets studied were in good condition, while 43% were serviceable after 12 months of use. The two categories depict that these nets were still in good use and able to provide protection to the users. The 12-month evaluation of Yorkool® brand has received positive approval on physical integrity in other study findings elsewhere with as high as 90.1% (Ahogni *et al.*, 2019), 96.8% (Ahogni *et al.*, 2020c) and 97.7% (Ahogni *et al.*, 2020a) categorized in good condition in Benin. Also, in Madagascar, 69.2% were in good condition and the rest were serviceable with none requiring replacement (Randriamaherijaona *et al.*, 2017). Similarly, the nets evaluate in Nepal after a median duration of use of 9 months found 80.7% were in good condition and 10% too torn needing replacement (Ghimire *et al.*, 2020).

The physical integrity is influenced by several environmental factors and handling practices. One of the two factors that were identified in this study was the use of wood fire for cooking in houses where cooking happened in the same room where there was a place to sleep that increased the risk of deterioration of nets 8-fold. This

risk was expected given that the population live in a rural setting where households use wood as source of fuel. Findings from Benin showed that households where wood was used as cooking medium had pHI-value that was 16 times higher than those that used coal (Ahogni *et al.*, 2020b). The other factor was related to the nets with horizontal tears at the bottom were 96 times more likely to need replacement. This may refer to the handing practices which is related to the common practice of stacking the net on the side between the bed and mattress hence causing wear and tear. The lower part of the net was the most affected with holes of all sizes. This was similar to findings in another study that reported a significantly higher total hole area in the lower half of the rectangular nets (Craig *et al.*, 2015).The reasons for deterioration in the lower part of the net is mainly due to the fact that handling more frequently occurs around this part compared to the other parts like the upper part or the roof which is almost untouched at all times.

### **5.1.2 Household Washing and Drying Practices of Yorkool® LLINs in Seme, Kisumu County**

Majority of the nets were washed more frequently than the current recommendations. In Seme community nearly 97% of nets were washed < 3 months prior to the survey. In less than a month nearly 82% of the nets were washed prior to the study. Variable washing frequencies have been reported in other research, with up to 8 washes per month in Mali (Leonard *et al.*, 2014). In Kwale,coastal region it was reported frequent washing of the nets as opposed to the recommended washing frequency by the WHOPEs (Mutuku *et al.*, 2013).A study in western Kenya reported similar results of frequent washing of the nets, majority of the nets were washed less than 3 months prior to the survey (Santos *et al.*, 2019).Nevertheless, frequent LLINs washing is associated with insecticide loss (Gichuki *et al.*, 2021).The different washing frequencies in these studies were contributed either due to social and structural factors or environmental factors. The current study was consistent with a study in Daresalam, whereby the nets were reported to be washed frequently due to dirt because dirty nets were termed as unhealthy and unacceptable in the society (Azizi *et al.*, 2023). The participants were well knowledgeable about taking care of the nets in terms of washing but due to their personal reasons regarding dirt and

social status surrounding dirty nets they are forced to wash the nets anytime its dirty. Monitoring and understanding household washing routines is crucial for LLINs lifespan, despite significant heterogeneity across research.

The kind and brand of soap may influence washing frequency, depending on the soap's pH level. In the current study the brand and the Ph levels of the soaps was not identified but the participants were referring to “toyo” (meaning local bar soap) when questioned on the type of the soap used. Bar soaps and mild detergents do less damage to insecticidal levels on nets than bleach. In the present study, no bleach-washed nets were recorded, this is consistent with a study in Uganda (Helinski *et al.*, 2015). The current study was consistent with a study in Mali, whereby majority of the nets were washed using bar soap and few of the nets were washed with a mixture of omo and bar soap (Cisse *et al.*, 2025). In Eastern Uganda (Helinski *et al.*, 2015), Western Uganda (Kilian *et al.*, 2021), and Southern Ethiopia (Tomass *et al.*, 2016) reported majority of correspondents used bar soap to net wash. Contrary to the present study, India reported majority of the respondents washed there nets using detergents because of its ease accessibility contrary to the WHO guidelines (Sahu *et al.*, 2020). Washing nets with a harsher detergent instead of a regular bar soap may affect insecticidal integrity of the nets. A study by Musa et al evaluated the effect of repeated washing of LLINs using local rural detergent on the feeding success and survival rates of *Anopheles gambiae* and reported that feeding success and survival rates increased with successive number of washes of the nets after the 5<sup>th</sup> wash (Musa *et al.*, 2020). This is a clear indicative that even after five washes of the nets using detergent deteriorates the insecticidal activity of the nets. It is not feasible to create uniform washing protocols in the field but continuous sensitization on recommending of usage of mild soaps when washing the nets should be emphasized.

Exposing mosquito nets to repeatedly hitting them against rocks can reduce their insect-repelling properties and lead to premature wear and tear, ultimately compromising their effectiveness in protecting against mosquito bites (Sharma *et al.*, 2021). A study in Western Kenya reported that in rural places LLINs are typically washed by hitting them against hard surfaces such as rocks. This approach has not been thoroughly examined, and no comparative research exist. This cleaning process

is frequently used on dirty white nettings (Santos *et al.*, 2019). This finding was consistent with a study in western Uganda (Kilian *et al.*, 2015), in India (Sahu *et al.*, 2020) whereby majority of the nets were not scrubbed on hard surface during washing but in contrast with a study in southern Ethiopia that reported 58.7% of scrubbing their LLINs on hard surface while washing (Tomass *et al.*, 2016). Soaking nets before washing is another factor that determines the ability to retain the efficacy of insecticide activity. This study showed that about 10% of the nets were soaked before washing the last time cleaning was done with majority not soaking at all. This report is similar to a study in India where majority of the nets were not soaked before washing (Sahu *et al.*, 2020), but in contrast with study in Southern Ethiopia they reported majority of the nets were soaked before washing. Some studies have reported that when impregnated nets are soaked for 30-60min and washed with soap powder and tap water in the usual way, the mortality drops significantly after 4 washes (Hiruy *et al.*, 2023).

Drying of nets in shade would be appropriate to preserve the effectiveness of insecticide in the net after washing. This message was given to the households in the villages at the time of distribution of nets to get full benefit of the nets. The community was aware of proper usage, adverse effect of net drying in direct sunlight from the ongoing health education programme of the Community Health Centre. During the survey, it was observed that majority 72.1% of nets were dried under the shade. This was because the community was sensitized pre net distribution and post net distribution by the community health volunteers. The study was consistent with another household survey conducted in Eastern Uganda in 2015 where the majority of the respondents dried their nets in the shade (Helinski *et al.*, 2015), also a similar study in Southern Ethiopia reported more than half of the nets were under dried under the shade (Tomass *et al.*, 2016). A study in Western Uganda (Kilian *et al.*, 2015) reported majority of the nets being dried under the shade. A study conducted in India by Sahu *et al.*, 2020 reported similar results as the current study whereby majority of the nets were dried under the shade thus all the nets were effective in terms of insecticidal efficacy of field use (Sahu *et al.*, 2020). This is in contrast with a study in western Kenya (Santos *et al.*, 2019), India (Sahu *et al.*, 2017), and Mali (Leonard *et al.*, 2014) reported majority of the nets being dried in direct sunlight. Drying nets in

direct sunlight is not advised since it can degrade insecticidal activity of the nets (WHO, 2022). Proper net drying is a key LLINs care component that needs addressing all the time during distribution. Previous research unrelated to this study demonstrated that direct sunlight can be damaging to insecticides based on pyrethroids due to the breakdown of pyrethrin molecules by UV rays, which results in the insecticide becoming ineffective (Samia *et al.*, 2024). A study in Western Kenya on methods of drying LLINs after washing, whereby it was reported that nets that were dried hanging under the shade significantly retained more insecticide and recorded higher percentage mortality compared to the nets that were dried in direct sunlight lost the most amount of insecticide and were least effective (Santos *et al.*, 2019).

### **5.1.3 Bio-efficacy of Yorkool® LLINs in Seme, Kisumu County after One Year of Use in Households**

The evaluation of the biological efficacy revealed that the Yorkool® LLINs brand studied had a good residual efficacy, since it induced a 92% rate of KD after 60 minutes and 86% mortality after 24 hours. WHO recommends  $\geq 95\%$  KD and/ or  $\geq 80\%$  mortality on vectors for an LLIN to be considered efficacious for use as a malaria prevention measure (WHO, 2022). The bioassay results in this study showed a mean KD in 1 hour of 92% and mortality of 86% in 24 hours against the susceptible *Anopheles gambiae* species. This means the nets fell short of the WHO cut off in KD criteria but passed the mortality cut off. However, a high proportion of Yorkool® LLINs met the WHO criteria with 70% of the nets meeting the KD cut off ( $\geq 95\%$  KD) and 83.3% were on target on mortality ( $\geq 80\%$ ). Various studies have shown varying bio-efficacy levels of Yorkool® nets in relation to knock-down and mortality threshold. A notably lower efficacy was reported in Benin after 12 months of use with KD cut-off of 58% and 72% for mortality (Ahogni *et al.*, 2019). However, another study in Benin reported efficacy of 98% on KD and 97.5% on mortality (Ahogni *et al.*, 2020a). In Eritrea, Yorkool® LN nets had a knock-down (KD) rate of 80.5% at 60 minutes and a 71% mortality rate after 24 hours of exposure (Yenus *et al.*, 2024). An evaluation in Madagascar on Yorkool® LLINs showed a very low mortality rate of 14% at 12 months use (Randriamaherijaona *et al.*, 2017). In Nepal,

only 10% of the Yorkool® nets evaluated had mosquito mortality of more than 80% (Ghimire *et al.*, 2020). Based on the several contradictory findings in relation to knock-down and mortality rates for Yorkool® nets after 12 months of use, there is need for systematic review to compare the different conditions of the evaluations to identify or rule out the possibility of extrinsic factors that may be influencing the bio-efficacy of these brand of nets.

Long lasting insecticide nets have played an important role in the remarkable success in reducing malaria burden over the past decade. They are a core prevention tool and are widely used by people at risk of malaria (WHO,2023). The current study found that the evaluation of the biological efficacy revealed that the Yorkool®LLINs brand studied had a good residual efficacy, since it induced a 92% rate of KD after 60 minutes and 86% mortality after 24 hours. After 12 months, 86% of LLINs on average met WHO cut-off for quality of at least 80% of nets achieving either 95% knock-down or 80% mortality (WHO,2022).WHO recommends  $\geq 95\%$  KD and/ or  $\geq 80\%$  mortality on vectors for an LLIN to be considered efficacious for use as measures for malaria prevention. The bioassay results in this study showed a mean KD of 92% in 1 hour and mortality of 86% in 24 hours against the susceptible *Anopheles gambiae* Kisumu strain species. This means the nets fell short of the WHO cut off in KD criteria but passed the mortality cut-off.

However, a high proportion of Yorkool® LLINs met the WHO criteria with 70% of the nets meeting the KD cut off ( $\geq 95\%$  KD) and 83.3% were on target on mortality ( $\geq 80\%$ ). Various studies have shown varying bio-efficacy levels of Yorkool nets in relation to knock-down and mortality threshold. A notably lower efficacy was reported in Benin after 12 months of use with KD cut-off of 58% and 72% for mortality (Ahogni *et al.*, 2019). However, another study in Benin reported efficacy of 98% on KD and 97.5% on mortality (Ahogni *et al.*, 2020a). In Eritrea, Yorkool®LLINs nets had a knock-down (KD) rate of 80.5% at 60 minutes and a 71% mortality rate after 24 hours of exposure (Yenus *et al.*, 2024). An evaluation in Madagascar on Yorkool® LLINs showed a very low mortality rate of 14% at 12 months use (Randriamaherijaona *et al.*, 2017). In Nepal, only 10% of the Yorkool® nets evaluated had mosquito mortality of more than 80% (Ghimire *et al.*, 2020).

Based on the several contradictory findings in relation to knock-down and mortality rates for Yorkkool® nets after 12 months of use, there is need for systematic review to compare the different conditions of the evaluations to identify or rule out the possibility of extrinsic factors that may be influencing bio-efficacy of these brands of nets.

The insecticidal properties of nets are affected by the washing and drying procedures used on them. Additionally, there is evidence that excessive washing may deteriorate the fabric's integrity (Syme *et al.*, 2025). Although it is unclear how much household owners can do to prevent some of these damages, it is likely that a better understanding of net care and awareness of potential net damage will extend the net's lifespan. Long-lasting insecticidal net maintenance behaviour with regard to net washing was somehow satisfactory in the study community. Frequent washing of the long-lasting insecticidal nets can degrade both the physical and chemical integrity of the nets. The current WHO recommendations stipulate that the LLINs are to be washed every 3 months (PMI Kenya MOP, 2022).

## **5.2 Limitations of the Study**

- **Lack of Baseline Data:**

Since no baseline (Day 0) measurements were obtained for the mosquito nets prior to distribution and use, potential initial variations arising from manufacturing differences could not be accounted for. To mitigate the lack of Day (0) measurements, the study utilized internal comparison groups with different net washing and drying practices to determine their effect. However, because of the quality control by the reputable manufacturer, difference in net quality would be expected to be minimal.

- **Reliance on Self-Reported Data:**

Information regarding the washing practices, drying practices and maintenance behaviors was collected through self-reported responses. Such data are inherently susceptible to recall bias, as participants may have had

difficulty accurately remembering past behaviors. Additionally, responses may have been influenced by social desirability bias, whereby participants provided answers, they perceived to be favourable or acceptable rather than fully accurate. These factors may have introduced reporting inaccuracies and potentially affected the reliability of the behavioural data collected.

### **5.3 Conclusion**

- **Yorkkool® LLINs Physical Integrity**

After one year household use, majority of Yorkkool® LLINs used in Seme sub-county had high physical deterioration rate with the evaluation showing about 60% of the nets needing replacement after 12 months of use. The LLINs that were exposed to wood fires were significantly more likely to require replacement. The current study showed that houses which had mud-brick walls and soil floors, contributed to net wear.

- **Yorkkool® LLINs Washing and Drying Practices**

Majority of households in Seme sub-county demonstrated good net care; the nets were washed gently with local bar soap and dried in the shade. However, it was noted that washing frequency exceeded WHO recommendations that stipulates the nets should be washed after every 3 months; this may contribute to physical and chemical degradation over time.

- **Bio-efficacy of Yorkkool® LLINs**

After 12 months of household use, Yorkkool® LLINs maintained high bio-efficacy, with vector mortality meeting the WHO threshold.

### **5.4 Recommendations**

- There is a need for routine monitoring of Yorkkool® LLINs physical integrity and bio-efficacy to guide timely replacement. Additionally, longitudinal studies should be done to examine net durability over time, providing

evidence to improve net distribution, maintenance, and replacement procedures.

- The Ministry of Health Malaria Control Programs should conduct more frequent LLIN replacement mass campaigns, particularly in households using wood fires or with lower-quality housing and prioritizing replacing nets with significant lower-part damage.
- There is need for Continuous Community Education reinforcing proper net handling, including avoiding placing the nets near open flames and minimizing mechanical stress.
- The Seme community should be sensitized on net washing frequency in accordance with World Health Organization guidelines, which stipulate after every three months. More emphasis should also be on drying of the nets as per WHO guidelines, which is under shade.
- The Seme community should be encouraged to separate cooking areas from sleeping areas to reduce heat and smoke exposure to the nets to prevent physical damage of the nets.
- The Ministry of Health Malaria Program should select Long-Lasting Insecticide Nets brands with higher durability that are suited to local conditions and integrate those findings into national malaria control strategies to enhance LLIN effectiveness and lifespan.

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## APPENDICES

### Appendix I: Household Survey Informed Consent Form

#### **PART 1. INFORMATION SHEET FOR NET USERS**

**Study title:** Assessing the bio-efficacy of Yorkool®a pyrethroid-based long-lasting insecticidal nets over a one-year period and determining net washing and drying practices in Seme, Kisumu County.

#### **Investigators**

<b>Name</b>	<b>Role</b>	<b>Institutional Affiliation</b>
Mercy Achulu	Principal Investigator	Jomo Kenyatta University of Agriculture and Technology (JKUAT)
Dr Perpetual Ndungu	CO-Investigator	Jomo Kenyatta University of Agriculture and Technology (JKUAT)
Dr Luna Kamau	CO-Investigator	Kenya Medical Research Institute, Centre for Biotechnology Research and Development (CBRD)
Dr Paul Gichuki	CO-Investigator	Kenya Medical Research Institute, Eastern and Southern Africa Centre of International Parasite Control (ESACIPAC)

**Study location:** The study will be conducted in Seme, Kisumu County.

You are being asked to take part in a research study. The box below tells you important things you should think about before deciding to join the study. We will provide more detailed information below the box. Please ask questions about any of the information before you decide whether to participate. You may also wish to talk

to others (for example, your family, friends, or your doctor) about this study, before agreeing to join.

### **Key Information for You to Consider**

**Voluntary Consent.** Your participation in the study and interviews is entirely voluntary. You are not under any obligation to participate, and you have the right to refuse this invitation. If at any time during the interview, you decide not to participate further, you are free to withdraw immediately, with no further discussion.

**Purpose.** This study seeks to assess the insecticidal activity of Yorkool®, a pyrethroid long-lasting insecticidal net and household factors that may affect its bio efficacy after one-year period of use.

**Duration.** Your part of the study will last for approximately 30 minutes.

**Procedures and Activities.** I shall ask you few questions and also check the condition of the nets which were given to you. In some households I will be taking the nets with me for purpose of carrying out bio efficacy test on them. In case I take a net from a household I shall give you a new one of the same type.

**Risks.** In this study, you are not likely to experience any risk or discomfort

**Benefits.** If you participate in this research, you are not likely to get any personal benefit or incentives.

**Alternatives.** Participation is voluntary and the only alternative is to not participate.

### **Purpose of research**

#### **Description of the research**

Malaria is a major public health issue in Kenya. It is spread from one person to another through infected bites of female anopheles' mosquitoes. Sleeping under mosquito nets protects one from mosquito bites. Further, when the nets are treated with some chemicals that kill insects(insecticides)then they will prevent the bites and

also kill the mosquitoes. The distributed nets are treated with chemical and thus do not require re-treatment until the end of their useful life, these are called long lasting insecticidal mosquito nets. The nets are supposed to offer protection to the person using the net for at least three years and a minimum of 20 washes. This study will assess these nets after one-year use to see whether they meet the minimum requirements in terms of its bio efficacy.

### **Procedure**

Your village has been selected to participate in this study and I would like to invite you to participate in this study. If you agree, I shall ask you a few questions and also check the condition of the nets which were given to you. The interview may last about 30 minutes. In some households I will be taking the nets with me for purpose of carrying out bio efficacy test on them. In case I take a net from a household I shall give you a new one of the same types.

### **How long will the study last?**

The duration of the study will be one year. The questionnaire will take approximately 30 minutes. The study team will keep you informed of any changes made to the study or should new information become available during the study period.

### **Risk and Discomfort**

By participating in this study, you are not likely to experience any risk or discomfort. However, I shall ask you to allow me to get into your house to see the nets which may be uncomfortable to you. I will try to decrease the chances of any uncomfortable events from occurring during my visits.

### **Potential benefits**

The study doesn't have direct benefits to the participants, but the information obtained will provide valuable information to the Division of National Malaria Control Programme (DNMCP). The information obtained can be used in making

decision on how frequent the mass net distribution should happen in areas where malaria is a problem.

### **Data Security and Confidentiality**

All information and records related to your participation will be kept confidential and will not be revealed to anyone, except if required by law, such as in a legal request for the list of beneficiaries. Your identity will not be revealed in any reports or publications resulting from the study. The information will not be used for any other purpose other than for this study and will be accessible only to the study investigators. The survey results will be stored in password protected computer accessed only by the principal investigator.

### **Participation**

Your participation in the study and interviews is entirely voluntary. You are not under any obligation to participate, and you have the right to refuse this invitation. If at any time during the interview, you decide not to participate further, you are free to withdraw immediately, with no further discussion. The study nets that have been given to your household belong to you and yours to keep.

### **Contact**

If you have questions or concerns about this study, please contact the principal Investigator, Mercy Achulu, P.O. Box 20873-00200, Nairobi; Mobile number 0722156054. If you have questions about your rights as a research participant, the contact person is: The Committee Chairperson, KEMRI Scientific and Ethics Review Unit, P. O. Box 54840-00200, Nairobi; Telephone numbers: 020-2722541, 0717719477; Email address: [seru@kemri.org](mailto:seru@kemri.org) **OR** [kemriseru18@gmail.com](mailto:kemriseru18@gmail.com)

### **PART 2. INFORMED CONSENT FORM**

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a householder in this study and am told that I have the right to withdraw from the study at any time

without in any way affecting my medical care. I am also told that the Principal Investigator of the study can exclude my household from the study without my consent. I have been provided with a copy of this consent form.

**Consent and Signature Options:**

**SIGNATURE OF THE PARTICIPANT**

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**Printed Name of the participant**

---

**Signature of participant**

**Date**

**If unable to read**

I have witnessed the accurate reading of the consent form to the potential participant, and the individual had the opportunity to ask questions. I confirm that the individual has given consent freely.

**SIGNATURE OF WITNESS TO CONSENT/CONSENT PROCESS (This individual can be a relative of the participant, but cannot be an individual involved with the research study)**

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**Printed Name of the witness**

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**Signature of witness**

**Date**

I have accurately read or witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

**SIGNATURE OF INDIVIDUAL ADMINISTERING CONSENT**

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**Printed name of Researcher**

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**Signature of Researcher**

**Date**

## Appendix II: Otas Mar Yie Timo Nonro Ei Ot

### BATHE MOKWONGO: OTAS MAR WECHÉ MAG JOMATIYO GI OCHUNGU

**Thororo mar nonro:** Nonro kuom ratil mar ochungu mar le matindo mafuyo mar Yorkool ma olosi gi pyrethroum ma dak kuom thuolo malach. Nonro itimo bang' thuolo mar higa achiel kendo nonro bende fuono weche mag luoko kendo tuoyo ochunguni ei gweng mar Seme ei Kisumu kaunti.

#### Investigators/Jotim Nonro

Nying	Tich	Tudruok kod migawo
Mercy Achulu	Jatim Nonro maduong'	Mbalariany mar Kenyatta kuom Pur kod Lony
Dr Perpetual Ndungu	Jalup jatim nonro	Mbalariany mar Kenyatta kuom Pur kod Lony
Dr Luna Kamau	Jalup jatim nonro	Migawo mar timo Nonro mar Thieth ei Kenya, Bade mar Lony kuom Ngima kod Dongruok
Dr Paul Gichuki	Jalup jatim nonro	Migawo mar timo Nonro mar Thieth ei Kenya, bade ma thiro le matindo mafuyo ei Africa ma Ugwe gi Masawa

**Kuma itimoe nonro:** Nonro ibi time i gweng' mar Seme ei kaunti ma Kisumu.

Ikwayi ni mondo iyie ibed achiel kuom joma itimonegi nonro. Sanduk mantiere piny kaeni oting'o weche mabeyo ma owinjo ipar ka pok iyie mar bedo achiel kuom joma itimonegi nonro. Wabiro medo weche mamoko matut e bwo sanduk. Akwayi ni mondo ipenj penjo kotenore gi nonroni ka pok iyie mar timo nonroni. Oyieni mar

wuoyo gi jomoko kaka anyuola mari, osiepegi, kata daktari kaluwore gi nonro ni ka pok iyie mar timo nonroni.

### **Weche maroteke mondo okonyi**

**Yie ma onge achune:** Yie mari kuom timo nonroni en ma onge achune moro amora. Oyieni mar timo nonroni kata tamori kwayoni ma onge wach moro amora. Ka opo ni e seche ma nonro dhi nyime, to iparo mar weyo gi e kore, oyieni mar weyo gi kanyo ma nonro chung' gi kanyo.

**Tich mar Nonro:** Nonroni itimo mondo ofweny kit tich mar ochungu mar Yorkool® ma olos gi pyrethrum to gi weche mag ot ma chando tich mar ochunguni bang tiyo kuom thuolo mar higa achiel.

**Thuolo:** Bedo mari e nonroni dhi kawo thuolo ma di rom dakika 30

**Chenro kod tije:** Adhi penji penjo moko machuok kendo adhi neno chal mar ochungu owuon mane omiyi. Kuom ute moko, adhi ting'o ochungugo mondo adhi atimnegi pimo mar fwenyo kit kaka gikonyo. Ka opo ni akawo ochungu eot, to adhi chiwo machielo ma olos machal kod ma akawono.

**Obadho:** Kuom nonroni, okikadho kata bedo e obadho kata luoro moro amora.

**Mich:** Ka oponi iyie mar timo nonroni, to onge mich moro amora ma idhi miyi kaka jal ma otimo nonro.

**Gima opogore:** Timo nonroni en mana kuom yie mari to ka opogore kod yie, en mana tamruok

### **Gima omiyo itimo nonro**

#### **Ler mar nonro**

Malaria en midhusi ma omako gwenge e piny mar Kenya. Olandore koa kuom ng'ato nyaka kuom ng'at machielo koluware kod kecho mar suna mamiyo makende.

Nindo e bwo ochungu geng'o dhano mondo kik suna kaye. Kendo ka ochungugi othiedhi gi kemikol ma nego suna, to gibiro konyo geng'o kecho mar suna kendo negogi. Ochungu mane opogi gin mane othiedhi gi kemikols omiyo ok gidwar thieth kendo nyaka chopi gibed ma oti ema omiyo iluongogi ni ochungu mabudho mar geng'o suna. Ochungugi owinjo ogeng' ng'at ma tiyo kode kuom thuolo mar higni adek ka tin kod luoko ma ok tin ne di piero ariyo (20). Nonroni biro temo nono ochungugi bang' higa achiel ka gitiyo mondo ofweny ka bende gichopo e ratil ma owinjo gitiye.

### **Chenro**

Gweng'ni oyier e yor timo nonroni kendo agombo mondo mi arwaki ibed achiel kuom jogo ma wadhi timogegi nonro. Ka iyie, to adhi penji penjo moko matin kendo adhi neno ochungu mari mane opogi mondo ane chalne. Nonroni nyalo kawo thuolo madirom dakika piero adek. Kuom ute moko, adhi ting'o ochungugo mondo adhi atimnegi pimo mar fwenyo kit kaka gikonyo. Ka opo ni akawo ochungu eot, to adhi chiwo machielo ma olos machal kod ma akawono.

### **Thuolo mar timo nonro**

Thuolo mar timo nonroni biro bedo mar higa achiel. Penjo biro kawo thuolo madirom dakika piero adek. Jogo ma biro timo nonro biro bedo ka keloni weche motudore gi lokruok moro amora ma di bedi kata ka opo ni nitiere weche mamoko ma omedore ka luwore kod nonroni e thuolo mar timo nonro.

### **Obadho**

Kuom yie mari mar timo nonroni, ok ibi bedo e obadho kata luoro moro amora. To abiro kwayi mondo iyiena adonji ei odi mondo ane kaka ochunguni chalo ma mano gima ok nyal miyo ibed thuolo. Adhi temo matek mondo kik amiyi luoro kata matin e thuolo ma alimieni.

### **Mich ma di bedie**

Nonroni onge kod mich ma inyal dhiali godo kuom yie mar timo nonro toweche ma iwachinwa dhi miyo migawo mar Sirkal ma thiro landruok mar Malaria. Weche ma wayudo koa kuomi kae bende dhi konyowa e ng'ado weche kuom kinde maromo nade ma wanyalo tiomoe poko mar ochungugi e gwenge ma malaria chendoe,

### **Arita mag weche mag nonro**

Weche duto ma otenore kod bedo mari e nonroni ibi kan mopondo kendook bi landi ne ng'ato ang'ata mak man aka chik nyalo dwaro mondo otimre kamano kaka ka opo ni chik dwaro neno otas mar joma nende okonyore koluwore kod poko mar ochungugi. Weche ma otenore kod in ok bi landi e lipot kata chapa moro amora koluwore kod nonroni. Weche ma wayudo kae ok bi ti godo e yo moro amora ka opogore kod nonroni kendo jogo ma timo nonroni ema nyalo neno kendo tiyo gi wehegi. Duoko mar nonroni ibi kan gi gima opondo ei komputa ma jatim nonro maduong' ema nyalo chopoe.

### **Timo nonro**

Yie mari mar timo nonroni en mana kuom yie mari. Onge chik ma otweyi mondo nyaka itim nonroni kendo in kod ratito mar tamruok timo nonroni. Ka opo ni e seche ma nonro dhi mbele to ing'ado ni iweyo timo nonroni, to oyieni mar weyo gi kanyo kendo penjo ma moko od adhi penji. Ochungu ma itimonegi nonrogi, gin magi kendo oyieni mar dong' kodgi.

### **Tudri gi:**

Ka opo ni dibed kod penjo kata weche mamoko koluwore gi nonroni, inyalo manyo jatim nonro maduong' ma en Mercy Achulu. P.O. Box 20873-00200, Nairobi; e namba mar 0722156054. Ka opo ni in kod penjo kuom ratiro magi kuom nonroni, ng'at ma inhyalo manyo en Jakom mar Aput mar KEMRI mar Sayans kod Loko Chike, P.O. Box. 54840-00200, Nairobi; e namba mar 020-2722541, 0717719477; Mbui: [seru@kemri.org](mailto:seru@kemri.org) **KATA**, [kemriseru18@gmail.com](mailto:kemriseru18@gmail.com)

### **BATHE MAR ARIYO: OTAS MAR YIE MAKENDE**

Asesomo weche duto mantiere malo kanyo, kata osesomna weche go. Abedo kod thuolo marpenjo penjo kuomgi kendo penjo mane a penjo ayudo duokogi ma oromo. Ayie ma onge achune mondo atim nonroni kaka ja ot kendo onyisa ni an kod ratiro mar weyo timo nonroni e saa asaya ma nonro dhi nyime ma di po ni ohinya godo kuom ngimana. Kendo ocha onyisa ni jatend timo nonro maduong' nyalo golo oda kuom nonro ni ma kata ok okwaya. Omiya otas mar yie machalo kod mae.

**Thuolo mar Yie kod Sai:**

**SAI MAR JAL MA ITIMONE NONRO**

---

**Nying ma otapi mar jal ma itimone nonro**

---

**Sai mar jal ma itimone nonro**

**Tarik**

**Ka ok onyal somo**

Abedo janeno kuom somo ma kare mar otas mar yie timo nonro ma osomne jal ma itimone nonro kendo jalno obedo kod thuolo mar penjo. An janeno ni jalno oyie nono mar timo nonro

**SAI MAR JANENO KUOM YIE TIMO NONRO/ CHENRO MAR YIE (Jali nyalo bedo wat ng'at ma itimone nonro, to ok onyal bedo jatim nonro)**

---

**Nying ma otapi mar janeno**

---

**Sai mar janeno**

**Tarik**

Asesomo maber/ abedo janeno kuom somo maber mar otas yie mar jal ma itimone nonro kendo jail obedo kod thuolo mar penjo. Ana janeno ni oyie mondo otimne nonroni ma onge chudo moro amora.

**SAI MAR JATIM NONRO**

---

**Nying ma otapi mar jatim nonro**

---

**Sai mar jatim nonro**

**Tarik**

## **Appendix III: Questionnaire for LLINs Use and Handling**

### **BACKGROUND HOUSEHOLD INFORMATION**

Name of data collector.....

Date.....

Name of county.....

Name of sub county.....

Name of village.....

### **SECTION 1: HOUSEHOLD INFORMATION**

I would like to ask you some questions about your household

#### 1.1 Respondent

Who is responding to the questions?

- Head of household
- Another adult in household

What is your name?

What is your age?

Education Level

What is the highest level of education of the head of the household?

- None (no formal education)
- Primary school
- Secondary school
- Higher education
- Other specify

Does your household have electricity?

- Yes
- No

How many sleeping places were used last night in your household?

(Including sleeping places outside and temporary spaces)

How many mosquito nets that can be used for sleeping does your household have?

Of the number of mosquito nets, how many were issued in the 2021 mass net distribution?

Of the total nets issued in the last mass net distribution, how many are still present in your house?

How many nets distributed in the last mass net distribution are not present in your house?

How many nets are in use in the household?

## **SECTION 2: LLIN HANDLING**

### 2.1 Net identification number (label)

Has the net ever been washed?

- Yes
- No
- Don't know

When was the last time you washed the net?

- 1 week ago
- 1 week to 1 month ago
- 1-3 months ago

- 3-6 months ago
- >6 months ago
- Don't know

What type of soap was used?

- None
- Local bar soap
- Detergent powder
- Mix (bar and detergent)
- Bleach
- Don't know

**How long did the net soak for?**

- Did not soak the net
- <1hr
- >1hr
- Don't know

**Was the net scrubbed hard or beaten on a hard surface (e.g rocks, with sticks)?**


- Yes
- No
- Don't know

**Where was the net dried?**

- Outside in the sun
- Outside in the shade
- Inside the house
- Don't know


**END**

## Appendix IV: NACOSTI License


  
**NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION.**

Date of Issue: 16/March/2023

**RESEARCH LICENSE**




**This is to Certify that Ms. mercy owendi Achula of Jomo Kenyatta University of Agriculture and Technology, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Kisumu on the topic: ASSESSING THE BIO-EFFICACY OF THE YORKKOOOL @A PYRETHROID- BASED LONG LASTING INSECTICIDAL NET OVER A ONE-YEAR PERIOD AND DETERMINING NET WASHING AND DRYING PRACTICES IN SEME, KISUMU COUNTY. for the period ending : 16/March/2024.**


License No: NACOSTI/P/23/23968

759807

Applicant Identification Number

  
 Director General  
**NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION**

Verification QR Code



NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

See overleaf for conditions

### THE SCIENCE, TECHNOLOGY AND INNOVATION ACT, 2013 (Rev. 2014)

Legal Notice No. 108: The Science, Technology and Innovation (Research Licensing) Regulations, 2014

The National Commission for Science, Technology and Innovation, hereafter referred to as the Commission, was established under the Science, Technology and Innovation Act 2013 (Revised 2014)

herein after referred to as the Act. The objective of the Commission shall be to regulate and assure quality in the science, technology and innovation sector and advise the Government in matters related thereto.

## **CONDITIONS OF THE RESEARCH LICENSE**

1. The License is granted subject to provisions of the Constitution of Kenya, the Science, Technology and Innovation Act, and other relevant laws, policies and regulations. Accordingly, the licensee shall adhere to such procedures, standards, code of ethics and guidelines as may be prescribed by regulations made under the Act, or prescribed by provisions of International treaties of which Kenya is a signatory to
2. The research and its related activities as well as outcomes shall be beneficial to the country and shall not in any way;
  - i. Endanger national security
  - ii. Adversely affect the lives of Kenyans
  - iii. Be in contravention of Kenya's international obligations including Biological Weapons Convention (BWC), Comprehensive Nuclear-Test-Ban Treaty Organization (CTBTO), Chemical, Biological, Radiological and Nuclear (CBRN).
  - iv. Result in exploitation of intellectual property rights of communities in Kenya
  - v. Adversely affect the environment
  - vi. Adversely affect the rights of communities
  - vii. Endanger public safety and national cohesion
  - viii. Plagiarize someone else's work
3. The License is valid for the proposed research, location and specified period.
4. The license any rights thereunder are non-transferable
5. The Commission reserves the right to cancel the research at any time during the research period if in the opinion of the Commission the research is not implemented in conformity with the provisions of the Act or any other written law.
6. The Licensee shall inform the relevant County Director of Education, County Commissioner and County Governor before commencement of the research.
7. Excavation, filming, movement, and collection of specimens are subject to further necessary clearance from relevant Government Agencies.
8. The License does not give authority to transfer research materials.
9. The Commission may monitor and evaluate the licensed research project for the purpose of assessing and evaluating compliance with the conditions of the License.
10. The Licensee shall submit one hard copy, and upload a soft copy of their final report (thesis) onto a platform designated by the Commission within one year of completion of the research.
11. The Commission reserves the right to modify the conditions of the License including cancellation without prior notice.
12. Research, findings and information regarding research systems shall be stored or disseminated, utilized or applied in such a manner as may be prescribed by the Commission from time to time.
13. The Licensee shall disclose to the Commission, the relevant Institutional Scientific and Ethical Review Committee, and the relevant national agencies any inventions and discoveries that are of National strategic importance.
14. The Commission shall have powers to acquire from any person the right in, or to, any scientific innovation, invention or patent of strategic importance to the country.
15. Relevant Institutional Scientific and Ethical Review Committee shall monitor and evaluate the research periodically, and make a report of its findings to the Commission for necessary action.

National Commission for Science, Technology and Innovation(NACOSTI),

Off Waiyaki Way, Upper Kabete,

P. O. Box 30623 - 00100 Nairobi, KENYA

Telephone: 020 4007000, 0713788787, 0735404245

E-mail: dg@nacosti.go.ke Website: www.nacosti.go.ke

## Appendix V: Seru Approval



*In Search of Better Health*

# KENYA MEDICAL RESEARCH INSTITUTE

## OFFICE OF THE DIRECTOR RESEARCH & DEVELOPMENT

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**KEMRI/RES/7/3/1** **January 17, 2023**

**TO: MERCY OWENDY ACHULU,  
PRINCIPAL INVESTIGATOR.**

**THROUGH: THE DEPUTY DIRECTOR, CBRD,  
NAIROBI.**

Dear Madam,

**RE: PROTOCOL NO. KEMRI/SERU/CBRD/479/4587 (RESUBMISSION II OF INITIAL SUBMISSION): ASSESSING THE BIO-EFFICACY OF YORKKOO<sup>®</sup> A PYRETHROID-BASED LONG LASTING INSECTICIDAL NET OVER ONE YEAR PERIOD AND DETERMINING NET WASHING AND DRYING PRACTICES IN SEME, KISUMU COUNTY. (VERSION 5 DATED 9 JANUARY 2023)**

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Reference is made to your letter dated January 9, 2023. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledges receipt of the following revised study documents on January 12, 2023;

1. The revised proposal (Version 5, 9th January 2023)
2. SERU review comments document
3. CBRD CSC approval letter

This is to inform you that the Committee notes that the issues raised during the 329<sup>th</sup> Committee A meeting of the KEMRI Scientific and Ethics Review Unit (SERU) held on **November 08, 2022** have been adequately addressed.

Consequently, the study is **granted approval** for implementation effective this day, **January 17, 2023**, through to **January 16, 2024**. Please note that authorization to conduct this study will automatically expire on **January 16, 2024**. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **December 05, 2023**.

Please note that only approved documents including (informed consents, study instruments, Material Transfer Agreement) will be used. You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) <https://oris.nacosti.go.ke> also obtain other clearances needed.

Yours faithfully,



**ENOCK KEBENEI,  
THE ACTING HEAD,  
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT**

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*In Search of Better Health*