

**Bio-efficacy of long lasting insecticidal nets (LLINs) on
Anopheles mosquitoes under field condition**

Principal investigators: Dr. Maung Maung Mya

Co – investigators : (1) Dr. Myat Phone Kyaw

(2) U Sein Thaung

(2) Dr. Yan Naung Maung Maung

Introduction

- Long lasting insecticide treated nets (LLINs) have emerged as a potent tool in lowering morbidity and mortality of mosquito borne diseases.
- In Myanmar, WHO recommended PermaNet 2.0 and Olyset net (LLINs) have been widely distributed to reduce malaria transmission in endemic areas.
- Recently, K-O-Tab 1-2-3, the advanced product of simple K-O-Tab, has been introduced as a long lasting insecticide kit for bed nets.

- Malaria is transmitted by *Anopheles mosquitoes*
- LLINs and ITNs lead to a reduction of human-vector contact and diminish mosquito population
- and also provide a physical barrier with high coverage levels that benefit the whole community.
- However, bio-efficacy of long term insecticidal action of LLINs depends on the exposure of climate, washing frequencies and duration of used during the recommended period.

- Moreover there is a lack of information about the impact of repeated washings and duration of use on insecticidal action of distributed LLINs against *Anopheles* mosquitoes in Myanmar.
- Therefore, bio-efficacy of distributed long lasting insecticidal treated nets in relation to **washing frequencies** and **duration of use** nets against *Anopheles* mosquitoes should be investigated to provide information gap concerning on retention of field used LLINs.

Aims and objectives

- The aim of the study is to assess the bio-efficacy of long lasting insecticidal nets (LLINs) with or without washing on *Anopheles* mosquitoes in field areas.

Specific objectives

- to evaluate the bio-efficacy of repeated washed and unwashed LLINs for prevention of mosquito bite
- to determine the knockdown and mortality effect of *Anopheles* mosquitoes among distributed LLINs
- to compare the long lasting insecticidal action between different types of distributed LLINs in field areas

Materials and Methods

Study areas:

- Mon State -2 areas
- Kayin State - 2 areas
- Pegu Region - 1 area
- Mandalay Region 1 area
- Laboratory of Medical Entomology Research Division, DMR

Study period :

- June 2016 to December 2017 (2 year study)

Study Design:

- Laboratory and field based experimental study

Sample size determination and sampling procedure

- Considering for difference is bio-efficacy of LLIN between those nets used for two year and more than two year being 20% ($P_1=90\%$ with two year; $P_2= 70\% >2$ year) at 95% confidence level and 80% power, the sample size real used will be 62. Therefore 7-10 LLINs will be randomly selected in each study site (70-100 nets).
- Ten houses from each site will be selected randomly. One LLIN each will be collected from all randomly selected houses.
- Therefore a total of 60 LLINs will be collected from 6 sites of the four States and Regions.

Data collection

Susceptible strain collection

- Susceptible strain of *Anopheles dirus* mosquitoes from Mudon Township Mon State will be collected
- and reared for mass production in Laboratory of Medical Entomology Research Division, will be used as control mosquito in field areas.

Field mosquito collection

- Field *Anopheles* mosquitoes will be collected from selected areas using animal bait big net and indoor and outdoor collection by light trap method at 18:00 to 06:00 hour in selected areas.
- Collected mosquitoes will be tested the susceptibility status of mosquitoes using WHO test kit.
- After susceptibility test, remaining mosquitoes will be tested against randomly collected LLINs nets.

Mosquito species identification

- Species identification of field collected adult and adult emerged from larval survey will be done by morphological methods according to different identification keys. (Harrison and Scanlon 1975, Harrison 1980 and Myo Paing et al., 1990).

Preparation of mosquito net samples

- Washed and unwashed 2 to 3 years old 10 LLINs nets per site will be collected randomly.
- Untreated standard polyester net was used as control.
- Susceptible strain of laboratory reared 3-5 days old 5 *Anopheles dirus* mosquitoes each will be used as control on each mosquito net for bio-efficacy testing.
- Field collected mosquitoes will be tested with five sites of mosquito nets as top , front, back and both lateral sides of each mosquito net by cone bioassay test.

Procedure of insecticide susceptibility test

- Susceptibility test will be performed using impregnated paper assay with WHO test kit (WHO 1998) to confirm the susceptible strains. Laboratory reared *An. dirus* will be used as control.
- Five each field collected female *Anopheles* mosquitoes (pool sample) will be exposed to each impregnated papers exposed tube for one hour.
- After one hour mosquitoes will be transferred to attached clean tubes from the testing tubes and will supply 10% glucose.
- Humidity will be maintained with water soaked towel covered with on it.
- The percentage knockdown will be measured at 60 min after exposure and mortality will be assessed 24hr after exposure.

- **Procedure of bioassay test**
- Bio efficacy of long lasting insecticidal nets will be evaluated according to standard method of WHO cone technique (WHO 2005).
- Insecticide susceptible strain of *An. dirus* (colony from Medical Entomology Research Division) will be used before the bioassays as control.
- Five cones (one top, 2 front and back, 2 lateral sites) will be placed horizontally on the nets and 5 each field collected adult female *Anopheles* mosquitoes (pool sample) will be exposed to each cones for 3 min.
- After 3 min mosquitoes will be removed from the cones and placed in individual paper cups with access to 10% glucose and humidity will be maintained with water soaked towel covered with on it.
- The percentage knockdown will be measured at 60 min after exposure and mortality will be assessed 24hr after exposure.

Data analysis

- Percentage knockdown and mortality against number of washes and duration of used LLINs nets curve will be drawn.
- If the percentage of mortality in the negative control will be above 5%, then a correction will be made using Abbotts formula (Abbot 1987).
- Maximum number of washes providing knockdown and /or mortality above the cut of point ($\geq 95\%$ Knockdown after 60 min and /or $\geq 80\%$ mortality after 24 hours post exposure) will be reported.
- The data will be analyzed with SPSS software applying Student '*t*' test and ANOVA (Analysis of variance) test.

Ethical consideration:

Ethical clearance will be obtained from DMR ethical review committee.

Expected Output:

- This study will provide further information on bio-efficacy of LLINs related to washing frequencies and will support the mosquito borne diseases control programme in Myanmar.

Estimation of detailed budgetary breakdown

No	Item	Unit Cost USD	Person/ Unit	Day	Total USD
1	Development of instrument for data collection (a) Files, Stationary,	20	6 sites		120
3	Supervisory Cost (a) travel, Per diem, Investigators) (a) travel (1) Mon State (round trip 200 USD x 2 areas =400USD (2)Kayin State (round trip 200 USD x 2 areas =400USD (3)Bago Region (round trip 200 USD x 1 areas =200USD (4)Mandalay Region (round trip 200 USD x 1 areas =200USD Per diem 2 insect collector x 10 days x 7 USD x6 sites =840USD	200 200 200 200 7	2 2 1 1 2 x 6 sites	10 10 10 10 10	400 400 200 200 840
4	Field and Laboratory expenses Field : experimental hut 1hut x8USD x10days x 6site Laboratory Mosquito rearing 10USD x 6sites	8 10	1 x 6 sites 6 sites	10	480 60
5	Data processing and analysis 1x150	150	1		150
6	Report Writing 1x150	150	1		150
	Total USD				3000

Thank you

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