



# **Comparison of standard and modified human landing collection (HLC) techniques for blackflies in the era of onchocerciasis elimination**

**Technical report**

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# Table of Contents

<b>Acknowledgements</b> .....	<b>3</b>
Authors .....	3
Contributors .....	3
Partners .....	3
<b>Executive summary</b> .....	<b>4</b>
<b>Background and rationale of the study</b> .....	<b>6</b>
<b>Methodology</b> .....	<b>6</b>
Area of implementation .....	6
Selection of collection sites and points .....	6
Selection and training of vector collectors .....	7
Blackfly collection and dissection .....	8
Data management and analysis .....	9
Ethnics .....	9
<b>Results and interpretation</b> .....	<b>9</b>
Fly catches .....	9
Calculating key entomological indicators .....	10
<b>Abbreviations and acronyms</b> .....	<b>11</b>
<b>References</b> .....	<b>11</b>

# Acknowledgements

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# Executive summary

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## Background

Entomological evaluations for Neglected Tropical Diseases (NTDs) such as onchocerciasis and lymphatic filariasis are required for stopping mass drug administration (MDA) in the context of the diseases' elimination. The human landing collection (HLC) technique is the standard sampling method for collecting human-biting blackflies for these purposes. Despite ethical concerns regarding the exposure of vector collectors to infective flies, the method is still commonly used as no suitable alternative has yet been found to replace it. With many countries entering elimination, alternative and safer methods are needed.

This study aims to evaluate a modified HLC technique where vector collectors are protected by wearing trousers with colours that are attractive to blackflies – this allows the flies to land on the collectors, while also preventing biting.

## Study design and methods

The study was experimental where both the standard HLC technique and a modification of the standard technique were performed in the same collection sites. Both techniques were then directly compared in terms of the volume of fly collection and parity.

The standard HLC technique involved collectors rolling up their trousers to expose their foot and lower leg, then using a mouth aspirator to catch flies that landed on them. In the modified technique, the collectors wore long coloured trousers of blue, black and a hybrid of blue and black (blue-black) and caught flies that landed on the trousers using an aspirator.

Collections were done from morning (7am) to evening (5pm) by two trained collectors who collected flies, interchanging the collection hours and sites. This was carried out four days per month for four months, from October to November (rainy season) and from January to February (dry season). The flies collected were counted and dissected to determine the entomological indices. The mean proportions of the number of flies caught and the physiological status (parous) were compared between the standard and modified techniques and between seasons using the non-parametric Mann-Whitney U test.

## Results

For all seasons, a total of 5,130 (29.7%) flies were collected by the standard HLC technique; 5,148 (29.9%) by the black trousers; 3,717 (21.6%) by the blue and 3,251 (18.9%) by the blue-black. There is no statistical difference between the modified technique using black trousers and the standard HLC. Black trousers performed equally well as the standard HLC technique, catching a similar number and physiological status of flies overall. The blue patterned trousers caught a significantly lower proportion of flies and of those parous in both seasons ( $p < 0.01$  and  $p = 0.02$ ).

**Conclusion and recommendations:** The findings suggest that the modified HLC technique using black trousers is a viable alternative. Not only does it address the ethical concerns raised by the standard HLC, but it also collects flies in the same physiological status as the

standard technique. Further studies are needed to confirm the generalisability in different eco-zones, transmission environments and among different blackfly species.

## Background and rationale of the study

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As the focus shifts from onchocerciasis control to elimination of onchocerciasis transmission, many countries are looking forward to achieving elimination by 2030 - thus the need for developing new tools and strategies to evaluate elimination (WHO 2020; WHO 2001). Entomological surveys are used to verify if onchocerciasis elimination has been achieved (WHO, 2016; WHO, 2010) and require the collection of large numbers of anthropophilic blackflies using the standard human landing collection (HLC) technique - this provides information on transmission indices to determine the interruption of transmission (WHO 2016; Walsh JF et al., 1978).

Although the HLC technique is considered the gold standard, this method of collecting blackflies for entomological studies raises ethical issues as it exposes the collector to potentially infective insect bites (Otabil et al., 2018; Gimnig et al., 2013; Dia et al., 2005). With the need to develop new sampling techniques to replace the standard HLC method, there are factors that need to be considered when choosing alternatives. Any substitute should be able to collect appropriate numbers of the same vector populations, the same physiological status as those biting humans and in a condition that enables age structures and entomological indices to be determined (Hendy A et al., 2017). Thus, to date no method has been found suitable to replace the HLC technique. The main purpose of the study is to evaluate the performance of a modified protective HLC technique using trousers in colours known to be attractive to flies (Bidlingmayer and Hem, 2012; Krčmar et al., 2014).

## Methodology

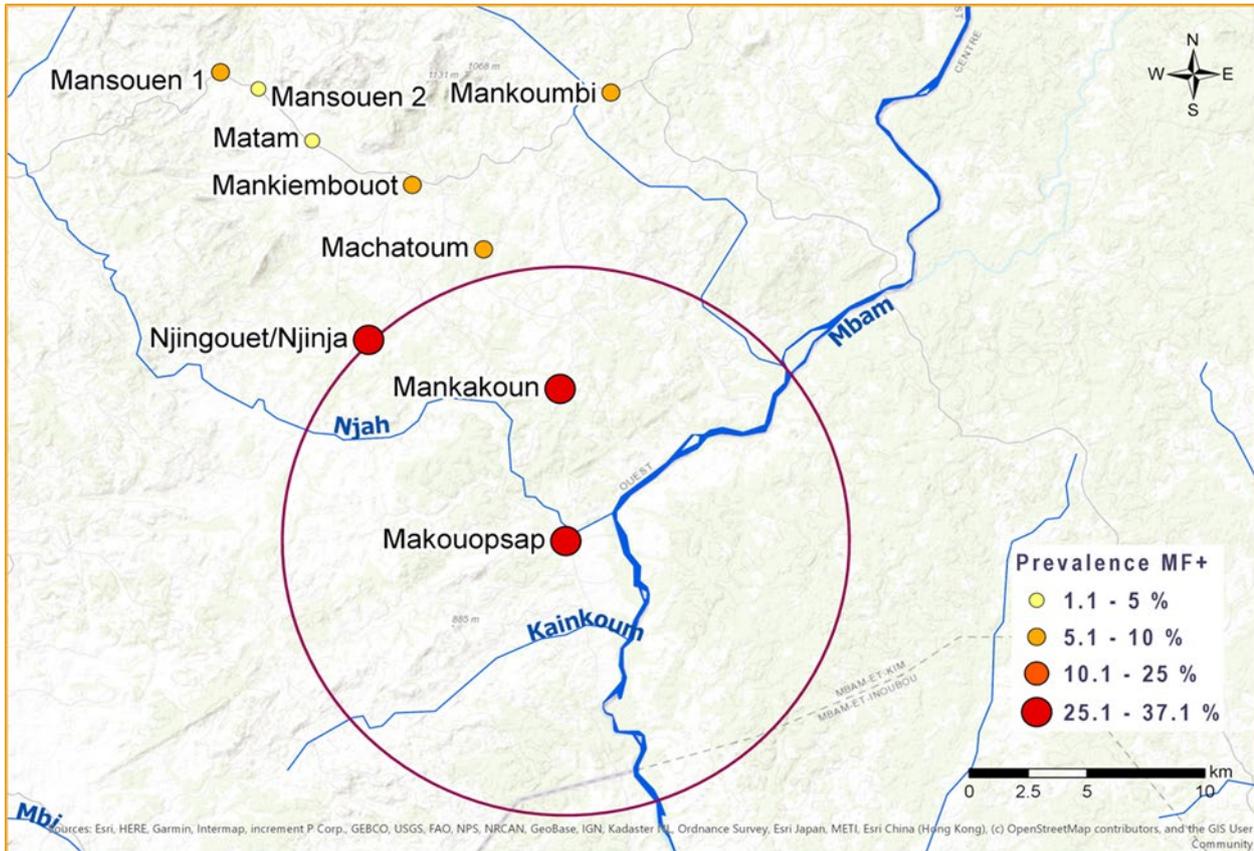
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### Area of implementation

The study was conducted in the Massangam health district in the West Region of Cameroon, which is known to have a high prevalence of onchocerciasis and continuous transmission despite more than 15 years of ivermectin (IVM) mass drug administration (MDA) (Katarawa et al., 2013). The main rivers in this area are the Mbam and Noun, with the Mbam having many tributaries of which the river Nja is the main one. The rainy season runs from August to November and the dry season from December to March; this is then followed by a short rainy season from April to June and a short dry season in July.

### Selection of collection sites and points

Blackfly collection data from a previous entomology study conducted in the Massangam area (Bakajika et al., 2018) was assessed to guide the choice of vector collection sites. Two breeding sites with the highest number of blackflies collected were selected for the study – one along the river Mbam and one along river Nja (Figure 1). Around each collection site on the rivers, four collection points 50 metres apart were selected corresponding to the various collection techniques (one for standard and three for modified); making a total of eight collection points for the two breeding sites.



**Figure 1: Massangam HD showing communities and the rivers Mbam and Njah**

## Selection and training of vector collectors

A total of 16 community members, aged at least 20 years old and living permanently in the community, were selected to be trained as blackfly collectors. Some of the collectors had experience in blackfly collection from previous collection activities in the community, and these individuals acted as mentors to new collectors.

The training session started with the explanation and justification of the study, and involved the active participation of selected community members with an emphasis on better data collection. The collectors were later trained on:

1. The collection technique (skills and ability for the collection).
2. The wearing of protective trousers.
3. The rotation of collectors among collection points.
4. The use of materials, especially the locally-made mouth aspirators.
5. The conservation of collected samples.

A practical session was organised, taking into consideration the five main steps of the catching procedure. Training lasted for one day and by the end of the exercise, the chronogram of activities - highlighting the days and months of the collection and a Latin square (Table 1) explaining the rotation across collection points - was developed and shared among all participants.

**Table 1: Rotation of collectors among collection techniques and collection days**

	Collection Point 1	Collection Point 2	Collection Point 3	Collection Point 4
Day 1	(1, HLC_S)	(2, HLC_B)	(1, HLC_N)	(1, HLC_BN)
Day 2	(2, HLC_BN)	(2, HLC_S)	(2, HLC_B)	(2, HLC_N)
Day 3	(3, HLC_N)	(3, HLC_BN)	(3, HLC_S)	(3, HLC_B)
Day 4	(4, HLC_B)	(4, HLC_N)	(4, HLC_BN)	(4, HLC_S)

**KEY:** HLC\_S = standard, HLC\_B = blue, HLC\_N = black, HLC\_BN = blue/black

## Blackfly collection and dissection

Blackflies were captured by two variations of the HLC technique: the standard method and the modified method.

**The standard method of HLC (HLC\_S)** This involved exposing the legs and lower limbs of the collectors during collection time. Flies that landed on exposed legs were captured with a locally-made mouth aspirator.

**The modified method of HLC (HLC\_X)** This involved covering the legs and lower limbs of the collectors during collection time with blue (HLC\_B), black (HLC\_N) or blue-black (HLC\_BN) trousers. Flies landing on the covered legs were captured with a mouth aspirator.

Collections were performed between 7am and 5pm during four days per month, from October to November (the rainy season) in 2018 and January to February (the dry season) in 2019. To minimise bias and fatigue, two collectors captured flies interchangeably at each point (Table 1), one from 7am to 12pm and the other from 12pm to 5pm on the days of collection.

Each collection point was geo-referenced using a GPS, and each collection tube was labelled with the collection site, collection technique and hour of collection. Due to the absence of flies in the river Nja during the second round (month) of collection, this site was subsequently suspended, and efforts were instead concentrated on the river Mbam for maximum collection.

After each hourly interval, the aspirators containing the caught flies were transported to the field laboratory. Fresh flies contained in each aspirator that were still alive were knocked down through contact with vapour of an ether solution impregnated on a cotton pad, which was placed on the opening of the aspirator. Knocked-down flies were transferred to a petri dish containing lightly-soaped distilled water, then counted and dissected for parity. The parous flies were further dissected in search of the parasitic stages of *Onchocerca volvulus* infections (L1, L2 and L3). The number of flies caught, dissected, infected (L1, L2, or L3) and infective (L3 in the head) were recorded on a data collection sheet for each hour, day, collection technique and site.

## Data management and analysis

Data from the collection sheets was entered into Excel and imported to STATA statistical package - version 13.0 (TX: StataCorp LP) - for analysis. The biting rate was calculated as the number of flies caught over the collection period; parous rate as the proportion of dissected flies that were parous; infection and infectivity rates as the proportion of parous flies with L1, L2 or L3; and parous flies with L3 (in the head) respectively. Mean proportions were compared across techniques and seasons using the non-parametric Mann-Whitney U test.

## Ethics

This study was approved by the National Ethics Committee (Comité National d’Ethique de la Recherche pour la Santé Humaine), approval No: 2020/12/1322/CE/CNERSH/SP.

## Results and interpretation

### Fly catches

Overall, 17,246 flies were caught over the four-month period: 62.4% (10,757) during the rainy season and 37.6% (6,489) during the dry season, with 3.9% (673) being parous. Infection rate for the collection period was at 0.8% (5) of parous flies, with 3 (1.0%) parous in the dry season and 2 (0.5%) in the rainy season. Infection rate was 0.0% for all flies dissected for both seasons. Only one fly was infective (dry season; standard technique) giving an infective rate of 0.1% of parous flies (Table 2).

**Table 2: Fly collection between techniques and seasons**

Collection technique	# Caught	# Dissected	# Parous (% of dissected flies parous)	# Infected (% of parous flies Infected)	# Infective (% of parous flies infective)
<b>Rainy season (October - November)</b>					
Standard	3,187	3,138	111 (3.5)	1 (0.9)	0 (0.0)
Blue	2,412	2,412	102 (4.2)	0 (0.0)	0 (0.0)
Black	3,330	3,330	105 (3.2)	1 (1.0)	0 (0.0)
Blue-black	1,828	1,828	63 (3.4)	0 (0.0)	0 (0.0)
<b>Total</b>	<b>10,757</b>	<b>10,708</b>	<b>381 (3.6%)</b>	<b>2 (0.5)</b>	<b>0 (0.0)</b>
<b>Dry season (January - February)</b>					
Standard	1,943	1,943	78 (4.0)	2 (2.6)	1 (1.3)
Blue	1,305	1,300	47 (3.6)	0 (0.0)	0 (0.0)
Black	1,818	1,817	87 (4.8)	1 (1.1)	0 (0.0)

<b>Blue-black</b>	1,423	1,423	80 (5.6)	0 (0.0)	0 (0.0)
<b>Total</b>	<b>6,489</b>	<b>6,483</b>	<b>292 (4.5%)</b>	<b>3 (1.0)</b>	<b>1 (0.3)</b>

## Calculating key entomological indicators

There was no statistical difference in the biting rates between the standard HLC (29.7%; n=5,130) and the black HLC (29.9%; n=5,148) during both the dry season (z=1.51; p=0.13) and rainy season (z=1.48; p=0.14). The standard technique had significantly higher biting rates when compared with the blue modified technique (21.6%; n=3717) in both seasons (p<0.01). The biting rate was significantly higher only during the rainy season (z=4.55; p<0.01) when comparing the standard and the blue-black techniques (18.9%; n=3251) (Table 3).

Amongst the various coloured fabrics, black recorded significantly more biting rates than blue during the rainy and dry seasons (p=0.03 and p=0.02), and more than blue-black during only the rainy season (p<0.01). There was no significant difference between blue and blue-black.

When comparing the physiological status (parous) of the flies caught between the standard and modified techniques, there was no statistically significant difference between the number of parous flies caught between black, blue-black and standard techniques for both seasons. However, the standard technique collected significantly more parous flies than blue during the dry season (z=2.40; p=0.02) but not during the rainy season (z=0.44; p=0.66).

**Table 3: Mean proportion of flies collected and parous between standard and modified collection techniques**

	All Flies Collected			Parous Flies Collected		
<b>Standard</b>	Dry Season: 1943 (0.285)			Dry Season: 78 (0.010)		
	Rainy Season: 3187 (0.318)			Rainy Season: 111 (0.011)		
<b>Trouser Colour</b>	Blue	Black	Blue-black	Blue	Black	Blue-black
<b>Dry season</b>						
<b>Colour n (prop)</b>	1305 (0.204)	1818 (0.254)	1423 (0.258)	47 (0.006)	87 (0.012)	80 (0.016)
<b>p-value</b>	p<0.01	p=0.13	p=0.12	p=0.02	p=0.60	p=0.70
<b>Rainy season</b>						
<b>Colour n (prop)</b>	2412 (0.214)	3330 (0.277)	1828 (0.191)	102 (0.117)	105 (0.010)	63 (0.010)
<b>p-value</b>	p<0.01	p=0.14	p<0.01	p=0.66	p=0.42	p=0.64

For all techniques, the rainy season collected significantly more flies than the dry season (z=4.19, p<0.01). However, when comparing the proportion of flies caught in each technique between the various seasons, only blue-black showed any significant difference (p=0.01).

## Conclusion

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The black modified HLC technique is a viable alternative for the standard HLC in addressing the ethical concerns, as there is no significant difference in the biting rates and physiological status of the flies collected between these two techniques. However, given that this is a small-scale study, it is necessary to conduct a similar study during a more extended period and using multiple transmission zones to enable a greater generalisability and comparability of the outcome.

## Abbreviations and acronyms

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HLC	Human landing collection
HLC_B	Human landing collection - blue trousers
HLC_N	Human landing collection - black trousers
HLC_BN	Human landing collection - blue/black trousers
IVM	Ivermectin
LF	Lymphatic filariasis
MDA	Mass drug administration
NTDs	Neglected Tropical Diseases
RSTMH	Royal Society of Tropical Medicine and Hygiene
WHO	World Health Organisation

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