GOVERNMENT OF SAMOA
MINISTRY OF HEALTH

Lymphatic Filariasis

Transmission Assessment Survey 2017

and

Soil Transmitted Helminthes Survey

Final report

CONTENTS

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- Last but not least the LF TAS survey teams

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Abbreviations

Alb	Albendazole
AUA	Apia Urban Area
CEO	Chief Executive Officer
DEC	Diethylcarbamazabine
DOTS	Directly Observed Treatment Strategy
EU	Evaluation Unit
FTS	Alere^{TM}Filariasis Test Strip
GPELF	Global Program for the Elimination of Lymphatic Filariasis
ICT	Immunochromatography
IDA	Ivermectin + DEC + ALB
LF	Lymphatic Filariasis
MESC	Ministry of Education, Sports and Culture (Samoa)
MDA	Mass Drug Administration
MOH	Ministry of Health (Samoa)
MWCSD	Ministry of Women, Community and Social Development
NHS & IHR	National Health Surveillance and International Health Regulations
NWU	North West Upolu
PacELF	Pacific Program for the Elimination of Lymphatic Filariasis
ROU	Rest of Upolu
SBS	Samoa Bureau of Statistics
SSBT	Survey Sample Builder Tool
STH	Soil Transmitted Helminthes
TAS	Transmission Assessment Survey
WHO	World Health Organization

Executive Summary

There has been much effort by the Samoa government through its Ministry of Health to control Lymphatic Filariasis (LF) disease in the country. It has proven to be a very debilitating, disfiguring and stigmatized neglected tropical disease affecting Samoa. Since the early 1950s Samoa has collaborated with development partners such as WHO and later JICA, in this endeavour. Since 1999, a renewed regional effort with PacELF to push LF elimination, had seen several rounds of Mass Drug Administration (MDA) country-wide with at least eight MDAs implemented in Samoa itself.

In accordance with surveillance methods recommended by WHO, the Ministry of Health carried out its first transmission assessment survey for LF in 2013. It discovered the North West Upolu (NWU) area, one of the three enumeration units (EU) in the country, continued transmission of LF microfilarial worms. Two further rounds of MDA were then implemented within this area alone, the first in February 2015 and the last one in April 2017.

This year, Samoa implemented its second transmission assessment survey, to assess whether or not LF had been interrupted in the failed EU of NWU, which had received two MDAs and also whether there was recrudesence in Savaii and other parts of Upolu. Methodology for the survey was kept as similar as possible for comparisons. However, for antigenic testing, ICT cards were replaced with Filriasis Test Strips (FTS). The sampled population were those of grades one and two (six and seven year old) children from all primary schools around the country. As an added feature of the LF TAS survey this year it was agreed to implement alongside it, the Soil Transmitted Helminthic (STH) survey as an initial attempt to gather much needed data for the status of Helminthic infestation amongst the Samoan population. This used primary school children in grades three and four (ages 8 and 9 years) and it utilized the Kato Katz test to identify the different helminthes that may be present.

Survey results for the 2017 LF TAS saw all Enumeration Units AUA + ROU; NWU and Savaii having rather significant recrudescence with LF prevalence rates of 1.43%; 6.79% and 5.25% respectively. Despite the difference in tests used and the slight drop in actual sampled number of the population used, LF prevalence rates were indeed profound and shows a definite rise in LF transmission continuing country-wide in Samoa. At this stage, there is much needed work to be done for the realization of LF elimination, in Samoa.

Survey results for STH survey indicates infestation amongst school children, which however needs further detailed data which was missing in the current survey. Prevalence results cannot be calculated as a significant number of samples were not processed due to unforeseen difficulties with Laboratory staff availability. At best, this survey serves to indicate the need for better planning and perhaps a separation of these two surveys. It also highlighted Laboratory inadequacies for the processing of stool specimen, as an injunction for other significant Public Health infectious disease surveillance such as typhoid contact tracing.

In effect, Public Health measures for control and elimination of LF as well as addressing other PH diseases of significance must be strengthened urgently and simultaneously.

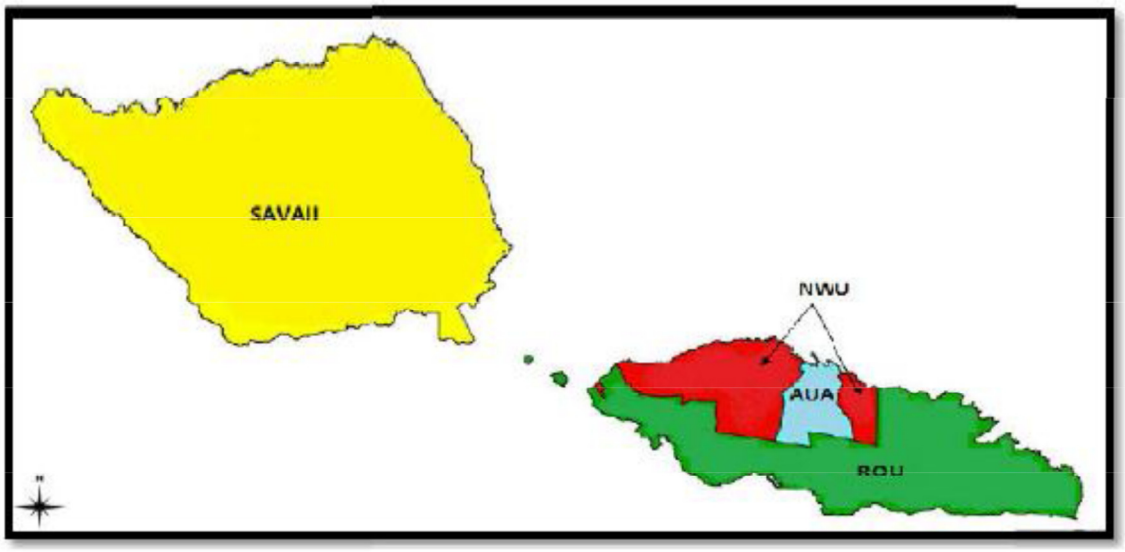
Background

Lymphatic filariasis (LF) is a parasitic disease transmitted by mosquitoes that can cause significant morbidity in humans. Filariasis in Samoa is caused by *Wuchereria bancrofti* transmitted by the Aedes mosquito species. The predominant mosquito species implicated has been *Aedes polyneniensis*. Samoa has had a long history of attempts to control LF, beginning in the 1950's, with fluctuating results. In 1999 the Pacific Program for the Elimination of Lymphatic Filariasis (PacELF) was established to eliminate the disease in the Pacific sub-region and Samoa was one of the first to implement this program (WHO 2006). PacELF program has several steps and the Mass Drug Administration (MDA) and surveillance phase together taking a minimum of 11 years. Fig 1 below shows these steps.

Figure 1. Steps in the lymphatic filariasis elimination strategy

Since joining PacELF Samoa had 5 rounds of MDA before a first survey in 2003/2004 showed that prevalence of LF by ICT had reduced from the 1999 baseline of 4.52% to 1.1%. The goal was <1% prevalence. Further rounds of MDA and another survey in 2007 found ICT prevalence had increased to 2.62%. Three more MDAs followed and in 2013 the first transmission assessment survey (TAS) was completed with Samoa divided into 3 Evaluation Units (EUs) (i) Apia Urban Area (AUA) combined with the Rest of Upolu (ROU) (ii) North West Upolu (NWU) and (iii) Savaii. The division into EUs utilized the regions predetermined by the Samoa Bureau of Statistics (SBS) for census purposes (Figure 2).

Figure 2 Map of Samoa showing the four regions which make up the 3 EUs



The results of this TAS are shown in Table 1. While two of the three EUs passed TAS the NWU EU showed a transmission level above the threshold level of 1% and therefore this EU was recommended for an additional two rounds of MDA. Savaii, AUA & the Rest of Upolu could stop MDA and go to the surveillance phase [MoH 2013].

Table 1: Summary of the LF Transmission Assessment Survey Results 2013

	Apia Urban Area plus Rest of Upolu	North West Upolu	Savai'i
Targeted sample population	1,214	1,188	1,042
Total population tested	1,216	1,271	1,098
Critical cut off of ICT positives	7	7	6
Total ICT positives found during field work	1	19	5
ICT prevalence (%)	0.08	1.49	0.46

This year Savaii, AUA & the Rest of Upolu were due for TAS 2 to see if there has been recrudescence of LF since the TAS 1 in 2013. As recommended then NWU has had two more MDAs, the last in April 2017, so from September 2017 this EU was eligible for another transmission assessment survey.

TAS is also an opportunity to study the STH/ intestinal worm status among children and WHO encourages such combined surveys for potentially endemic countries with guidelines available (WHO 2015). Four species of nematodes are collectively referred to as soil-transmitted helminths: the roundworm, *Ascaris lumbricoides*; the whipworm, *Trichuris trichiura*; and the hookworms, *Necator americanus* and *Ancylostoma duodenale*. These four species are frequently considered together because infection is diagnosed by the same laboratory method and treated with the same drugs.

In many parts of the world STH infections are responsible for deteriorating nutritional status due to iron deficiency and protein malnutrition. Children and women of reproductive age are typically the ones most severely affected by hookworm anaemia. Chronic STH infections can affect the physical and mental growth of children. School-age children typically have the highest intensity of intestinal worms and are most adversely affected by chronic STH infections.

Data on STH specifically for Samoa has not been published but Pacific countries in general have been identified as having a moderate to high prevalence of *Trichuris trichiura* before the ELF programs were implemented (Hugh et al 2002). The LF MDA could have had an impact on STH but it has not been assessed and also no separate deworming of school-aged children continued after stopping the MDA. Anecdotally pharmacies are dispensing worm tablets to people who have consulted GPs and the Patient information (PATIS) has a few records of outpatient/ ED visits for worms other than LF in 2016 and 2017 YTD. The new WHO guidelines for STH (2011b) recommend annual mass deworming when the prevalence of any STH infection is > 20% and twice annual deworming when the prevalence is >50%.

Methods

The three EUs used in the 2013 TAS were kept for comparison to the 2017 TAS 2 survey. Since 2013 NWU had had two more MDAs, the last in April 2017, so any TAS had to take place at least 4 months after this MDA round. From the Education Statistical Digest 2016 the number of children in Grades 1 & 2 was 11,490 with 5,484 students in AUA + RoU, 3,262 students in NWU, and 2,744 in Savai'i.
School enrollment was reported to be up to 98% *(Population Census 2011)* and all children in grades 1, 2, 3 & 4 were eligible to participate.

Lymphatic Filariasis

Using the Education Statistical Digest 2016 and the WHO recommended survey sample builder (www.ntdsupport.org/resources) with a 10% non response rate the potential sample sizes for the three EUs were 1,042 children in Savaii, 1,350 in AuA +RoU and 1,188 in NW Upolu for the LF TAS 2 [Table 2]. Individual students were systematically selected from the school rolls provided by MESC and school principals in combination with sampling interval provided in the survey sample builder.

Table 2: Summary Sampling of LF TAS in Samoa 2017

LYMPHATIC FILARISAIS TRANSMISSON ASSESSMENT SURVEY 2017				
	Apia Urban Area (AUA) plus Rest of Upolu (ROU)	**North West Upolu (NWU)**	**Savai'i**	**Total**
Survey design	Systematic sample of 82 schools	Systematic sample of 34 schools	Systematic sample of 54 schools	
Sample Size	1,350	1,188	1,042	3,580
Sampling fraction	0.27	0.40	0.43	
Sampling Interval	3.66	2.47	2.34	
Critical Cut-off value	8	7	6	

Soil Transmitted Helminthes

For the STH the sample size was 400 per EU. This covered an expected non response rate of 50% as this survey required a stool specimen [Table 3]. Twenty schools considered representative of the EU geographical area were selected with 20 stool specimens jars handed out to Grade 3 and 4 students present on the day.

Table 3: Sample design for STH and critical cut off values

TAS sampling design			Critical cut-off values (number of STH-positive children) for concluding the STH prevalence range is:				
Sampling design	STH survey population (*N*)	STH target sample size	< 2%[a]	2% to < 10%[b]	10% to < 20%[c]	20% to < 50%[d]	≥ 50%
Systematic sampling	any	166	0	1-9	10-22	23-66	≥66

Training and Implementation

Training was given by WHO consultants to the FTS and STH team members from the MoH NHS & IHR, HPED and QA Divisions in the use of the FTS kits and Lab staff in the Kato-Katz technique for estimating worms. Training covered:

- Consent, using unique identifiers, and completing questionnaire
- Specimen collection, using and interpreting rapid tests and stool tests
- Roles of surveyor, supervisor
- How to counsel parents about positive results

Details of these techniques are in Appendices 2 & 3. A pilot study for both FTS and STH was conducted at the Apia Primary School.

Consent and information sheets for parents of selected students were distributed to schools about a week before the LF or STH team would visit the school. This was also to ensure the day and a place to finger prick students would be suitable.

On the day of the visit, the team would check in with the Principal and meet the class teachers. The STH team would collect any stool specimens while the LF team set up for finger pricking, usually in one of the Year 1 or 2 classrooms or a room with good lighting and tables and chairs. The LF teams consisted of three people (a team leader who also carried out the finger prick; a person to do the registering of the student and check consent and a third to time and record the FTS result). Provision for transport was included in the LF TAS budget and the drivers were used to time and record the FTS result, after consultation with their LF team.

Children who were absent for the LF survey were followed up the next day at the school, if they were in attendance. This was the only follow up. Students who had left/transferred schools were not included but other students, with parents consent, did replace absent children. All participating children received a small gift. For STH, collection happened on the same day as fingerprick and the following day.

LF positive children, their family/household members and current visitors to the family home were all offered treatment of Albendazole and Dec. As well impregnated bednets and insect repellent were offered. Information on how to reduce adult mosquitoes and larvae was also given.

Survey Results

Lymphatic Filariasis

There were a total of 3,353 children tested (1263 in AuA + RoU, 1059 in Savai'i and 1031 in NWU). Overall there were 133 positives with a FTS prevalence of (18/ 1.43% in AuA, 45/ 5.25% in Savai'i and 70/ 6.79% in NWU) [Table 4]. All EUs had more positives than their critical cutoff values, so all failed. This means that at least another cycle of two more National Mass Drug Administrations (MDAs) need to happen for the whole country, before another LF TAS.

Though the school rolls allowed for over sampling the number of students tested, except for Savai'i, did not reach the targeted numbers (93.6% for AuA and 86.8% for NWU, Savai'i 101.6%). Using the target number as denominator the positives found in the survey would be enough, however, to keep the FTS prevalence in AuA + RoU and NWU above 1% and 0.5% levels - refer Table 4. At many schools there were selected students who had transferred elsewhere in Samoa or migrated overseas since the rolls (mostly showing enrollment in the January- April term) were collected by NS &IHR in late June. One school was closed during the week for NWU school testing and In Apia, some parents from certain private schools refused consent.

Of the positive cases in NWU, where an MDA was held in April 2017, the families reported that they had been missed during the MDA or had not been given/ taken all tablets (especially their children). There were a few families who stated that they had taken the tablets. It was noted that to reduce the effort in collecting pandanus leaves for mat weaving, families had planted pandanus close to homes. These plants as well as bromeliads (also common near homes) are ideal places for larvae to breed.

Table 4: Summary of LF TAS results by EU

	Apia Urban Area plus Rest of Upolu	**North West Upolu**	**Savai'i**
Target population	1,350	1,188	1,042
Tested population	1263	1031	1059
Critical cut off values	8	7	6
Total positives found during field work	18	70	45
FTS LF prevalence (%)	1.43	6.79	5.25

Table 5 LF TAS survey and positive FTS by age, sex, class and EU

	Apia Urban Area plus Rest of Upolu		**North West Upolu**		**Savai'i**	
Age	N*	%	N*	%	N*	%
4 yrs	11	0.86	2	0.19	4	0.38
5 yrs	362	28.66	186	18.04	215	20.30
6 yrs	485	38.40	439	42.58	373	35.22
7 yrs	328	25.97	326	31.62	305	28.80
8 yrs	52	4.12	59	5.72	54	5.10
9 yrs	11	0.87	4	0.39	10	0.94
10 yrs	0	0	0	0	2	0.19
11 yrs	1	0.08				
Unknown	13	1.03	15	1.45	96	9.07
Average age (yrs)	6.06		6.26		6.25	
Gender						
Male	643	50.9	542	52.6	564	53.3
Female	620	49.1	489	47.4	495	46.7

Class						
Grade 1	666	52.7	526	51.0	566	53.4
Grade 2	597	47.3	504	48.9	493	46.6
Total positive FTS						
Positive FTS by age						
unknown	0		0		4	
5 yrs	4		18		9	
6 yrs	5		28		20	
7 yrs	7		23		8	
8 yrs	2		1		2	
9 yrs	0		0		2	
Average age	6.38		6.10		6.22	
Positive FTS by gender						
Male	9		36		22	
Female	9		34		23	
Positive FTS by class						
Grade 1	7		36		25	
Grade 2	11		34		20	
All results (as recorded)						
Positive	18		70		45	
Negative	1200		923		983	
Invalid	19		0		4	
No blood / not migrate	24		16		4	
Refused	2		0		0	

*Missing data for age not included in average age

There was no difference by age, sex, class between the 3 EUs, though the proportion of Savai'i students missing their age (9.07%) was quite high. There were slightly more males and more Grade 1 students overall. For positive cases little difference was found by gender, age or class. With the FTS results more invalid and no blood/ not migrate were seen in AuA + RoU which was the first EU completed in the survey.

Soil Transmitted Helminthes

While the STH survey was a smaller survey, unfortunately there were more difficult issues in collecting (valid) stool samples, and problems processing samples due to inavailability of laboratory staff and getting them tested within the required time period (for hookworm eggs). In the end much effort in collection was wasted and many specimens were discarded. From a target of 200 specimen per EU, there was a total of 433 returned specimen jars from across both islands and of these only 186 had a valid sample (others had urine, leaves, earth and earthworms). Of the testable samples, 152 were

students in Savai'i and 34 from students in AUA + RoU so no conclusion can be reached about the prevalence of STH in Samoa from this study.

Additionally the data collected with samples was mostly missing age (136 missing), school grade (153 missing) and no village details were recorded. Even more frustrating was that the information recorded about the type of worms seen was often only for example 8 eggs or ascaris eggs, so calculating intensity and type of egg was difficult. Overall there were 16 students stools that had eggs identified as ascaris, 3 with Trichuris worms, 4 with hookworms and 2 with pinworms. As well, 15 students stool samples had unidentified eggs seen. Two students had more than one type of worm recorded.

Table 6: Summary of STH results by EU

	Apia Urban Area plus Rest of Upolu	North West Upolu	Savai'i
Target population	166	166	166
# Specimen Collected	130	151	152 +
Tested population	34	0 (lab was unable to test spp)	152
Total positives found during field work	7	0 (lab was unable to test spp)	28

Villages and LF Positive cases

Table 7 presents the overall positive cases from the LF TAS.The 133 LF positive students came from 65 villages across Samoa. Across Upolu and Savai'i islands, 6 was the greatest number of positives in a village (Tufulele in Upolu and Salelologa in Savai'i). On Upolu villages from NWU had the top 10 positions and more, with Vailele and Vaitele and VaiteleUta situated in NWU. The next villages with the most positives, Salua and Apolima, though in AuA&RoU, are also on the western end of Upolu.

Table 7: Number of positive LF by village and EU

Village	AuA	NWU	Savai'i	Total
Salelologa			6	6
Tufulele		6		6
Faleasiu		5		5
Leauva'a		5		5
Mulifanua		5		5
Satapuala		5		5
Faleula		4		4
Laulii		4		4
Nofoalii		4		4
Saipipi			4	4
Sapapalii			4	4

Village	AuA	NWU	Savai'i	Total
Tuvao		4		4
Afega		3		3
Saleimoa		3		3
Salua	3			3
Vailele	1	2		3
Vaitele/ VaiteleUta		3		3
Aele		2		2
Apolima	2			2
Asaga			2	2
Asau			2	2
Faleatiu		2		2
Fasitoo tai		2		2
Gagaemalae			2	2
Levi		2		2
Malie		2		2
Neiafu			2	2
Pu'apu'a			2	2
Salailua			2	2
Siusega		2		2
Tafua			2	2
Toamua		2		2
Aopo			1	1
Faleapuna	1			1
Falefa	1			1
Falelima			1	1
Faletagaloa			1	1
FasitooUta		1		1
Fogapoa			1	1
Fogatuli			1	1
Fusi			1	1
Gautavai			1	1
Lalomalava			1	1
Lano			1	1
Leone	1			1
Letogo		1		1
Lotofaga	1			1
Luatuanuu	1			1
Lufilufi	1			1
ManonoUta	1			1
Matautu	1			1
Paia			1	1

Village	AuA	NWU	Savai'i	Total
Puleia			1	1
Saasaai			1	1
Samata-i-uta			1	1
Samatau	1			1
Sataoa	1			1
Sili			1	1
Solosolo	1			1
Suifaga			1	1
Taelefaga	1			1
Vaiola			1	1
Vaipua			1	1
Vaiusu		1		1
Grand Total	**18**	**70**	**45**	**133**

Other observations

The teams all remarked when visiting schools that many of the children had coughs, runny noses or sniffles and very few had a handkerchief or used one and skin diseases for example scabies and boils. Some team members remembered their days at school when having a pinned hankie was a requirement and they were all instructed in the uses and health benefits of having one.

The schools were generous in providing drinks and food. While water and towels were available to teams to wash hands before eating, less than a handful of schools provided soap.

Discussion

The LF TAS survey found that all LF TAS EUs clearly failed the WHO prevalence standard of 0.5% for which no transmission should be possible. LF positives cases were well distributed around both islands of Savaii and Upolu with NWU having the highest prevalence still. This suggests resurgence in infected mosquitoes and therefore continued transmission of LF microfilaria to a new generation in Samoa. Similarly Samoa's nearest neighbour American Samoa recently (in 2016 Lau & Graves 2017) had its third LF TAS which also failed. Both community (people aged 8 and over) and school students were sampled as part of last steps to be eligible for elimination of LF. Both surveys resulted in many more positives than the critical cutoffs allowed. This was unexpected and means that American Samoa would return to MDAs. However Tonga, located near both Samoas was declared LF free in 2017 and therefore may have pointers for the two Samoas.

While the current Samoa study did not test families of positive children, but only offered treatment to all, the American Samoan study did test the whole household and found that household members had significantly higher rates of positive tests than the random community selection which suggested strong

household clustering of transmission. This justifies treating the household and suggests that greater effort is needed around addressing the home as a focus for transmission.

Since the last Samoa nation-wide MDA in 2011, mosquito control measures have included a cabinet endorsed multi-sectoral Integrated Vector Control Committee (IVCC) activities; media awareness for source reduction, chemical control and continued Lab and Clinical Surveillance (though mainly for other vector borne diseases like dengue, Zika and chikungunya). An integrated response has seen communities and organizations work with MOH to use chemical spraying in their respective locations. Schools are also included in spraying. The SIDS conference in 2014 and Commonwealth Youth Games in 2015 focused on vector control around public spaces such as Tuanaimato, and following chickungunya (2014), dengue (2015) and Zika (2016) outbreaks.

Source reduction has been recommended for control of mosquito breeding sites in the leadup to and during the rainy seasons. How vigorously this is repeated and compliance assured is unknown. The findings from home visits of pandanus and other plants growing close to homes and / or containers/ rubbish present that are potential breeding sites as well as the open nature of Samoan homes (with few using bed nets or repellent) makes it imperative to address this and reduce Samoans risk of exposure to not only lymphatic filariasis but also dengue, chikungunya and Zika.

With the dengue outbreak beginning August/ September 2017 a National Clean Up campaign has been suggested as the most sustainable and cost effective way to combat mosquitoes. This would also put accountability and responsibility for mosquito control on both government and the community.

NWU had the most positive cases, nearly 4 times those in the rest of Upolu and 1.5 times the number in Savai'i. NWU also has had greater numbers of other vector borne diseases. Using 2016 census data the population density of NWU is much higher compared to the rest of Samoa (excluding Apia Urban Area) and this possibly contributes to the continuing spread of such diseases as well as other communicable diseases such as typhoid. While there were few LF positive cases in Apia its urban landscape possibly is a factor compared to the more rural and generally low lying, swampy NWU. Other factors in play for less number of positives in AUA + ROU may also be the technique with which the teams used as AUA + ROU provided more practice in finger pricking technique and the use of the new FTS test.

Non participation in the 2017 MDA was also a factor in the high number of positive cases found in NWU. Many of the positive cases and families had relayed that they were not aware and therefore never took the MDA treatment in April 2017. The next MDAs will need to ensure that participation is much higher, at least 90% or higher. Additionally the American Samoan study questioned the MDA dosage based on age, given the very high rates of overweight and obesity, and recommended future use of weight based or an age based schedule appropriate for the average weight of the local population. Here some families of positive cases did say they had participated in the MDA and as overweight and obesity are also a major problem in Samoa consideration should be given to dosage rates in the next MDA.

In November 2017 WHO released Guidelines for alternative mass drug administration in response to country requests to WHO for alternative regimens so that their national programs target is still LF elimination by 2020. In these guidelines WHO recommends triple drug therapy (Ivermectin + DEC + ALB)

(IDA) for potentially shortening the number of MDA rounds required to meet pre-TAS and TAS criteria. Two of the special settings where WHO recommends this regimen fit Samoa: 1) for EUs that have not met epidemiological targets in sentinel and spot-check site surveys or in TAS despite meeting drug coverage targets, and 2) for communities where post-MDA or post-validation surveillance identified infection suggesting local transmission.

While the actual school rolls allowed oversampling, the target number for AUA + ROU and NWU weren't reached. Large numbers of student transfer to other schools; migration; absenteeism; MESC programs that interrupted school days so that many classes were cancelled; also a large proportion of refusal to participate and finding replacement students was sometimes difficult especially in the larger schools, were some of the factors that were in play. However, the number of positive cases were so high this probably isn't such an issue. Follow up was also done but the LF schedule and school hours also limited this.

Other issues arising during the time of the survey were in particular staffing ones. While the team leaders remained unchanged, as other staff had their routine roles to also fulfill, changes were necessary, as the NHS & IHR was already understaffed. As well those who did the data entry had their core responsibilities and data entry was compromised. Backfilling for the duration of the LF TAS and/or using nursing orientees (for whom it could be considered community experience) is required. It is also suggested that each team has an electronic device – laptop or tablet where registration and results can be directly entered and then at the end collated for analysis. This would save time and effort and an earlier final report.

The STH survey had been a rather disappointing feat, due to many issues which do overlap with LF TAS survey. Though positive stools for the STH survey were found, but because of the overall poor response and missing data, no conclusion can be reached from this study about the prevalence of STH and therefore the need for a regular deworming program in Samoa. The capacity and the capabilities of the survey staff was the biggest disappointment. Reluctance and excuses for both collection and not processing specimens was disappointing and future surveys must establish well staff to carry this out to the end. Also due to MESC programs and the many issues listed above, it was most difficult to collect stool samples. Coupled with the general mindset of people in general concerning stool specimen as a dirty affair, we believe caused many to refuse. Perhaps separation of the two surveys may give more attention and the needed time to concentrate on issues for proper STH survey. Other options for STH data collection may be worked into requirements for school entry which would oblige parents and students to comply. Still for this time round, some children with worms were able to be treated. As for the LF TAS staffing was problematic and additionally the Kato-Katz technique is time limited, to find hookworm especially, so prompt stool collection and testing is needed.

In effect, Public Health measures for control and elimination of LF as well as addressing other PH diseases of significance must be strengthened urgently and simultaneously. In particular, with the recent rise in arboviral outbreaks in the country and region, multi-faceted approaches in control of mosquito-borne diseases requires sustained and continued efforts: the triple therapy IDA now recommended by WHO; integrated vector control methods of both **source reduction and chemical control** in the community as well as inter-governmental committees; targeted Public Health awareness; enhanced LF and mosquito surveillance as well as enforcement of laws governing Public and Environmental Health must come together.

Conclusion

Based on the WHO on the WHO guidelines for Transmission Assessment Surveys (TAS) Samoa has failed the 2017 school survey as all three EUs had well over their critical cut-off points and more national MDAs are required once again. That almost all the positive cases in NWU reported not being a part of the April 2017 MDA, only 4 months previously, shows that achieving 71% coverage, though above the WHO required 65% coverage rate, is not nearly enough to prevent transmission. Future MDAs need to aim for 90% and more.

The concept and procedure for any further STH surveys needs to be revised by WHO in consultation with Samoa MoH as the response was much lower than expected and there were staffing, data collection and recording concerns.

Recommendations

Strategic

1. *That two additional MDAs across all Samoa be conducted before any further LF TAS survey. The first MDA should be in the first half of 2018. Required coverage in villages should aim for 90%. That consideration be given to the use of IDA as recommended by WHO.*

   The LF TAS survey found that all EUs clearly failed, with positives distributed around both islands. This suggests a resurgence in infected mosquitoes and therefore transmission of LF to a new generation.

   Included with MDA should be a promotion of community action around mosquito control as the mosquitoes that cause LF (and dengue, chikungunya and Zika) are day biting, urban/ home based.

2. *That Samoa and American Samoa work in conjunction with WHO in aligning LF programs in the two countries.*

   Preliminary meetings have been held to discuss the way forward and this is likely to be further progressed towards the end of 2017.

3. *That awareness raising, education and program(s) are developed between responsible Ministries to encourage action by the Samoan community to minimize mosquitoes and mosquito bites.*

   The mosquitoes that deliver LF as well as Zika, chikungunya and dengue are ones that prefer urban/ home settings and bite from dawn to dusk. Therefore eradication, vector control and mosquito bite prevention begin at home. Samoans and other residents need to be aware of the critical role they play in this, particularly as the Samoan culture and way of life is one open to the outdoors, without screens. This makes it easy for mosquitoes to feed on human blood. People need to be reminded of what they can do – repellent, bed nets, clothing, not planting shrubs and trees like pandanus or bromeliads close to fales, keeping a tidy, rubbish (old cars, tyres, buckets etc) free garden. This would be done in conjunction with help from the MWSCD Sui I Nuu and Sui I Tamatai committees.[see recommendation 4]

4. *That the Ministry of Women, Community and Social Development reenergize and strengthen the Sui o Nuu and Sui Tamatai o Nuu (SN and STN) committee members to follow up on environmental issues related to mosquito control and also general hygiene concerns raised in this report. These committees should work with appropriate MoH staff in Divisions of Health Promotion, NHS& IHR and PEN Fa'aSamoa to deliver comprehensive health information*

   Assessment of family living conditions and the home environment of positive cases found that most were living in traditional style open homes conducive to mosquitoes and mosquito bites, such as unscreened fales with no bed nets. There was also potential for larvae breeding sites close to homes from discarded containers, tyres and plants such as pandanus. No one seemed to apply insect repellent, few mosquito coils.

   As well other hygiene issues were noted by LF survey teams such as some homes without their own toilet, animals (such as chickens, cats) able to walk anywhere in the living quarters etc. These are

under the remit of the Sui o Nuu to advise and aid with improving the living conditions for those in their community and reinforce the need for mosquito control.

5. *Health promotion through MESC and MoH to encourage the use of hankies/ tissues for coughs, sniffles, and reminder that using soap is essential in hand washing/ cleaning practice.*

   These were from observations made by team members during their time at each school. While not related to lymphatic filariasis, these missing/ forgotten aspects of staying healthy should be routine as they are very simple and easy to implement and make routine.

6. *The concept and procedure for any further STH surveys needs to be revised by WHO*.

   STH testing was much lower than required for the survey. The difficulty is getting stool specimens from apparently well children was difficult and many of the "stools" collected contained no stool, or instead urine or even contained earthworms. There were also laboratory staffing movements that added to data collection and data recording concerns and the need to bring in appropriate equipment and materials.

Operational

7. *Adequate staffing for teams with backfill is required. This applies to both the LF TAS and STH surveys. Equiping the LF TAS teams with an electronic device to record registration details and results at the school, with collation of all teams' data at the end (of an EU).*
8. *Use of 4WD vehicles for easier access to some schools and positive cases whose families live well off the main roads.*
9. *The use of nursing orientees should be considered as gaining practical experience in an aspect of community nursing, understanding the level of health literacy in a community and general "patient" interaction.*

   This is with the concerns of Public Health issues in many homes visited during this survey for which Nurses in their orientation should be exposed to the very grassroots of health issues in the community.

10. *Consider separation of the two surveys LF TAS from STH survey for the future.*

Appendix 1 LF TAS Survey results for Individual schools and EUs

EU: AUA + RoU Target 1350	Students selected (N)	Students tested (N)	% Coverage	Positive FTS (N)
Ah Mu Academy - Pesega	47	35	75	0
All Saints Anglican Primary School	13	8	62	0
Apia Primary	150	88	59	0
Apolima-uta Primary	22	21	96	2
Aufaga Primary	15	14	93	0
Divine Mercy Primary School	18	17	94	1
Faleapuna Primary	8	8	100	1
Falease'ela Primary	12	11	92	0
Falefa Primary	15	11	73	1
Falefitu Primary	25	25	100	1
Falelatai Primary	28	22	79	0
Faleu Primary	8	8	100	0
Falevao Primary	10	7	70	0
Fusi Primary School	9	8	88.9	0
Lalomanu Primary	11	10	90.9	0
Lalomauga Primary	10	10	100	0
Lepa Primary	11	12	109	0
Lepea Primary	20	12	60	0
Lona Primary	4	4	100	0
Lotofaga Primary (Lepa/LotofagaDist)	18	13	72	1
Lotofaga Primary (Safata District)	13	13	100	0
Lotopue Primary	8	7	88	0
Luatuanuu Primary	17	8	47	1
Lufilufi Primary	12	12	100	1
Magiagi Primary	24	23	96	0
Manono Primary	38	33	87	1
Manumalo Baptist School	38	29	76	0
Manunu Primary	6	5	83	0
Marist Brothers Primary School	57	46	81	0
Matatufu Primary	10	10	100	0
Matautu Primary	28	22	79	0
Moata'a Primary	23	18	78	0
Mulivai Primary	8	5	63	0
Nene Primary	8	8	100	0
Pata Primary	11	10	91	0
Peace Chapel Christian School	20	18	90	0
PesegaFou Primary	25	18	72	0
Robert Louis Stevenson	33	22	67	0

EU: AUA + RoU Target 1350	Students selected (N)	Students tested (N)	% Coverage	Positive FTS (N)
Primary				
Saanapu Primary	25	23	92	0
Safa'ato'a Primary	12	10	83	0
Salamumu Primary	9	9	100	0
Salani Primary	10	10	100	0
Saleaaumua Primary	13	9	69	0
Saleapaga Primary	13	10	77	0
Saleilua Primary	17	11	65	0
Salelesi Primary	16	9	56	0
Salesatele Primary	6	6	100	0
Salua Primary (Manono)	8	23	288	3
Samatau Primary	20	10	50	1
Samoa Adventist School	40	25	63	0
Samoa Primary School	27	12	44	0
Samusu Primary	7	7	100	0
Saoluafata Primary	13	11	85	0
Sapoe Primary	8	6	75	0
Sapunaoa Primary	9	9	100	0
Satalo Primary	6	2	33	0
Sataoa Primary	26	24	92	1
Satitoa Primary	10	8	80	0
Sauano Primary	7	7	100	0
Sauniatu Primary - LDS	8	8	100	0
Savaia Primary	11	11	100	0
Siufaga Primary	12	9	75	0
Siumu Primary	34	30	88	0
Solosolo Primary	30	29	97	1
St Peter's Falefa	29	11	38	0
St. Theresa's School - Lepea	25	10	40	0
St.Mary's - Savalalo	50	43	86	0
Taelefaga Primary	7	7	100	1
Tafitoala Primary	13	4	31	0
Tanugamanono Primary	15	10	67	0
Tiavea Primary	16	16	100	0
Uafato Primary	4	4	100	0
Ulutogia Primary	3	3	100	0
Vaiala Beach School	16	6	38	0
Vaie'e Primary	19	18	95	0
Vailima Primary	24	19	79	0
Vailoa Primary (Aleipata District)	6	6	100	0
Vailoa Primary (Faleata District)	17	13	76	0
Vaimea Primary	57	46	81	0
Vaimoso Primary	45	13	29	0
Vaivase Primary	64	41	64	1

EU: AUA + RoU Target 1350	Students selected (N)	Students tested (N)	% Coverage	Positive FTS (N)
Vaovai Primary	15	14	93	0
Total	**1666**	**1263**	**76**	**18**

EU: NWU Target 1188	Students selected (N)	Students tested (N)	% Coverage	Positive FTS (N)
Aele Primary School	40	40	100	2
Afega Primary	40	40	96	3
Aleisa Primary	49	23	47	
AogaFaamasani Amosa	12	-	-	-
Baptist Church Primary School	17	17	100	2
Fagali'i Primary	19	19	100	0
Faleasi'u Primary	55	49	89	3
Faleatiu Primary	11	17	155	6
Fale'ula Primary	34	30	88	5
Fasito'o tai Primary	23	20	87	2
Fasito'outa Primary	28	22	79	1
George Brown Primary School	63	39	62	0
Lauli'i Primary	40	40	100	4
Le'auva'a Primary	30	15	50	1
Letogo Primary	34	32	94	3
Leulumoega Primary	9	8	89	0
Levi Primary	20	19	95	2
Malie Primary	50	40	80	2
Moamoa&Tauao'o Primary	37	35	95	3
Mulifanua Primary	38	37	97	5
Nofoalii Primary	43	43	100	3
Saina/Toamua Primary School	34	20	59	3
Sale'imoa Primary	30	28	93	3
Satapuala Primary	34	34	100	5
Satuimalufilufi Primary	41	24	59	0
St. Joan of Arc School	50	46	92	1
St. Joseph's Primary - Leauvaa	34	32	94	1
Tuana'i Primary	20	20	100	0
Utuali'i Primary	33	31	94	6
Vaigaga Primary	45	31	69	0
Vailele Primary	23	23	100	0
Vailu'utai Primary	31	15	48	0
Vaitele Primary	112	102	91	4
Vaiusu Primary	45	34	76	1

EU: NWU Target 1188	Students selected (N)	Students tested (N)	% Coverage	Positive FTS (N)
Total	**1224**	**1031**	**84**	**70**

EU: Savai'i Target 1042	Students selected (N)	Students tested (N)	% Coverage	Positive FTS (N)
Aopo Primary	12	12	100	1
Asaga Primary	15	10	75	2
Asau Baptist & Christian Academy	12	12	100	0
Asau Primary	31	24	77	2
Auala Primary	12	12	100	0
Faga Primary	25	23	92	0
Fai'a'ai/Fogatuli Primary	19	19	100	1
Falealupo Primary	23	19	83	0
Falelima Primary	19	7	37	
Fogasavai'I Primary	16	16	100	1
Gagaemalae Primary	31	23	74	2
Gataivai Primary	33	30	91	0
Gautavai Primary	8	5	63	1
Iva Primary	37	35	95	0
Lalomalava Primary	26	25	96	1
Lano Primary	20	20	100	1
Laumoli Primary	23	20	87	0
Letui Primary	9	9	100	
Manumalo Baptist (Savaii)	21	16	76	1
Neiafu Primary	25	19	76	2
Paia Primary School	12	12	100	1
Palauli Primary	60	61	102	0
Papa/Sataua Primary	14	13	93	0
Patamea Primary	22	17	77	0
Pu'apu'a Primary	15	15	100	2
Puleia Primary	31	20	65	1
Sa'asa'ai Primary	24	19	79	1
Sacred Heart - Safotu	17	13	76	0
Safotu Primary	20	20	100	0
Safotulafai Primary	30	32	107	0
Safune Primary	15	7	47	1
Sagone Primary	12	11	92	0
Saipipi Primary	19	18	95	3
Salailua Primary	42	24	57	
Saleaula Primary	15	12	80	0
Salelavalu Primary	31	31	100	0
Salelologa Primary	73	60	82	6
Samalaeulu Primary	20	15	75	0

EU: Savai'i Target 1042	Students selected (N)	Students tested (N)	% Coverage	Positive FTS (N)
Samata-i-tai Primary	22	15	68	0
Samata-i-uta Primary	26	24	92	1
Samauga Primary	27	23	85	0
Sapapalii Primary	29	24	83	3
Sasina Primary	13	13	100	
Sataua/Fagasa Primary	12	12	100	0
Satupaitea Primary	41	26	63	0
Sili Primary	25	25	100	1
Siufaga Primary - SDA	13	11	85	1
St. Theresa's School - Fusi	25	16	64	0
Tafua Primary	22	21	95	2
Taga Primary	26	21	81	0
Tufutafoe Primary	12	11	92	0
Tutaga Primary	23	23	100	0
Vaiola Primary	27	26	96	4
Vaisala Primary	13	13	100	0
Total	1236	1059	86	46

Appendix 2 :Filariasis test - FTS

Basic Guidelines

i. Kits should be stored at 2-37°C. Test strips should NOT be frozen. The Alere™ Filariasis Test Strip kit is stable until the expiration date marked on its outer packaging when stored as specified. Kits should NOT be used past the expiration date.

ii. Before beginning field surveys, two strips from each lot of kits should be tested using a positive control that can be obtained from the Filariasis Research Reagent Repository Center (www.filariasiscenter.org). DO NOT use strips that are negative when tested with the control.

iii. When transporting strips for use in the field, a cool box is not required. However, care should be taken not to expose strips to extreme heat for prolonged periods of time.

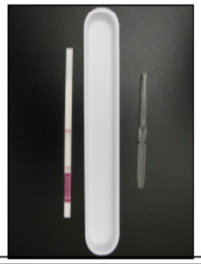
iv. Strips must be read using bright unfiltered light. Faint lines can be difficult to see when lighting is not adequate. This is especially important when reading strips at night.

Test Procedure

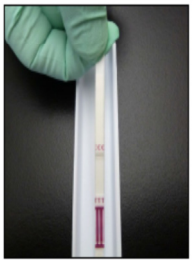
1

Allow all kit components to equilibrate to ambient temperature (15-37°C) before testing.

Remove contents from the foil pouch just prior to use. Provided materials include one test strip, plastic work tray and fixed volume (75µL) micropipette.



2



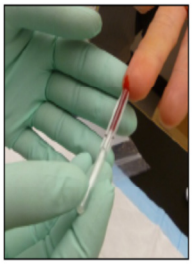
Strips should be handled carefully and held only at the end without the arrows. DO NOT apply pressure to the sample pad at the bottom of the strip.

Strips should be labeled with appropriate patient identifiers. Strips can be labeled directly (preferred) (A). Alternatively, the work tray can be labeled (B).

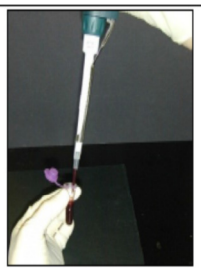
The strip should be placed in the work tray before the sample is added.

NOTE: It is advisable to secure the strip to the work tray with a sticker -type patient identifier label or tape.

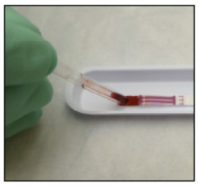
3



Collect 75µL of fingerstick blood by holding the supplied micropipette slightly above the horizontal plane. **DO NOT** squeeze the bulb end of the micropipette when collecting the sample. Alternatively, measure 75µL of anti-coagulated blood from a microcentrifuge tube using a calibrated micropipettor. **DO NOT** add blood directly from the finger to the strip.



4



Slowly add the blood sample to the lower half of the sample pad by gently squeezing the bulb.

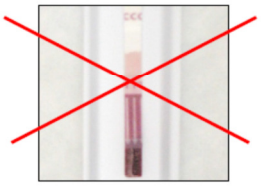
Set a timer for 10 minutes.

NOTE: It is helpful to record the reading time on the work tray.

5

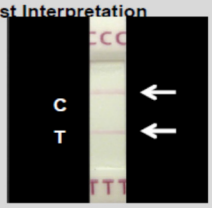
Read test results 10 minutes after the sample has been added.

NOTE: Record the appropriate result on the strip (preferred) or work tray.

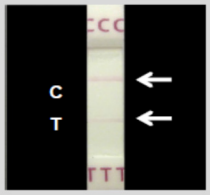


DO NOT read tests if the sample has not migrated ALL the way up the strip.

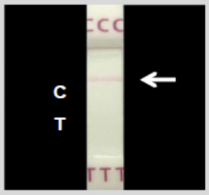
Test Interpretation



POSITIVE*
Any visible pink line in the test area should be interpreted as a positive result

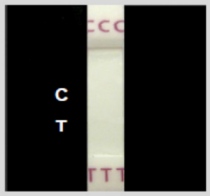


POSITIVE (weak)*

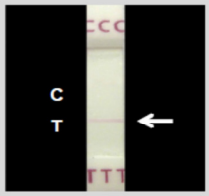


NEGATIVE*
Control line only

C = control
T = test



INVALID
No lines appear



INVALID
Test line only

**Scoring of test strips is useful but optional. Negative, 0; test line weaker than control line, 1; test line equal to control line, 2; test line stronger than control line, 3*

17

An electronic version of this bench aid can be found at: http://www.ntdsupport.org/resources

Alere Filariasis Test Strip

EN

INTENDED USE

The Alere™ Filariasis Test Strip is an in vitro immunochromatographic assay for the qualitative detection of Wuchereria bancrofti antigen in human capillary whole blood samples collected by fingerstick. It is intended to aid in the rapid diagnosis of lymphatic filariasis caused by W. bancrofti. It is recommended that negative test results be confirmed by standard diagnostic methods such as thick smear microscopy, membrane filtration, or another antigen detection method.

Negative results do not preclude infection with W. bancrofti and should not be used as the sole basis for treatment or other management decisions.

SUMMARY AND EXPLANATION OF THE TEST

Lymphatic filariasis is a parasitic disease, caused by microscopic, thread-like worms. The adult worms live in the human lymph system and release microscopic worms, called microfilariae, into the blood. Approximately 90% of lymphatic filariasis is caused by W. bancrofti.[1] If the infection is left undiagnosed and untreated, it can cause lymphedema and, ultimately, the disfiguring and debilitating condition known as elephantiasis. W. bancrofti infection can also cause hydrocele, or swelling of the scrotum. Lymphatic filariasis is widely considered to be a Neglected Tropical Disease; it affects over 120 million people in 80 countries in the tropics and sub-tropics around the world.[2]

Diagnosis of lymphatic filariasis using traditional microscopy methods can be difficult and requires precise and meticulous techniques, as it involves the identification of microfilariae in the blood of the infected person. Microscopy requires skilled handling of the sample preparation and an experienced technologist for interpretation of the blood smear. In addition, due to nocturnal periodicity, the microfilariae are only present in the peripheral circulatory system at night, so collection of an acceptable sample for microscopy must be done at night.

The Alere™ Filariasis Test Strip is a very simple, rapid method for the diagnosis of lymphatic filariasis, using whole blood collected by fingerstick. The easy-to-use format and rapid results allow for its use in settings in which traditional microscopy methods may not be readily available, where it can provide information to assist with treatment decisions.

PRINCIPLES OF THE TEST

The Alere™ Filariasis Test Strip is an in vitro, immunochromatographic, membrane assay for the detection of W. bancrofti antigen in capillary whole blood, collected via fingerstick. The test uses two different antibodies. One antibody is attached to colloidal gold and impregnated into a pink pad on the Test Strip. A second antibody is immobilized on the membrane. The blood sample is added to the lower half of the exposed white Sample Pad. The sample flows through the pink area of the pad and up the membrane, allowing any antigen in the sample to be labeled with the colloidal gold-labeled antibody. The antigen-label body complex is then immobilized in the test result area of the Test Strip.

Test results are interpreted at 10 minutes, based on the presence or absence of a pink-to-purple colored Test Line. A pink-to-purple procedural Control Line will appear in a valid test.

REAGENTS AND MATERIALS

Materials Provided

Test Strips: A membrane support combined with other reagents/pads to construct a Test Strip; each Test Strip is individually packaged with a desiccant and a plastic work tray.

Micropipettes: Fixed volume (75 µL) micropipettes used to transfer whole blood samples obtained via fingerstick to the Test Strips.

Patient Result Stickers: Stickers for adhering Test Strips to work surface and for recording patient results (optional).

MATERIALS REQUIRED BUT NOT PROVIDED

Lancets capable of delivering a minimum volume of 75 µL whole blood; sterile wipes or pads; clock, timer or stopwatch

PRECAUTIONS

1. For in vitro diagnostic use.
2. Ensure that all kit components are equilibrated to ambient temperature (15-37°C) before use.
3. The Test Strip is sealed in a protective foil pouch; do not use if pouch is damaged or open. Leave Test Strip sealed in its foil pouch until just before use. Keep storage boxes dry.
4. Do not use kit past its expiration date.
5. Optimal results will be obtained by strict adherence to the instructions provided herein.
6. Handle the Test Strip carefully. Hold it only at the top, which is the end without the arrows. Do not touch or apply pressure to the functional areas of the Test Strip, including the white Sample Pad at the bottom of the Test Strip.
7. If using the Patient Result Sticker, place it at the top of the Test Strip, as described above. Do not place it over the functional area of the Test Strip, including the white Sample Pad.
8. If using the plastic work tray, do not let the Test Strip touch the side of the tray, as doing so could cause false results.
9. When interpreting test results, use a bright unfiltered light.
10. Patient samples and used Test Strips should be handled as potentially infectious materials. Observe established precautions against microbial hazards. The use of lab coats, gloves and safety eye glasses is strongly recommended.
11. When collecting blood samples, use the micropipettes provided in the kit. See the detailed instructions in the Specimen Collection and Handling section below that explain how to properly use the micropipettes.
12. Micropipettes are single use items – do not use with multiple specimens.
13. Excessive air circulation and direct sunlight may slow the flow of the sample. During testing, protecting the devices from excessive air circulation and direct sunlight is recommended.

STORAGE AND STABILITY

Store kit at 2-30°C. The Alere™ Filariasis Test Strip kit is stable until the expiration date marked on its outer packaging when stored as specified.

QUALITY CONTROL

Daily Quality Control:

The Alere™ Filariasis Test Strip has a built-in (internal) procedural control. For daily quality control, Alere suggests that you record the results of this control for each test run.

Procedural Controls:

The pink-to-purple line in the top half of the result area of a tested strip (close to the area labeled with "C") can be considered an internal positive procedural control. If the sample flows and the reagents are working properly, this line will always appear.

The clearing of background color on a tested strip is a negative background control. Background color should not interfere with the reading of the test result.

External Positive and Negative Controls:

Good laboratory practice suggests that external positive and negative controls be run to ensure that:

- test reagents are working, and
- the test is correctly performed.

At a minimum, Alere recommends that external controls be run once with each new shipment. Recommended external controls are listed below. Alternatively, laboratories may use different controls, providing control results are as expected. Each lab should establish its own positive and negative external quality control samples and procedures.

For a negative control, a whole blood sample from a presumed W. bancrofti non-infected individual can be tested, following the specimen collection and test procedures described below.

For a positive control, a sample from a confirmed W. bancrofti positive individual or a sample containing W. bancrofti antigen can be used.

External Control Procedure:

If pipetting a control, use a calibrated pipette and apply 75 µL of the control to the lower half of the Sample Pad. See the Result Interpretation section for instructions on interpreting the results.

Other controls may be tested in order to conform with:

- local and/or federal regulations,
- accrediting groups, and/or,
- your laboratory's standard Quality Control procedures

If the correct control results are not obtained, do not report patient results. Contact Alere™ Technical Support.

SPECIMEN COLLECTION AND HANDLING

1. Prepare patient for fingerstick sample collection. The best locations for fingersticks are the 3rd and 4th fingers. Do not use the tip of the finger or the center of the finger pad. Avoid the side of the finger where there is less soft tissue, where vessels and nerves are located, and where the bone is closer to the surface. The 2nd (index) finger tends to have thicker, calloused skin. The fifth finger tends to have less soft tissue overlying the bone. Avoid puncturing a finger that is cold or cyanotic, swollen, scarred, or covered with a rash. Avoid fingers with rings on.
2. Warm up the fingers if needed. Have the patient hold their hand downwards to increase blood flow to the finger. **Note.** The patient should always sit higher than the person performing the fingerstick.
3. Wipe the tip of the appropriately selected finger with an alcohol swab and let the alcohol air dry.
4. Use a sterile lancet to make a skin puncture just off the center of the finger pad. Do not squeeze or apply strong repetitive pressure (milking) to the site; this may result in hemolysis or tissue-fluid contamination of the specimen. If necessary, gentle massaging of the finger may be conducted in order to ensure a steady blood flow.
5. Wipe off the first drops of blood with a dry cloth or gauze. Ensure steady blood flow that generates large enough drops of blood. If necessary, wipe off another drop, until blood flows freely.
6. Allow blood to flow freely from the pricked finger directly into the micropipette provided in the test kit by following the instructions provided below. Test immediately.

Inadequate specimen collection or improper sample handling may yield false or invalid results.

Instructions for using the micropipettes provided in the Alere™ Filariasis Test Strip kit:

Filling is automatic. **DO NOT** squeeze the bulb end of the micropipette when collecting sample. The micropipette uses capillary action to collect sample and will stop filling when the required volume has been sampled.

1. Hold the micropipette horizontally and touch the tip of the micropipette to the blood sample. Capillary action will automatically draw the sample to the fill line and stop. Avoid getting air bubbles in the micropipette.
2. To expel the sample, align the tip of the micropipette with the Sample Pad and gently squeeze the bulb. If the sample won't expel, the micropipette may not be adequately filled. Touch the tip to the blood sample again and allow it to fill completely.

TEST PROCEDURE for CAPILLARY WHOLE BLOOD SAMPLES

Allow all kit components to equilibrate to ambient temperature (15-37°C) before testing.

Remove the Test Strip from the foil pouch immediately prior to use. Place the Test Strip in the plastic work tray, so the indicator arrows point toward the operator. If not using the work tray, lay the Test Strip on a flat surface. Discard the desiccant included in this pouch. If using the Patient Result Sticker, place it at the top of the Test Strip, which is the end without the arrows.

Do not touch the exposed Sample Pad.

1. Using a micropipette provided in the test kit, slowly add the patient sample to the lower half of the exposed white Sample Pad by gently squeezing the bulb (see Step 2 of the instructions for using the micropipettes above). **Note.** See the indicator arrows on the bottom of the Test Strip that show where the Sample Pad is located. Avoid applying excessive pressure on the Sample Pad during sample addition and do not apply pressure to the functional areas of the Test Strip. **Important.** Incorrect addition of sample may cause erroneous results.
2. Start a timer for 10 minutes. When possible, do not disturb or move the Test Strip after the sample has been added. If it is necessary to move the Test Strip, keep it flat and move it very carefully by sliding it. Do not hold the Test Strip in a vertical position after the sample has been added.
3. Interpret the results in the result area of the Test Strip 10 minutes after adding the sample. **Note.** See the Procedure Card for an actual size graphic of the Test Strip that shows the location of the Control and Test Lines.

Note. When reading test results, tilt the Test Strip to reduce glare on the Test Strip, if necessary. Individuals with color-impaired vision may not be able to adequately interpret test results.

RESULT INTERPRETATION

Positive Test Result

A **positive** test result produces a pink-to-purple Control Line in the top half of the result area of the Test Strip and a pink-to-purple Test Line in the lower half of the result area. Any pink-to-purple visible Test Line is positive. Do not interpret the test result until 10 minutes have elapsed from when the sample was added.

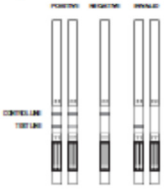
Negative Test Result

A **negative** test result produces a pink-to-purple Control Line in the top half of the result area of the Test Strip and the absence of a Test Line in the lower half of the result area. To ensure that low positive samples have had sufficient time to develop, a negative result should not be recorded until 10 minutes have elapsed from when the sample was added.

Invalid Test Result

The test is **invalid** if the Control Line in the top half of the result area of the Test Strip does not appear, whether a Test Line is present or not. If this occurs, the test should be repeated with a new Test Strip and a fresh sample. Contact Alere™ Technical Support if the problem persists.

Examples of Results:



REPORTING OF RESULTS

Result	Suggested Report
Positive	Positive for W. bancrofti antigen. This result does not rule out co-infections with other pathogens.
Negative	Negative for W. bancrofti antigen. W. bancrofti infection cannot be ruled out, as antigen levels in the sample may be below the detection limit of the test. Alere suggests that negative test results be confirmed by standard diagnostic methods such as thick smear microscopy, membrane filtration, or another antigen detection method.

LIMITATIONS

A negative test result does not exclude infection with W. bancrofti. Therefore, the results obtained with the Alere™ Filariasis Test Strip should be used in conjunction with standard diagnostic methods to make an accurate diagnosis.

The Alere™ Filariasis Test Strip detects antigen from both viable and non-viable W. bancrofti. Test performance depends on antigen load in the specimen and may not directly correlate with microscopy performed on the same specimen.

Performance of the Alere™ Filariasis Test Strip has not been established for monitoring treatment of Lymphatic Filariasis. Residual antigen may be detected following elimination of the parasite by anti-filarial treatment.[3]

The Alere™ Filariasis Test Strip prospective clinical study, summarized in the section below, was conducted in a W. bancrofti endemic area. Performance of the Alere™ Filariasis Test Strip has not been established in non-endemic, low infection level areas.

Performance of the Alere™ Filariasis Test Strip has not been established in children 5 years old and younger.

PERFORMANCE CHARACTERISTICS

Alere™ Filariasis Test Strip Test Performance vs. Comparator Method – Prospective Clinical Study

The clinical performance of the Alere™ Filariasis Test Strip was established in a multi-site, prospective, clinical study conducted in Liberia in a W. bancrofti endemic area in April and May of 2012.

A total of 614 prospective blood specimens, collected from children (ages 6 to 16 years of age) and adults (16 years or older), were evaluated in the Alere™ Filariasis Test Strip test and compared to a commercially available rapid immunochromatographic test. Evaluated specimens were capillary whole blood samples, collected via fingerstick. Twenty-three percent (23%) of the population tested was greater than 5 years of age but less than 10 years, 19% was 10 - 15 years of age, and 58% was greater than 16 years.

Alere™ Filariasis Test Strip test performance versus the comparator method, for all subjects combined, is detailed below. There were 614 Alere™ Filariasis tests performed, and 16 were invalid (3% - 16/614), leaving 598 tests available for the analysis presented below. One of the 16 invalid Alere™ tests was also invalid on the comparator method.

Alere™ Filariasis Test Strip Test Performance versus Comparator Method

	Comparator Method +	Comparator Method -	
Alere™ Filariasis +	97	27*	124
Alere™ Filariasis -	1	378	379
	98	405	503

Positive percent agreement: 97/98 = 99% (95% CI: 94.4-99.8%)

Negative percent agreement: 378/405 = 93% (90.1-95.4%)

* The true antigen status of the 27 samples that tested positive on the Alere™ Filariasis Test Strip, but were negative on the comparator method, was not definitively determined; however, all samples were collected from people residing in W. bancrofti endemic areas of Liberia.

Specificity (Cross-Reactivity):

To further evaluate the specificity of the Alere™ Filariasis Test Strip, retrospective testing of 132 well characterized, archived clinical serum / plasma samples was conducted in two research laboratories in the U.S. The samples included 44 confirmed to be positive by microscopy for various parasitic organisms, other than W. bancrofti and expected to be prevalent in the geographic areas where the Alere™ Filariasis test is intended to be used. The positive samples tested were:

- 10 *Strongyloides stercoralis* samples,
- 10 *Brugia timori* samples,
- 10 *Mansonella perstans* samples,
- 13 *Onchocerca volvulus* samples, and
- 1 *Onchocerca volvulus* and *Loa loa* co-infection samples.

Eighty-four (84) samples, collected from individuals residing in W. bancrofti non-endemic areas and presumed to be negative, were also tested. Ninety-eight percent (98% - 126/132) of the samples tested generated negative results on the Alere™ Filariasis Test Strip; one *Strongyloides stercoralis* positive sample and 2 *Brugia timori* positive samples generated positive results on the Alere™ Filariasis test. Each of these 3 samples was collected from people living in W. bancrofti endemic areas.

Interfering Substances:

The following substances that may be artificially introduced into whole blood were evaluated in the Alere™ Filariasis Test Strip at the concentrations listed below and were found not to affect test performance. **Note.** The analytical effects of the drugs on the Alere™ test were evaluated using whole blood samples containing high therapeutic concentrations. The effects on test performance of each drug's clinical metabolites are unknown.

Substance	Concentration
Albendazole	240 µg/mL
Ivermectin	20 µg/mL*
Diethylcarbamazine (DEC)	0.600 mg/kg*
Albendazole and Ivermectin	As above
Albendazole and DEC	As above
Mebendazole	0.060 µg/mL
Praziquantel	6 mg/mL*
Quinine	149 µmol/L
Acetylsalicylic acid (Aspirin)	3.62 mmol/L
Acetaminophen	1325 µmol/L
Ibuprofen	2425 µmol/L
Doxycycline	67.5 µmol/L
Cephalexin	337 µmol/L
Amoxicillin	206 µmol/L
Erythromycin	61.6 µmol/L
Kanamycin	124 µmol/L
Heparin	3000 U/L
EDTA	3.4 µmol/L

*Dosage calculated using patient weight equal to 166.67 kg

Reproducibility Study:

The reproducibility of the Alere™ Filariasis Test Strip was evaluated in an in-house study using panels of blind coded samples containing moderate positive, low positive and negative samples. Samples were delivered to the Test Strips using calibrated pipettes. Six (6) participants tested each sample type two times on three different days. There was 100% (72/72) agreement with expected test results with the positive samples, and 100% (36/36) agreement with expected test results with the negative samples. Two tests generated invalid results (3% - 2/108).

ORDERING AND CONTACT INFORMATION

Reorder number:

625-000: Alere™ Filariasis Test Strip

	US	1 877 441 7440
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Technical Support

Advice Line

Further information can be obtained by contacting Alere™ Technical Support on:

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Appendix 3.Kato-Katz Procedure

The Kato-Katz technique involves a microscopic examination of the feces to detect and count the number of helminthic eggs. This procedure should be performed the same day that the specimen was collected.

Preparation of cellophane clippings

Note: This preparation must occur at least 24 hours in advance

1. Prepare a 1% malachite green stock solution:
    a. Add 1 gm of Malachite green crystals to 100 ml of bottled water in a sealable glass bottle
    b. Mix thoroughly
    c. Label with content, concentration and date of preparation
2. Prepare a working solution of glycerol-malachite green solution:
    a. Add 100ml of bottled water to a clean 500 ml glass bottle
    b. Add 1ml Malachite green (1% stock solution above) to the glass bottle
    c. Add 100ml Glycerol (100%) to the glass bottle above
3. Prepare cellophane clipping
    a. Cut cellophane into appropriately-sized clippings (same width as microscope slide; length 30-35mm)
    b. Soak cellophane clippings in working glycerol-malachite green solution at least 24 hours before use

Preparing Kato-Katz Slides[1]

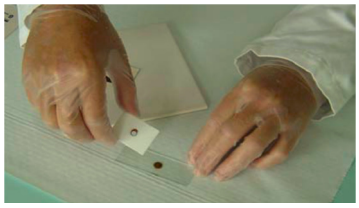
1. Label the microscope slide with the child's full name.
2. Prepare area with newspaper; lab technicians should be wearing latex gloves
3. Place the Kato-Katz template on the slide

4. Place a small amount of fecal material on the newspaper
5. Press the screen on top of the feces so that some of the feces filters through
6. Scrape with the flat spatula across the upper surface to collect the filtered feces

[1] These instructions were adapted from "Methods in Parasitology" presented by the Swiss Tropical Institute in Basel, 2005 and the 2011 Standard Operating Protocol of the DOLF (Death to Oncho and LF) Project, at Washington University in St. Louis, PI Dr. Gary Weil: http://www.dolf.wustl.edu/

7. Fill the hole in the template with the feces, smoothing it out so that the hole is completely filled, all the while pressing the template into the slide

8. Remove any excess feces with the spatula
9. Remove the Kato-Katz template by lifting it vertically (avoid any horizontal movement so as not to disturb the specimen)



10. Cover the fecal matter on the slide with a glycerol-malachite soaked cellophane clipping over the stool on the slide

11. Spread the stool aliquot by applying pressure from above and evenly distributing the stool underneath. For best results, use a clean microscope slide on top of the cellophane and press down or invert the slide and press it on a smooth and flat surface, such as a tile. The thick smear should be symmetrically round, evenly spread and of a diameter slightly smaller than the breadth of a microscope slide. Avoid lifting, wrinkling or moving

the cellophane when spreading the smear. ***Attention: Support the slide from beneath to avoid cracking***

12. Let the slide sit for 30-60 minutes then read immediately (see below)
13. All health team members should properly wash their hands with soap and water after touching the specimen containers

Reading of Kato-Katz Slides

Note: it is important that the slides be read within one hour of preparation, as hookworm eggs may become transparent and difficult to identify over time.

1. An experienced microscopist should examine the slide under a microscope using 40x or 100x magnification; it is important the entire fecal smear slide be read in a systematic manner.
2. The number of eggs of each species (Ascarislumbricoides, hookworm and Trichuristrichiura) should be tallied in a notebook or with a handheld counter.
3. The results of the tally will be entered into the smart phone after scanning the barcode found on the slide.
4. The WHO guidelines recommend a template that holds 41.7mg of feces, which corresponds to a multiplication factor of 24.Multiply the number of eggs counted by 24 to obtain the number of eggs per gram (epg).
5. Using the table below, look up the epg value to determine the intensity threshold. *Note: if someone has 0 eggs then they have no infection*

Helminth	**Intensity Threshold**		
	Light	**Moderate**	**Heavy**
Ascarislumbricoides	1-4999 epg	5,000 - 49,999 epg	>50,000 epg
Hookworm	1-1999 epg	2000 – 3999 epg	>4000 epg
Trichuristrichiura	1-999 epg	1000 – 9999 epg	>10,000 epg

Quality Control

The consistency of microscopic results during the survey should be verified by quality control. Before the survey is undertaken, a day should be spent evaluating the consistency of egg counting among laboratory technicians. Each day during the survey, the team leader should read 10% of the slides handled by each microscopist without prior knowledge of the results. In the case of a discrepancy larger

than 10%, the slide should be discussed by two readers and further slides examined to avoid repeat error.

4.4 Materials

Materials for Stool Collection:

1. Stool collection container: Purchased locally (if possible)
2. Screw-cap of stool collection container, with spatula
3. Container Label
1. Cut up newspaper for stool collection
2. One (1) Plastic bag per container
3. "Stool Sample" bar code sticker

Materials for Kato-Katz Test:

1. Kato Katz kits:
    a) Templates with hole
    b) Spatulas
    c) Nylon screen fabric
    d) Cellophane (see steps above for preparing cellophane)
2. Glycerol 100% (Locally purchased)
3. Bottled water (Locally purchased) Malachite green (crystals)
4. Wide Mouthed-Glass bottle with screw cap 500 ml (2)
5. Permanent marker
6. Latex gloves
7. Microscope slides
8. Microscope
9. Labels with barcode ("Kato-Katz")
10. Tweezers
11. Paper wipes
12. Newspaper
13. Tongue depressors
14. Permanent marker
15. Notebook or paper pad for tallying # of eggs

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