Malaria-LAMP:
A Highly Sensitive Molecular Diagnostic Method for Detecting *Plasmodium* DNA

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Outline

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  - World Malaria Situation
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  - How to Diagnose Malaria

- Novel DNA Detection Kit for Malaria ‘Malaria-LAMP’

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Background World Malaria Situation

In 2021, nearly **half of the world's population** was at risk of malaria.

There were an estimated **247 million cases** of malaria worldwide.

The estimated number of **malaria deaths** stood at **619,000** in 2021.

WHO Malaria (who.int) Key facts

Background World Malaria Situation

Malaria mostly spreads to people through the bites of infected female *Anopheles* mosquitoes.
There are main 5 *Plasmodium* parasite species that cause malaria in humans

5 *Plasmodium* parasite species

P. falciparum ***P. vivax*** *P. malariae* *P. ovale* *P. knowlesi*

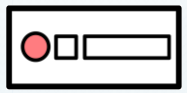
Left untreated, *P. falciparum* malaria can progress to severe illness and death within 24 hours.

The first symptoms may be mild, similar to many febrile illnesses, hence it is **difficult to diagnose malaria definitively**.

WHO Malaria (who.int)

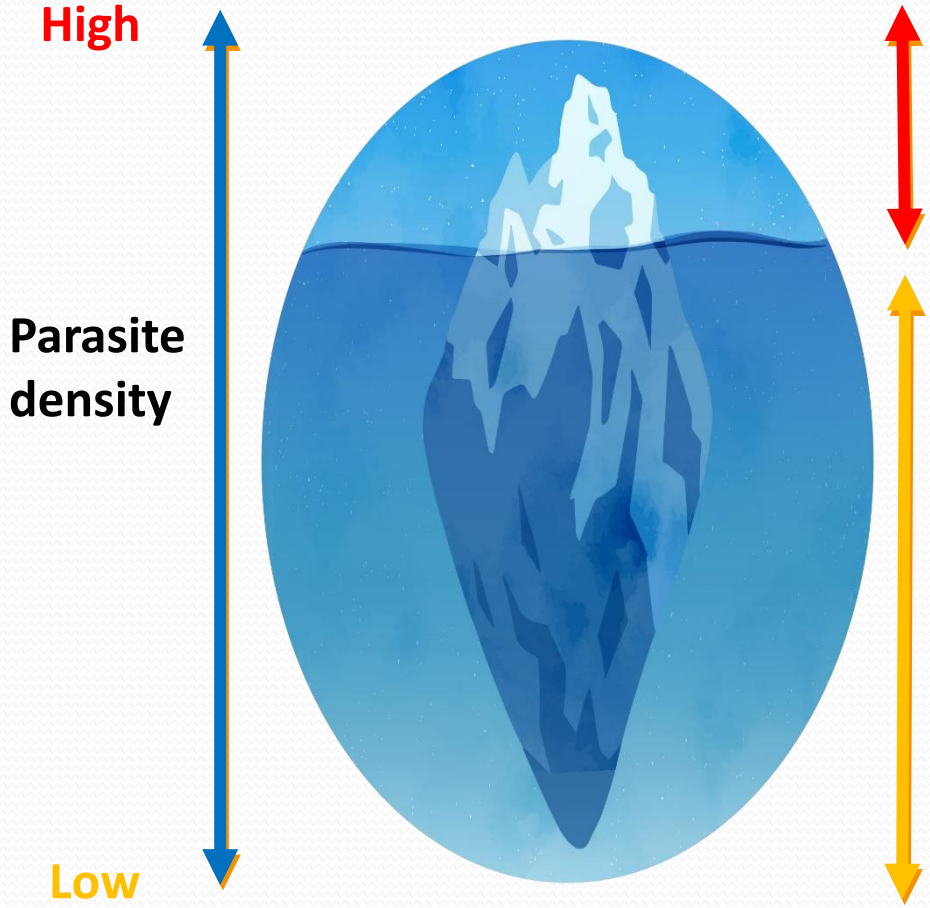
Background How to Diagnose Malaria

Conventional Malaria Diagnostic Methods

	Strong Points	Weak Points
Rapid Diagnostic Test 	✓ Rapid ✓ Easy to Use ✓ Affordable	✓ Low sensitivity ✓ Results depend on operators ✓ Difference of quality among test devices
Microscopy 	✓ Rapid ✓ Easy to Use ✓ Affordable	✓ Low sensitivity ✓ Results depend on operators
Molecular Test 	✓ High sensitivity ✓ Fewer differences between operators	✓ Complex procedure ✓ Highly demanding operating environment ✓ Expensive

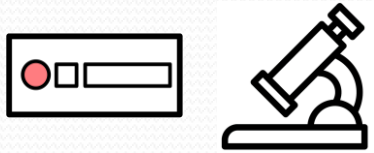
Background How to Diagnose Malaria

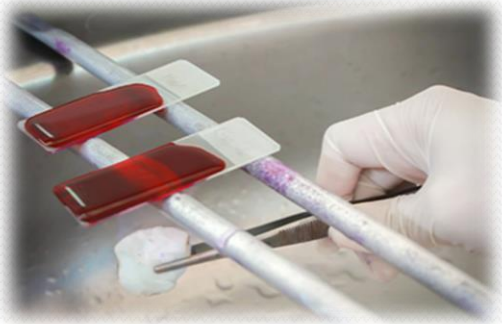
It is well known that there exist a large population of asymptomatic patients with low parasite density, so they cannot be detected by microscopy or RDT.



Symptomatic

RDT, Microscopy





Asymptomatic

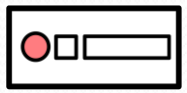
NAAT

(e.g. PCR, LAMP)

Correlation of Parasite Density, Symptom and Diagnostics

Novel DNA Detection Kit for Malaria 'Malaria-LAMP'

Conventional malaria diagnosis methods

	Strong Points	Weak Points
Rapid Diagnostic Test 	✓ Rapid ✓ Easy to Use ✓ Affordable	✓ Low sensitivity ✓ Results depend on operators ✓ Large differences among reagent manufacturers
Microscopy 	✓ Rapid ✓ Easy to Use ✓ Affordable	✓ Low sensitivity ✓ Results depend on operators
Molecular Test 	✓ **High sensitivity** ✓ **Fewer differences between operators**	✓ **Complex procedure** ✓ **Highly demanding operating environment** ✓ **Expensive**

Easier, faster and more robust!!

Novel DNA Detection Kit for Malaria 'Malaria-LAMP'

Malaria-LAMP is the Novel Solution for Malaria Elimination.

What 's good about Malaria-LAMP?

- **Sensitivity**
  Malaria-LAMP is more sensitive than microscopy and RDT
- **Specificity**
  Pan Malaria-LAMP: 5 *Plasmodium* parasite species detection
  Pf Malaria-LAMP: Only *P. falciparum* detection
  Pv Malaria-LAMP: Only *P. vivax* detection
- **Easy**
  3 Days Training
- **Fast**
  70 Tests/day
- **Robust**
  Minimum Instrument → Implementation Solar panel & Battery system

◀ Instrument

▲ Reagent

▲ Solar panel

Novel DNA Detection Kit for Malaria ‘Malaria-LAMP’

Malaria-LAMP Specification

LAMP Test Kit

Extraction	PURE
Store at	2-30 ºC (Room Temperature)
Shelf life	18 months
Regulation	IVDR (Class C)

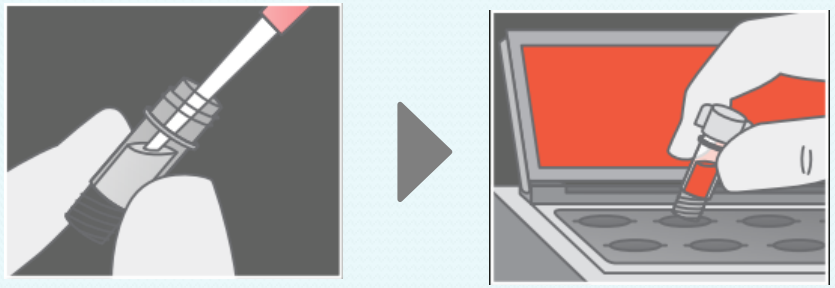
Instrument and Solar Panel

Maintenance	Free
Life expectancy	5 years
Regulation	IVDR (Class A)
Target	Resource limited setting

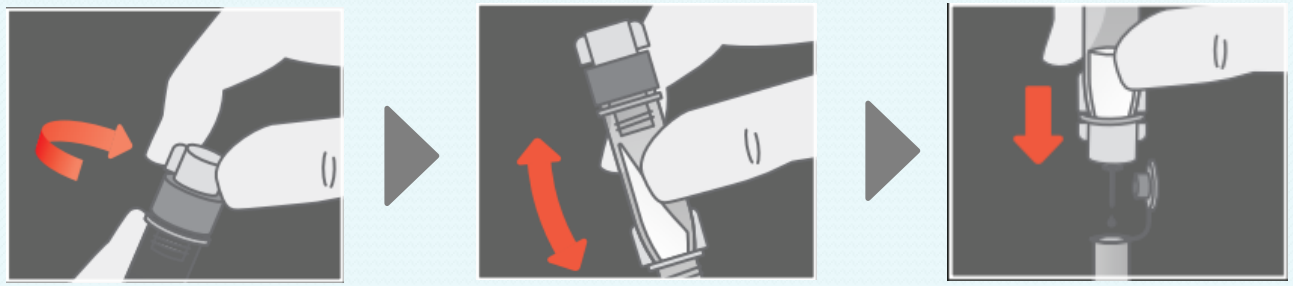
Novel DNA Detection Kit for Malaria ‘Malaria-LAMP’

How to use ‘Malaria-LAMP’

Step1. Sample Transfer and Lysis

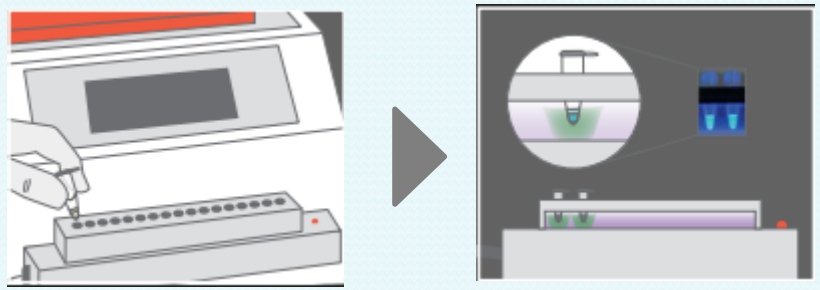


Step2. PURE DNA Extraction



Step3. Loop-mediated isothermal amplification

Step4. Result Reading



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Evaluation Studies of ‘Malaria-LAMP’

Case1 Haiti

Performance of the procedure for ultra-rapid extraction and loop-mediated isothermal amplification (PURE-LAMP) methods to detect malaria in Haiti

Target	902 febrile people and 172 afebrile people
Division	Nippes, Sud and Grand’Anse in Haiti
Study period	Summer season (2017 and 2018)
Method	Microscopy, RDT, Malaria-LAMP and nested PCR
Gold standard	Nested PCR
Analysis	Sensitivity, specificity, positive and negative predictive values and kappa statistics

Performance of the procedure for ultra-rapid extraction and loop-mediated isothermal amplification (PURE-LAMP) methods to detect malaria in Haiti (nih.gov)

Evaluation Studies of 'Malaria-LAMP'

Performance of PURE-LAMP, RDT and microscopy against nested PCR as reference

	Sensitivity, n/d % (95% *CI*)	Specificity, n/d % (95% *CI*)	PPV, n/d % (95% *CI*)	NPV, n/d % (95% *CI*)	*Kappa*
PURE-LAMP Pan	89/89 100 (95.9–100)	976/985 99.1 (98.3–99.6)	89/98 90.8 (83.3–95.7)	976/976 100 (99.6–100)	0.95
PURE-LAMP Pf	88/89 98.9 (93.9–100)	977/985 99.2 (98.4–99.6)	88/96 91.7 (84.2–96.3)	977/978 99.9 (99.4–100)	0.95
RDT Pan	54/89 60.7 (49.7–70.9)	983/985 99.8 (99.3–100)	54/56 96.4 (87.7–99.6)	983/1018 96.6 (95.2–97.6)	0.73
RDT Pf	76/89 85.4 (76.3–92.0)	980/985 99.5 (98.8–99.8)	76/81 93.8 (86.2–98.0)	980/993 98.7 (97.8–99.3)	0.88
Microscopy	44/89 49.4 (38.7–60.2)	978/985 99.3 (98.5–99.7)	44/51 86.3 (73.7–94.3)	978/1023 95.6 (94.1–96.8)	0.60

Malaria-LAMP showed the high performance to detection *Plasmodium*.
This study recommends Malaria-LAMP's use in targeted mass screening and treatment activities in low endemic areas of malaria.

Performance of the procedure for ultra-rapid extraction and loop-mediated isothermal amplification (PURE-LAMP) methods to detect malaria in Haiti (nih.gov)

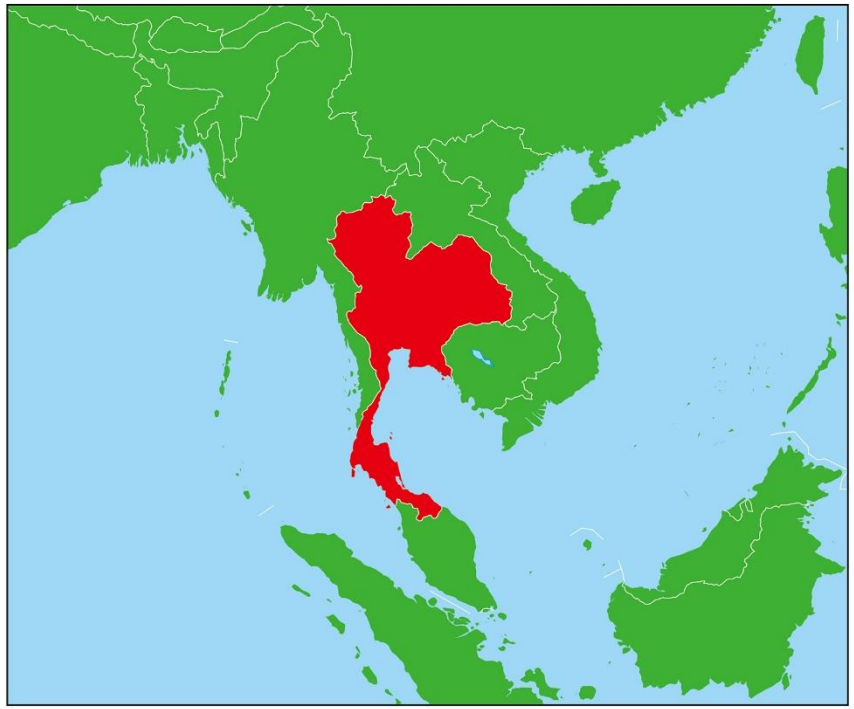
Evaluation Studies of ‘Malaria-LAMP’

Case2 Thailand

Evaluation of Malaria Detection Function Leveraging New Products in Thailand

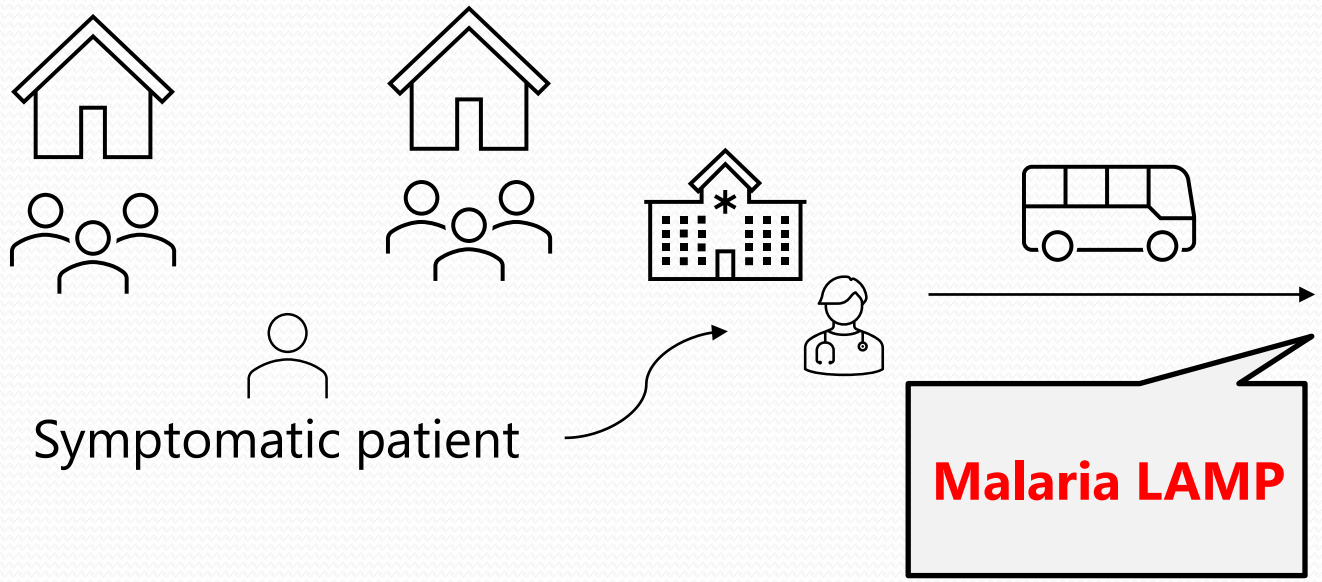
Unpublished data of NCGM (Principal Investigator: Professor Shigeyuki Kano, MD., Ph.D.)

Target	**3,315** Asymptomatic malaria-infected patients
Division	the malaria endemic areas and the service areas of the Faculty of Tropical Medicine
Study Period	December 2019 - February 2022
Method	Microscopy, RDT, XN-31 (Sysmex Corporation), Malaria-LAMP and Nested PCR
Analysis	Sensitivity, specificity, positive and negative predictive values

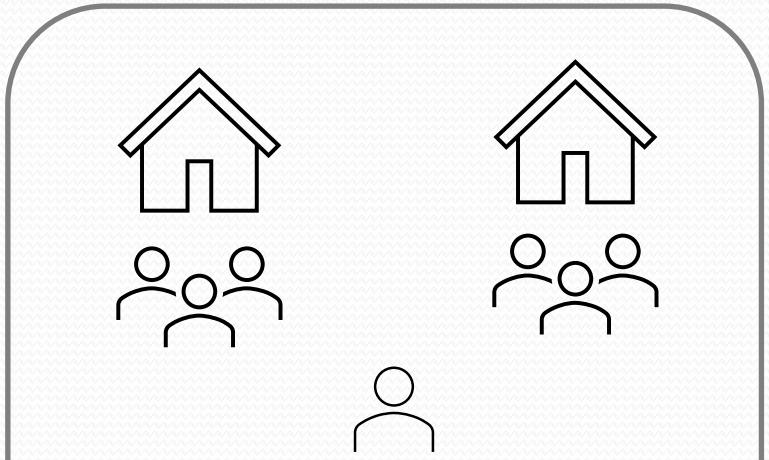


Evaluation Studies of ‘Malaria-LAMP’

Usual Diagnosis



Index Case Finding



Malaria Elimination and Reemergence Prevention Campaigns

Evaluation Studies of ‘Malaria-LAMP’

Comparison between Pan Malaria LAMP and Microscopy Test

		Microscopy		Total
		Positive	Negative	
Pan Malaria-LAMP	Positive	13	41	54
	Negative	0	3261	3261
Total		13	3302	3315

Accuracy	98.76%
Sensitivity	100.00%
Specificity	98.76%
PPV	24.07%
NPV	100.00%

Pan Malaria-LAMP test shows higher sensitivity than the microscopic examination

Conclusion and Discussion

- ✓ Malaria elimination requires highly sensitive tests to detect asymptomatic parasite carriers.
- ✓ Malaria-LAMP is a novel molecular test that enables highly sensitive test **easier, faster and more robust.**
- ✓ Two different studies have shown that **Malaria-LAMP test is highly sensitive.**

⇩

Malaria-LAMP is helpful for healthcare workers with limited experience in microscopic examination, especially in the areas where malaria is being eliminated or where there is no malaria, and for population screening for asymptomatic parasite carriers under malaria elimination and reemergence prevention campaigns.

Thank you very much for your attention!!