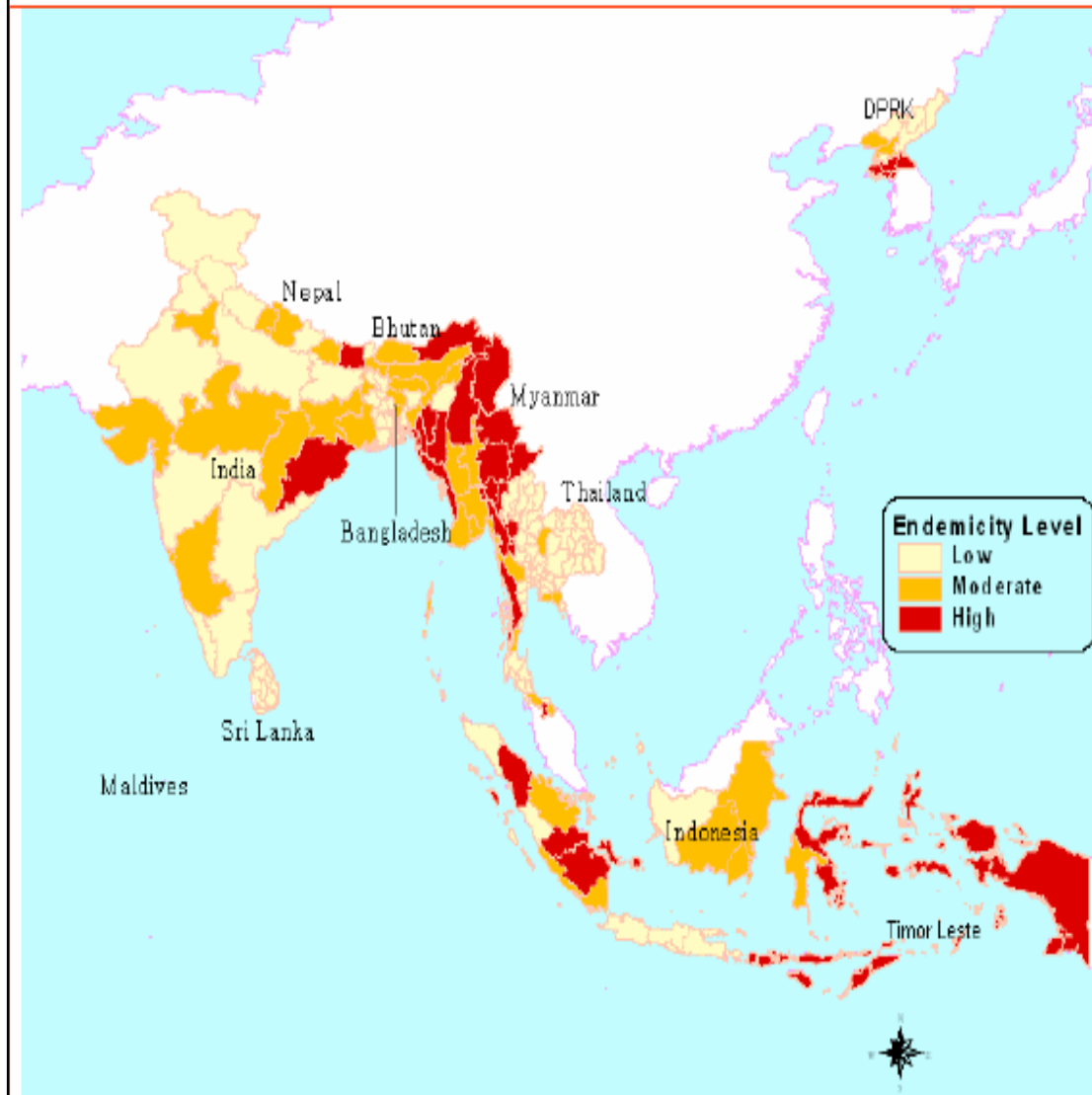


Malaria Infections in Thailand, North Region: Comparative Diagnosis of Human Malaria Infections Using Microscopy, PCR and LAMP

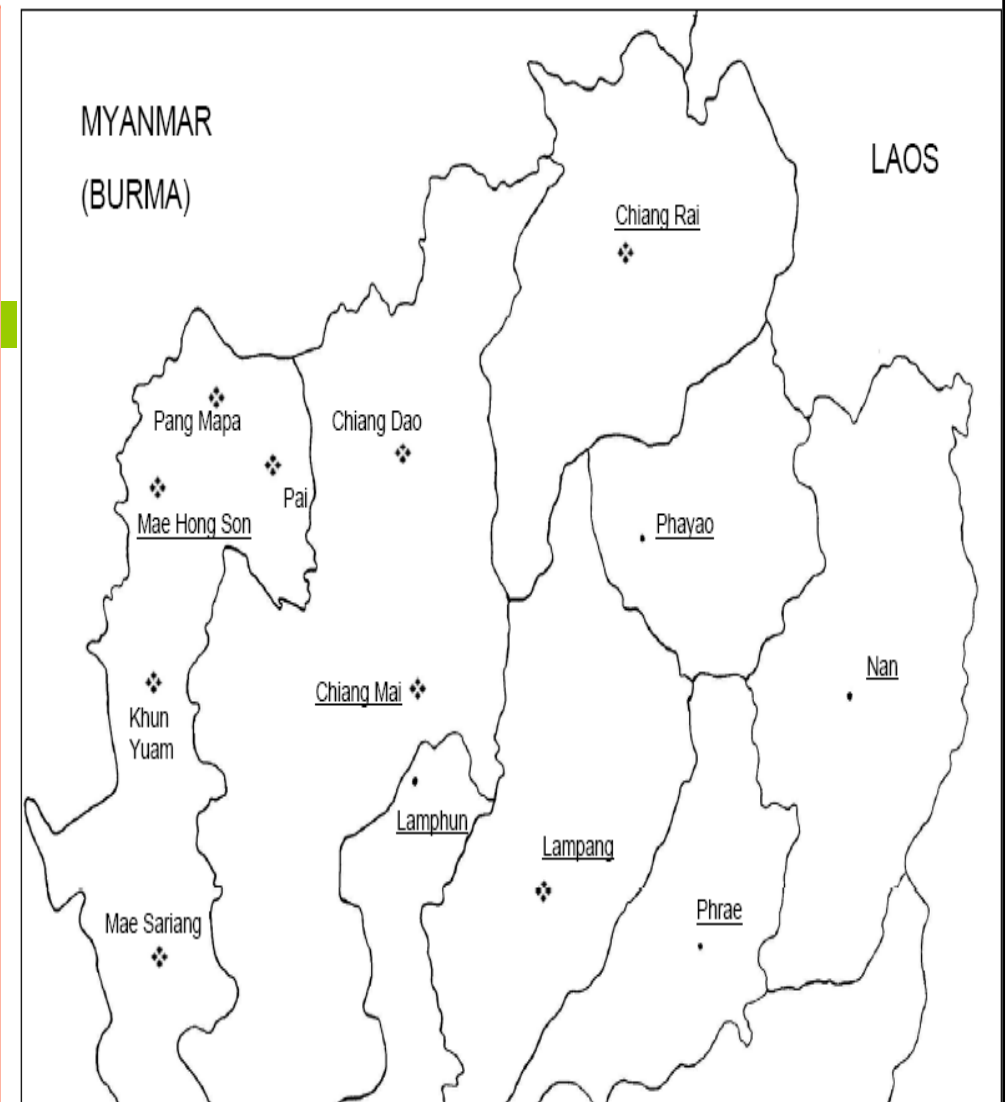
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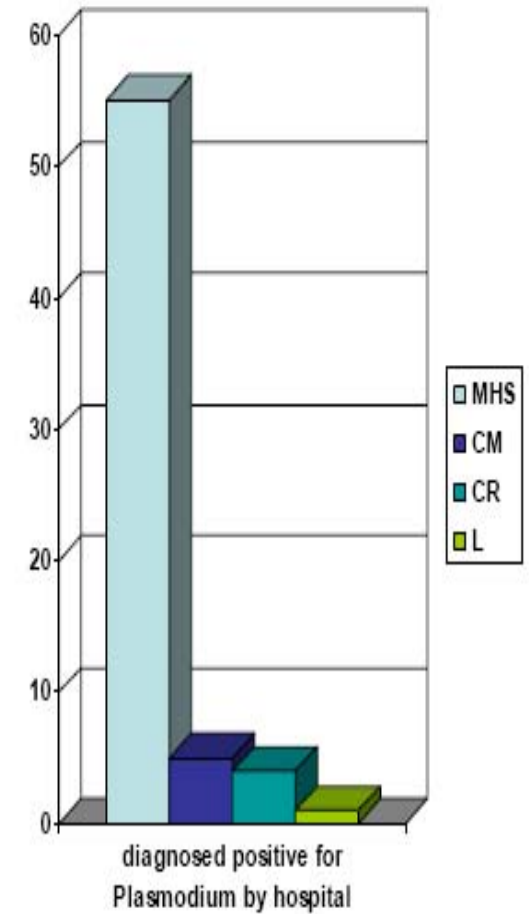
Malaria endemicity map of SEA Region 2006 WHO Regional office SEA



Malaria in Northern Thailand



Collaborative Center and Investigation Areas

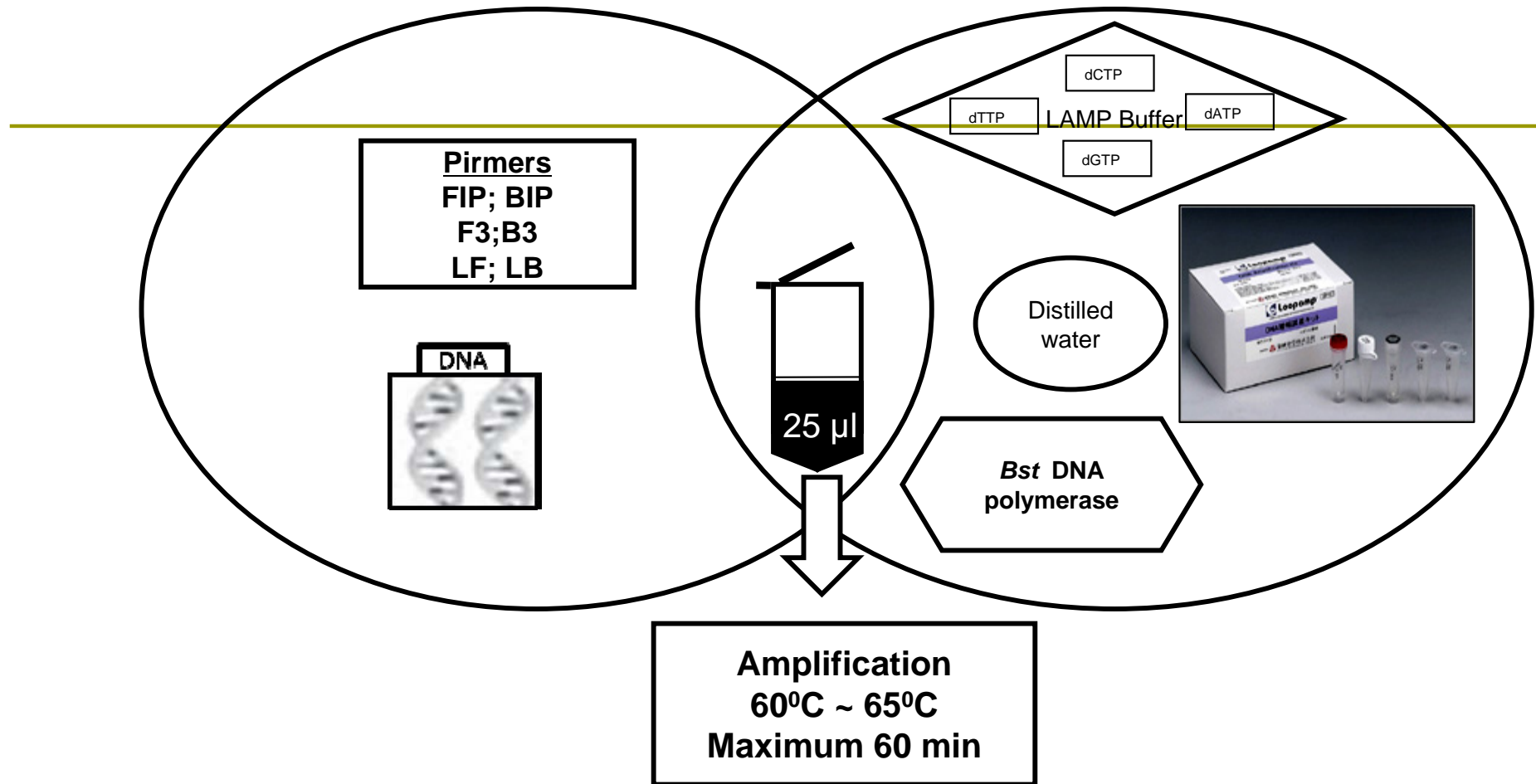


Comparative detection on the prevalence of *Plasmodium* infections in blood samples collected from N. Thailand

	Microscopy	PCR	LAMP	Any method
<i>P. falciparum</i>	37%	41%	37%	44%
<i>P. vivax</i>	16%	22%	21%	24%

Differences statistically not significant

LAMP Reagents and Heating Devices



Loop-mediated Isothermal Amplification (LAMP)

Nucleic acid amplification method developed by Notomi et al (2000) at Eiken Chemical Co. Ltd, Japan.

■ **Simple**

Single step isothermal amplification and visual detection of amplified products

■ **Rapid**

Final test results within 15-60 minutes

■ **Specific and Sensitive**

Use of 4 or 6 primers recognizing 6 or 8 distinct regions on the target

■ **Cost effective**

No special reagents and sophisticated equipment is required

■ **Amplified products**

Extremely large amount (10^9 - 10^{10} times within 15-60 minutes)

■ **Amplification of RNA**

Single step isothermal amplification of RNA by just adding reverse transcriptase

LAMP specificity for *Plasmodium*

Table 5: Specificity of LAMP shown by using DNA of different Protozoan.

DNA	Result
<i>Plasmodium falciparum</i> (FCR-3 strain)	+
<i>P. berghei</i> (ANKA strain)	+
<i>P. vivax</i> (Thai isolate)	+
<i>Cryptosporidium</i> (bovine genotype 2, Tokachi isolate)	-
<i>Trypanosoma cruzi</i> (Tulahmen strain)	-
<i>T. brucei gambiense</i> (IL2343)	-

(+): positive
(-): negative

Only DNA of *Plasmodium* species could be amplified

Detailed comparison: LM, PCR, LAMP Malaria Investigations in Chiang Mai

Parasite(s) detected by each method (no. of samples) ^a		
Nested PCR	Microscopy	LAMP
<i>P. falciparum</i> (50) ^b	<i>P. falciparum</i> (42)	<i>P. falciparum</i> (42)
	<u>negative (6)</u>	<u>negative (4)</u> ^c <u><i>P. falciparum</i> (2)</u>
	<u><i>P. vivax</i> (2)</u>	<u><i>P. falciparum</i> (2)</u>
<i>P. vivax</i> (20)	<i>P. vivax</i> (14)	<i>P. vivax</i> (14)
	Negative (2)	<i>P. vivax</i> (2)
	<i>P. falciparum</i> (4)	<i>P. vivax</i> (4)
<i>P. falciparum</i> + <i>P. vivax</i> (3)	<u><i>P. falciparum</i> (2)</u>	<u><i>P. falciparum</i> + <i>P. vivax</i> (1),</u>
	<i>P. vivax</i> (1)	<u><i>P. falciparum</i> (1)</u>
		<i>P. vivax</i> (1) ^c
Negative (32)	Negative (32)	Negative (32)

a Results that were nonconcordant between microscopy and LAMP are underlined

b Each row provides the result obtained with identical DNA samples

c DNA of these samples probably underwent degradation since nested PCR yielded concordant results to LAMP, when samples were retested 2 months after extraction, at the same time when LAMP was carried out.

Clinical sensitivity and specificity of three methods

Species	Method	Specificity	Sensitivity
Pf	LAMP	100%	91% (100%) ^a
	Microscopy	100%	91%
Pv	LAMP	100%	99%
	Microscopy	98%	65%

^a Sensitivity calculated by including the results of 5 samples that yielded distinct results when they were retested by nPCR in Japan.

LAMP: threshold time using DNA from different life cycle stages of *Plasmodium* species

Tab.1: Threshold time (Tt) of four *Plasmodium* life stages using *Plasmodium* genus specific LAMP, each sample containing a DNA concentration of 10ng/ μ l.

<i>Plasmodium</i> species and stage	Threshold time (min) (mean of 3 tests)
<i>P. falciparum</i> , mixed stages ¹⁾	28:16
<i>P. falciparum</i> , trophozoites	30:56
<i>P. falciparum</i> , schizonts	29:52
<i>P. berghei</i> , mixed stages ²⁾	38:34
<i>P. berghei</i> , sporozoites	45:30

1) trophozoites, schizonts and merozoites

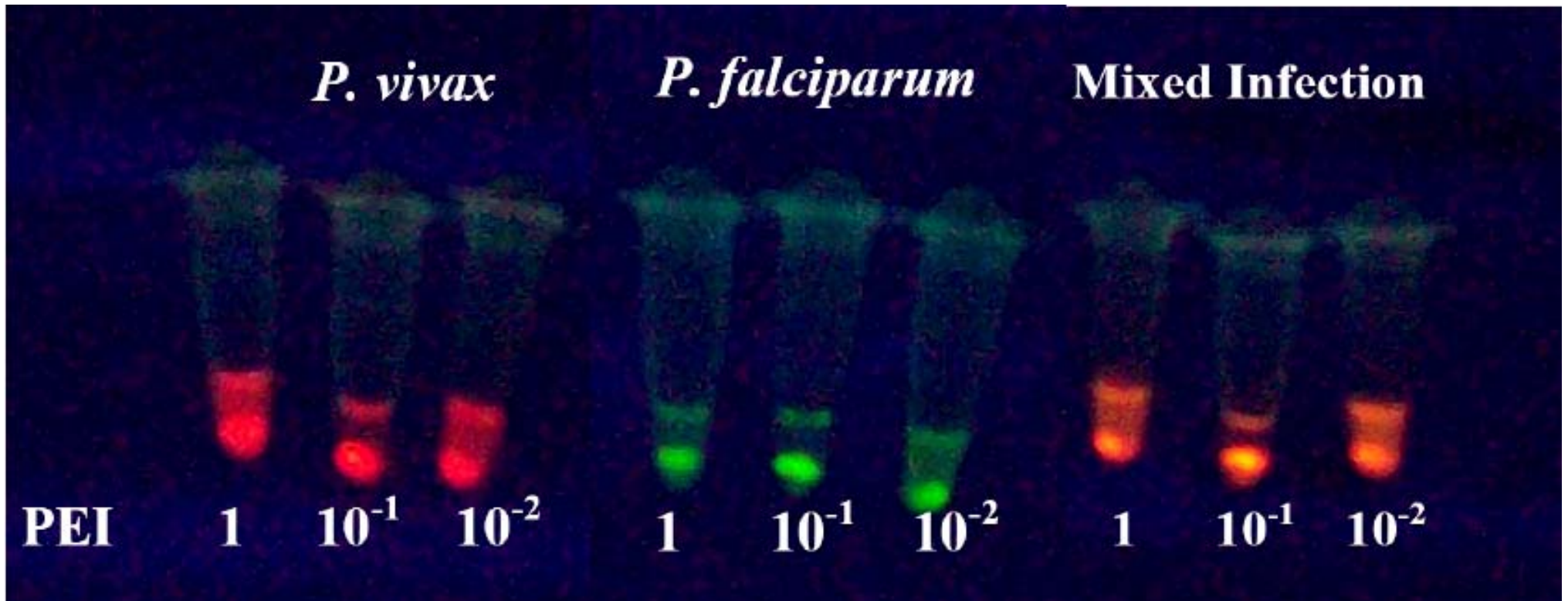
2) not known which stages

Multiplex LAMP



For the diagnosis of *P. falciparum* and *P. vivax* infections

Determination of best concentration of PEI for *Plasmodium* multiplex LAMP



THANK YOU

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