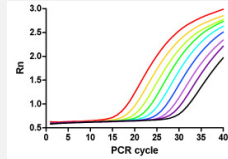
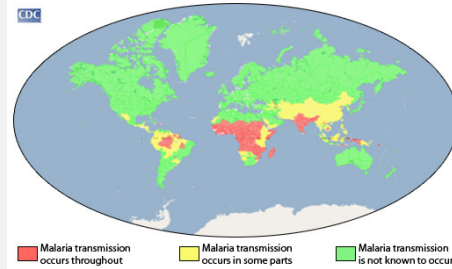
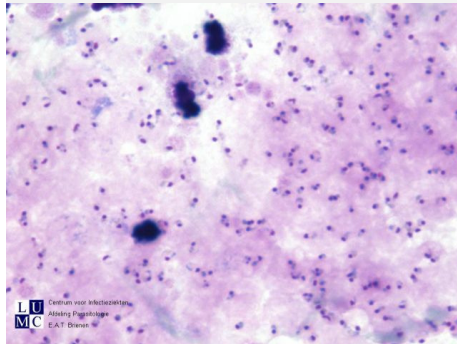


Molecular diagnostics malaria



Malaria; nieuwe diagnostische methoden

J.J. Verweij



Future?

- Antigen test 15 minutes



- DNA isolation 1-6 samples 15-20 minutes

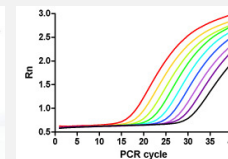


- Reduce Your Run Times to Less Than 40 Minutes



Molecular diagnostics malaria

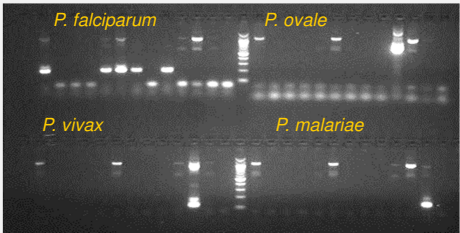
- Evolution of PCR
 - Some technical aspects
 - Quantification
 - Sensitivity!
- Loop-mediated isothermal amplification (LAMP)
- Conclusions
- Future perspectives



MOLBIO 01934

Identification of the four human malaria parasite species in field samples
by the polymerase chain reaction and detection
of a high prevalence of mixed infections

Georges Snounou^a, Suganya Viriyakosol^{a*}, William Jarra^a,
Sodsri Thaithong^b and K. Neil Brown^a



Nested PCR targeting SSU rRNA gen

MOLBIO 01934

Identification of the four human malaria parasite species in field samples
by the polymerase chain reaction and detection
of a high prevalence of mixed infections

Georges Snounou^a, Suganya Viriyakosol^{a*}, William Jarra^a,
Sodsri Thaithong^b and K. Neil Brown^a

26-02-2002 Malaria differentiatie nested PCR (Snounou et al)

2500553 Microscopisch P. ovale, geen amplificatie in geen van de vier specifieke PCR's
wel plasmodium bandje.

Sequencing of (partial) SSU rRNA gen

NOTES

Sequence Variation in the 18S rRNA gene, a Target for
PCR-Based Malaria Diagnosis, in Plasmodium ovale
from Southern Vietnam

FUMIHIKO KAWAMOTO,^{1*} HIROFUMI MIYAKE,² OSAMU KANEKO,³ MASATSUGU KIMURA,⁴
NGUYEN THI DUNG,⁵ NGUYEN THE DUNG,⁵ QING LIU,¹ MIAN ZHOU,¹ LE DUC DAO,⁶
SHINTARO KAWAI,⁷ SHIN ISOMURA,¹ AND YUSUKE WATAYA²

Development of a Real-Time PCR Assay for Detection of Plasmodium
falciparum, Plasmodium vivax, and Plasmodium ovale
for Routine Clinical Diagnosis

F. Perandin,¹ N. Manca,^{1*} A. Calderaro,² G. Piccolo,² L. Galati,² L. Ricci,³ M. C. Medici,²
M. C. Arcangeletti,² G. Snounou,⁴ G. Dettori,² and C. Chezzi²

P. vivax, and P. ovale 18S rRNA gene, in accordance with the sequences quoted
by Snounou et al. (23).

Plasmodium species specific PCR



- Multiplex real-time PCR
- *Plasmodium*-species-specific forward primers and *Plasmodium*-specific reverse primer
- An approx. 150-bp fragment *Plasmodium*-specific 18S sequences
- *Plasmodium*-species-specific detection probes
- Detection limit 20 parasites/mL (tested for *P. falciparum*)

1. Adegnika, A. A., J. J. Verweij, S. T. Agnandji, S. K. Chai, L. P. Breitling, M. Ramharter, M. Frolich, S. Issifou, P. G. Kremsner, and M. Yazdanbakhsh. 2006. Microscopic and sub-microscopic *Plasmodium falciparum* infection, but not inflammation caused by infection, is associated with low birth weight. *Am J Trop Med Hyg* **75**:798-803.
2. Muller-Stover, I., J. J. Verweij, B. Hoppenheit, K. Gobels, D. Haussinger, and J. Richter. 2008. *Plasmodium malariae* infection in spite of previous anti-malarial medication. *PARASITOL.RES.* **102**:547-550.
3. Niesters, H. G. 2002. Clinical virology in real time. *J.Clin.Virol.* **25 Suppl 3**:S3-12.
4. Rougemont, M., M. Van Saanen, R. Sahli, H. P. Hinrikson, J. Bille, and K. Jaton. 2004. Detection of four *Plasmodium* species in blood from humans by 18S rRNA gene subunit-based and species-specific real-time PCR assays. *J Clin Microbiol* **42**:5636-5643.
5. Shokoples, S. E., M. Ndao, K. Kowalewska-Grochowska, and S. K. Yanow. 2009. Multiplexed real-time PCR assay for discrimination of *Plasmodium* species with improved sensitivity for mixed infections. *J Clin Microbiol* **47**:975-980.
6. Wiria, A. E., M. A. Prasetyani, F. Hamid, L. J. Wammes, B. Lell, I. Ariawan, H. W. Uh, H. Wibowo, Y. Djuardi, S. Wahyuni, I. Sutanto, L. May, A. J. Luty, J. J. Verweij, E. Sartono, M. Yazdanbakhsh, and T. Supali. 2010. Does treatment of intestinal helminth infections influence malaria? Background and methodology of a longitudinal study of clinical, parasitological and immunological parameters in Nangapanda, Flores, Indonesia (ImmunoSPIN Study). *BMC Infect Dis* **10**:77.



Improved malaria real-time PCR (1,2,4-6)

Plasmodium primers (1,2,5,6)

PFal-F	5'-CCG ACT AGG TGT TGG ATG AAA GTG TTA A-3'	M19172
PViv-F	5'-CCG ACT AGG CTT TGG ATG AAA GAT TTT A-3'	X13926
POva1-F	5'-CCGACTAGGTTTTGGATGAAAGATTTTT-3'	L48986/L48987
POva2-F	5'-CCAACTAGGTTTTGGATGAAAAGTTTTT-3'	X99790/J001527
PMal-F	5'-CCGACTAGGTGTTGGATGATAGAGTAAA-3'	M54897
Plas-171R	5'-AACCCAAAGACTTTGATTCTCATAA-3'	All above

P. falciparum(1,2,6)

XS_Pfal114	Yakima Yellow 5'-CTTTCGAGGTGACTTTTAGAT-3' XSQ	M19172
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P. vivax(2,6)

XS_vivax133	FAM 5'-TTTTCTCTCGGAGTTTATT-3' XSQ	X13926
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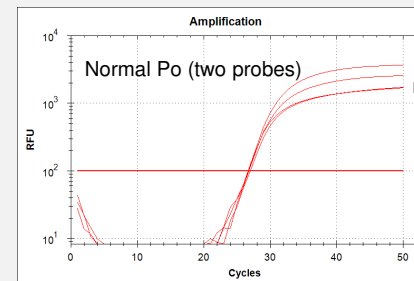
P. ovale

XS_oval90(2,6)	Texas Red 5'-CCCAGAAAGGAATTTCTTATT-3' XSQ	L48986/L48987
XS_oval90-type2(6)	Texas Red 5'-TCCAAAAGGAATTTCTTATT-3'XSQ	X99790/J001527

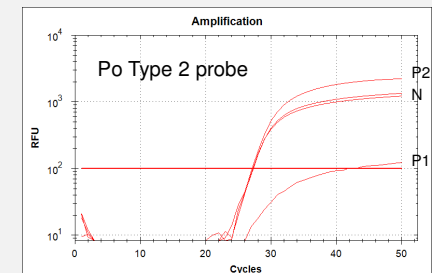
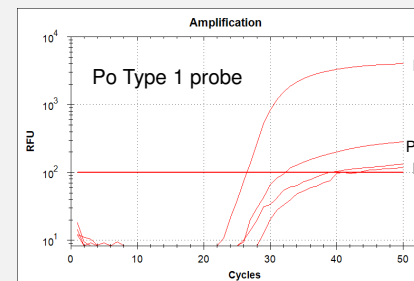
P. malariae(2,6)

XS_malariae89	Quasar705 5'-AGCTATCTAAAGAAACACTCAT-3' XSQ	M54897
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Different results with different *P. ovale* probes



N: Nijmegen in duplo



Two Nonrecombining Sympatric Forms of the Human Malaria Parasite *Plasmodium ovale* Occur Globally

Colin J. Sutherland,^{1,2,3} Naowarat Tanomsing,⁴ Debbie Nolder,¹ Mary Oguike,² Charlie Jennison,² Sasithon Pukrittayakamee,^{5,6} Christiane Dolecek,¹⁰ Tran Tinh Hien,¹⁰ Virgilio E. do Rosário,¹¹ Ana Paula Arez,¹¹ João Pinto,¹¹ Pascal Michon,¹² Ananias A. Escalante,¹³ Francois Nosten,^{4,13} Martina Burke,¹ Rogan Lee,¹⁵ Marie Blaze,¹ Thomas Dan Otto,⁴ John W. Barnwell,¹⁴ Arnab Pain,¹ John Williams,¹ Nicholas J. White,^{1,7} Nicholas P. J. Day,^{1,7} Georges Snounou,^{16,17,18} Peter J. Lockhart,¹⁹ Peter L. Chiodini,^{1,3} Mallika Imwong,⁹ and Spencer D. Polley²

- Type 1: *P. ovale curtisi* Christopher F. Curtis (1939–2008)
- Type 2: *P. ovale wallikeri* David Walliker (1940–2007)

An observational study of malaria in British travellers: *Plasmodium ovale wallikeri* and *Plasmodium ovale curtisi* differ significantly in the duration of latency

Debbie Nolder,¹ Mary C Oguike,² Hector Maxwell-Scott,^{2,3} Hatoon A Niyazi,² Valerie Smith,¹ Peter L Chiodini,^{1,3} Colin J Sutherland^{1,2,3}

The geometric mean latency period in *P. ovale wallikeri* was 40.6 days (95% CI 28.9 to 57.0), whereas that for *P. ovale curtisi* was more than twice as long at 85.7 days (95% CI 66.1 to 111.1; $p=0.002$).



MINIREVIEW

Recent Advances in Detection of *Plasmodium ovale*: Implications of Separation into the Two Species *Plasmodium ovale wallikeri* and *Plasmodium ovale curtisi*

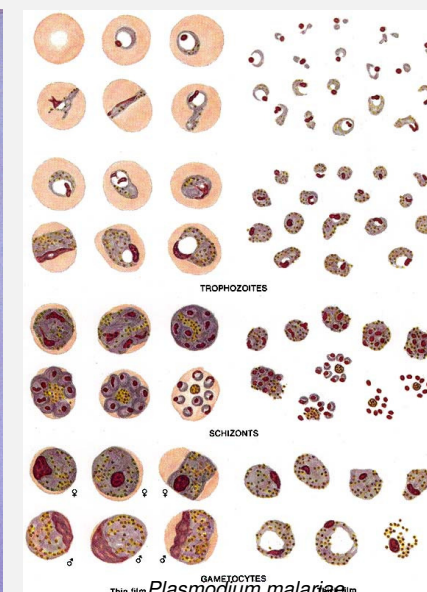
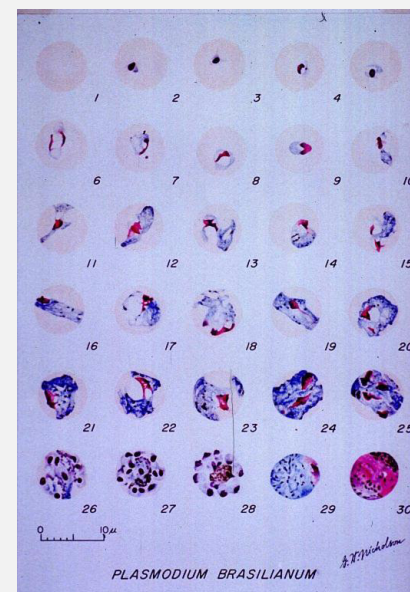
Hans-Peter Fuehrer,^a Harald Noedl^b

Institute of Parasitology, Department of Pathobiology, University of Veterinary Medicine Vienna, Vienna, Austria^a; Institute of Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Vienna, Austria^b

Cross-reaction *P. malariae* PCR with other *Plasmodium* species?

- NCBI blast search probe sequence (18S)
 - *P. malariae*
 - *P. malariae* type 1
 - *P. malariae* type 2
 - *P. brasilianum*

(1316) 1316 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420
 Pbras_AF130735 (530) CACTAGGTTGGATGATAGAGTAAAAATAAAGGACATTGATATATAGAGTGTTCCTTTAGATAGCTTCCTTCAGTACCTTATGAGAAATCAAAGCTTTGGGTT
 Pm_1_AF487999(1141) CACTAGGTTGGATGATAGAGTAAAAATAAAGGACATTGATATATAGAGTGTTCCTTTAGATAGCTTCCTTCAGTACCTTATGAGAAATCAAAGCTTTGGGTT
 Pm_AB489195(1068) CACTAGGTTGGATGATAGAGTAAAAATAAAGGACATTGATATATAGAGTGTTCCTTTAGATAGCTTCCTTCAGTACCTTATGAGAAATCAAAGCTTTGGGTT
 Pm_2_AF488000(1141) CACTAGGTTGGATGATAGAGTAAAAATAAAGGACATTGATATATAGAGTGTTCCTTTAGATAGCTTCCTTCAGTACCTTATGAGAAATCAAAGCTTTGGGTT
 Pm_AF145336 (530) CACTAGGTTGGATGATAGAGTAAAAATAAAGGACATTGATATATAGAGTGTTCCTTTAGATAGCTTCCTTCAGTACCTTATGAGAAATCAAAGCTTTGGGTT
 Pm_M54897(1312) CACTAGGTTGGATGATAGAGTAAAAATAAAGGACATTGATATATAGAGTGTTCCTTTAGATAGCTTCCTTCAGTACCTTATGAGAAATCAAAGCTTTGGGTT
 Pma89_XS_Q705 (1) CACTAGGTTGGATGATAGAGTAAAAATAAAGGACATTGATATATAGAGTGTTCCTTTAGATAGCTTCCTTCAGTACCTTATGAGAAATCAAAGCTTTGGGTT
 Pma89_XS_Q705 (3) CACTAGGTTGGATGATAGAGTAAAAATAAAGGACATTGATATATAGAGTGTTCCTTTAGATAGCTTCCTTCAGTACCTTATGAGAAATCAAAGCTTTGGGTT
 Pma89_XS_Q705 (1) CACTAGGTTGGATGATAGAGTAAAAATAAAGGACATTGATATATAGAGTGTTCCTTTAGATAGCTTCCTTCAGTACCTTATGAGAAATCAAAGCTTTGGGTT



The Microscopical Diagnosis of Human Malaria, 1963, Field et al

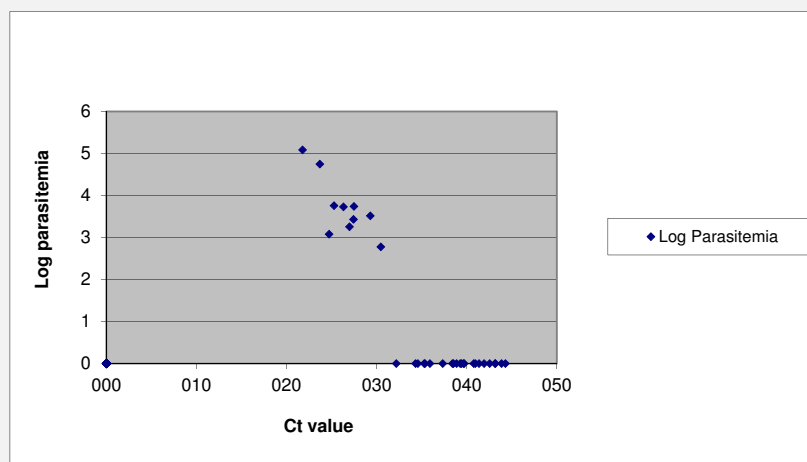
- Malaria parasites of primates resembling *P. malariae*
 - *P. hylobati* (Java gibbon)
 - *P. inui* (Asian macaque)
 - *P. giarardi* (Madagascar lemur)
 - *P. brasilianum* (Brazilian monkey)
- Malaria parasites of man through mosquitoes to monkeys or apes
 - *P. malariae* (Laveran, 1881)
- Malaria parasites of lower monkeys through mosquitoes to man
 - *P. brasilianum* (Gender & von Berenberg-Gossler, 1908)

Monkeys of the rainforest in French Guiana are natural reservoirs for *P. brasilianum*/*P. malariae* malaria
Parasitology (2000), **120**, 11–21

T. FANDEUR^{1*}, B. VOLNEY¹, C. PENEAU² and B. DE THOISY³

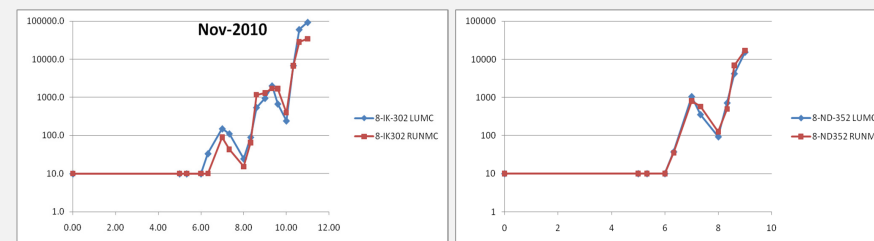
- The high degree of DNA homology in the sequences of the SSUrRNA and *msp1*-like genes is consistent with other characterizations demonstrating a taxonomic relationship between *P. brasilianum* and *P. malariae* species.
- Our results provide further evidence that *P. brasilianum* and *P. malariae* are virtually identical and should probably be considered to be a single malaria species.
- *P. malariae* = *P. brasilianum*

Real-time *P. falciparum* PCR in pregnant women from Gabon (n=140)



Akim Adegnika, Jaco J. Verweij et al

Malaria PCR in human volunteers studies



Rob Hermesen et al

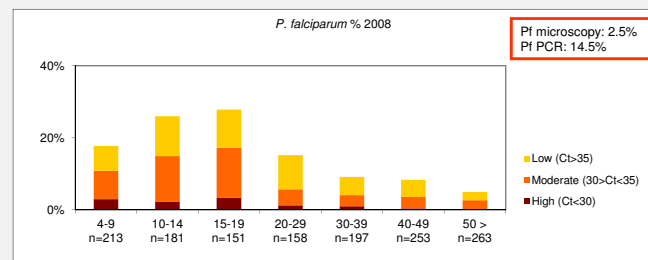
Method	sensitivity		
Thick blood film (expert)	50000 parasites/ml		0.001%
Thick blood film (routine labs UK)*	500000 parasites/ml		0.01%
PCR (LUMC/Nijmegen)	20-50 parasites/ml		0.0000002%
* Milne et al.			

RESEARCH

Open Access

Epidemiology of *Plasmodium* infections in Flores Island, Indonesia using real-time PCR

Maria MM Kaisar^{1,2}, Taniawati Supali¹, Aprilianto E Wiria^{1,2}, Firdaus Hamid^{1,3}, Linda J Wammes², Erliyani Sartono², Adrian JF Luty⁴, Eric AT Brienen², Maria Yazdanbakhsh², Lisette van Lieshout^{2*} and Jaco J Verweij^{2,5}

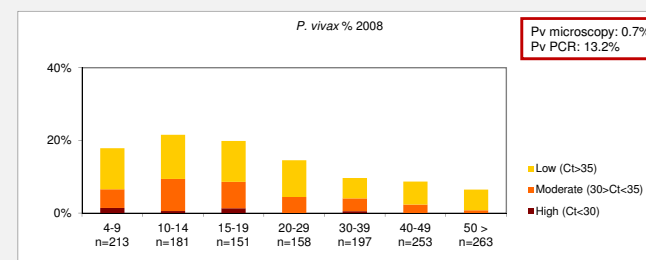


RESEARCH

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Epidemiology of *Plasmodium* infections in Flores Island, Indonesia using real-time PCR

Maria MM Kaisar^{1,2}, Taniawati Supali¹, Aprilianto E Wiria^{1,2}, Firdaus Hamid^{1,3}, Linda J Wammes², Erliyani Sartono², Adrian JF Luty⁴, Eric AT Brienen², Maria Yazdanbakhsh², Lisette van Lieshout^{2*} and Jaco J Verweij^{2,5}



Immunobiology 218 (2013) 706–711



Contents lists available at SciVerse ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio



Parasitic infections and immune function: Effect of helminth infections in a malaria endemic area

Anna G.C. Boef^a, Linda May^b, David van Bodegom^a, Lisette van Lieshout^b, Jaco J. Verweij^{b,1}, Andrea B. Maier^a, Rudi G.J. Westendorp^a, Ulrika K. Eriksson^{a,*}

Malaria PCR n=610 (% positives)

<i>P. falciparum</i>	85.6
<i>P. ovale</i>	4.3
<i>P. malariae</i>	9.2
<i>P. vivax</i>	0

85.6% of apparently healthy participants testing positive
All participants under 30 years of age testing positive

Multiplex real-time PCR for diagnosing malaria in a non-endemic setting: a prospective comparison to conventional methods

RHT Nijhuis, L. van Lieshout, JJ Verweij, ECJ Claas, E Wessels

EJCMID in press

	PCR +	TS +	TS -	QBC +	QBC -	RDT +	RDT -
Pf	39	35	4	34	5	37	2
Pv	6	6	0	6	0	5	1
Po	7	7	0	7	0	1	6
Pm	4	2	2	2	2	1	3
Total	56	50	6	49	7	44	12

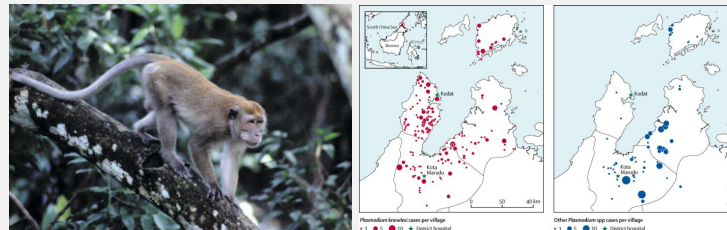
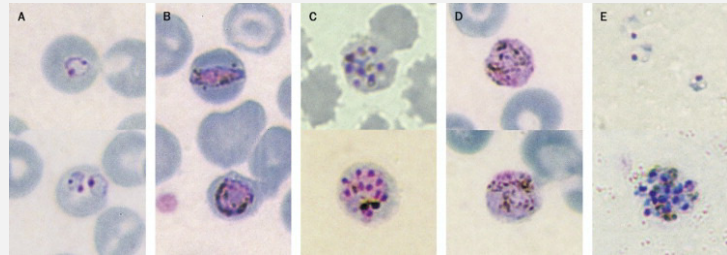
PCR negative/RDT+ n=6 (one patient after treatment, five no history of malaria)

PCR negative and other methods positive n=1 (one patient after treatment)

PCR negative and other methods negative n=776

A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings

Balbir Singh, Lee Kim Sung, Asmad Matusop, Anand Radhakrishnan, Sunita S G Shamsul, Janet Cox-Singh, Alan Thomas, David J Conway



THE LANCET • Vol 363 • March 27, 2004 • www.thelancet.com

www.thelancet.com/planetary-health Vol 1 June 2017

ETZ Multiplex real-time malaria PCR

P. falciparum, *P. vivax*, *P. ovale* c/w, *P. malariae*, *P. knowlesi*, PhHV

1. Adegnika AA, Verweij JJ, Agnandji ST, Chai SK, Breitling LP, Ramharther M, Frolich M, Issifou S, Kremsner PG, Yazdanbakhsh M. 2006. Microscopic and sub-microscopic *Plasmodium falciparum* infection, but not inflammation caused by infection, is associated with low birth weight. Am. J. Trop. Med. Hyg. 75:798-803.
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3. Wiria AE, Prasetyani MA, Hamid F, Wammes LJ, Lell B, Ariawan I, Uh HW, Wibowo H, Djuardi Y, Wahyuni S, Sutanto I, May L, Luty AJ, Verweij JJ, Sartono E, Yazdanbakhsh M, Supali T. 2010. Does treatment of intestinal helminth infections influence malaria? background and methodology of a longitudinal study of clinical, parasitological and immunological parameters in nangapanda, flores, indonesia (ImmunoSPIN study). BMC Infect. Dis. 10:77-2334-10-77.
4. Rougemont M, Van Saanen M, Sahli R, Hinrikson HP, Bille J, Jatou K. 2004. Detection of four plasmodium species in blood from humans by 18S rRNA gene subunit-based and species-specific real-time PCR assays. J. Clin. Microbiol. 42:5636-5643.
5. Shokoples SE, Ndao M, Kowalewska-Grochowska K, Yanow SK. 2009. Multiplexed real-time PCR assay for discrimination of plasmodium species with improved sensitivity for mixed infections. Journal of Clinical Microbiology. 47:975-980.
6. Bauffe F, Desplans J, Fraissier C, Parzy D. 2012. Real-time PCR assay for discrimination of plasmodium ovale curtisi and plasmodium ovale wallikeri in the ivory coast and in the comoros islands. Malaria Journal. 11:307.
7. Divis PC, Shokoples SE, Singh B, Yanow SK. 2010. A TaqMan real-time PCR assay for the detection and quantitation of plasmodium knowlesi. Malar J. 9:344-2875-9-344. doi: 10.1186/1475-2875-9-344; 10.1186/1475-2875-9-344.
8. Van den Bijllaardt W, Ritmeester W, Verweij JJ. Multiplex real-time malaria PCR, Unpublished.

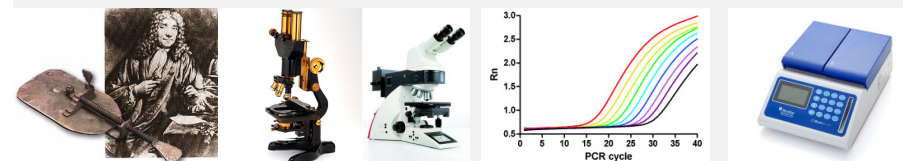
Validation in-house real-time PCR

	In-house real-time PCR						
	Pf	Pf/Pm	Pv	Poc/w	Pm	Pk	neg
Micr/PCR							
Pf	17						
Pf/Pm*		3					
Pv			9				
Po				4/0			
Pm					2		
Pk						5	
Neg/dub							13

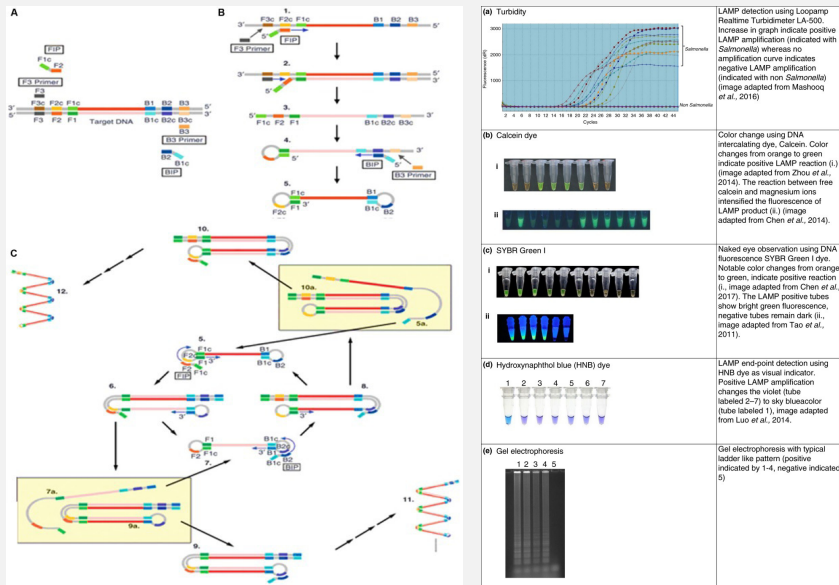
* Mixed infection PCR only

Molecular diagnostics malaria

- ✓ Evolution of PCR
 - Some technical aspects
 - Quantification
 - Sensitivity!
- Loop-mediated isothermal amplification (LAMP)
- Conclusions
- Future perspectives



loop-mediated isothermal amplification (LAMP)



magnesium pyrophosphate, $Mg_2P_2O_7$

Malaria LoopAMP®

OPEN ACCESS Freely available online

PLOS ONE

Loop Mediated Isothermal Amplification (LAMP) Accurately Detects Malaria DNA from Filter Paper Blood Samples of Low Density Parasitaemias

Berit Aydin-Schmidt^{1,8,*}, Weiping Xu^{1,9}, Iveth J. González², Spencer D. Polley^{3,4}, David Bell⁵, Delér Shakely¹, Mwinyi I. Msellem⁷, Anders Björkman¹, Andreas Mårtensson^{1,6,9}

Morris et al. *Malaria Journal* (2015) 14:205
DOI:10.1186/s12936-015-0731-2

MALARIA JOURNAL

RESEARCH

Open Access

Field deployment of loop-mediated isothermal amplification for centralized mass-screening of asymptomatic malaria in Zanzibar: a pre-elimination setting

Ulrika Morris¹, Mwinyi Khamsi², Berit Aydin-Schmidt¹, Ali K Abass², Mwinyi I Msellem⁷, Majda H Nassor², Iveth J González², Andreas Mårtensson^{1,5}, Abdullah S Ali², Anders Björkman¹ and Jackie Cook^{1,6}

Cuadros et al. *Malar J* (2017) 16:20
DOI:10.1186/s12936-016-1608-8

Malaria Journal

RESEARCH

Open Access

LAMP kit for diagnosis of non-falciparum malaria in *Plasmodium ovale* infected patients

Juan Cuadros¹, Alexandra Martín Ramírez², Iveth J. González², Xavier C. Ding³, Ramon Perez Tanoira^{1,5}, Gerardo Rojo-Marcos¹, Peña Gómez-Herruz² and Jose Miguel Rubio²

Serna et al. *Malaria Journal* (2010) 14:44
DOI:10.1186/12936-015-0559-9

MALARIA JOURNAL

RESEARCH

Open Access

Evaluation of non-instrumented nucleic acid amplification by loop-mediated isothermal amplification (NINA-LAMP) for the diagnosis of malaria in Northwest Ethiopia

Mesio Serna¹, Abebe Alemu², Abebe Genetu Bayih¹, Siyay Getie³, Gebeyaw Getnet⁴, Dylan Guelig⁵, Robert Burton⁶, Paul LaBarre⁷ and Dylan R Pillai^{1,8}

Diagnostic Microbiology and Infectious Disease 91 (2016) 149–155

Diagnostic Microbiology and Infectious Disease
journal homepage: www.elsevier.com/locate/diagmicrobio

Parasitology

NINA-LAMP compared to microscopy, RDT, and nested PCR for the detection of imported malaria

Abu Naser Mohon^{1,8}, Lydia Da-Yeong Lee¹, Abebe Genetu Bayih¹, Asongna Folefac¹, Dylan Guelig⁵, Robert A. Burton⁶, Paul LaBarre⁷, Wilson Chan¹, Bonnie Meathrell¹, Dylan R. Pillai^{1,2,3,4,5,6,7,8}

OPEN ACCESS Freely available online

PLOS ONE

Loop Mediated Isothermal Amplification (LAMP) Accurately Detects Malaria DNA from Filter Paper Blood Samples of Low Density Parasitaemias

Berit Aydin-Schmidt^{1,8,*}, Weiping Xu^{1,9}, Iveth J. González², Spencer D. Polley^{3,4}, David Bell⁵, Delér Shakely¹, Mwinyi I. Msellem⁷, Anders Björkman¹, Andreas Mårtensson^{1,6,9}

Fever patients ABI-extracted DNA (n = 865)

	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Kappa value
Pan-LAMP	91.5 (84.8–95.8)	100 (99.5–100)	100 (96.6–100)	98.7 (97.6–99.4)	0.95
Pf-LAMP	86.3 (78.7–92.0)	100 (99.5–100)	100 (96.4–100)	97.9 (96.6–98.8)	0.92

Fever patients: including Chelex re-extracted DNA (n = 823+42)

	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Kappa value
Pan-LAMP	98.3 (94.0–99.8)	100 (99.5–100)	100 (96.8–100)	99.7 (99.0–100)	0.99
Pf-LAMP	98.3 (94.0–99.8)	100 (99.5–100)	100 (96.8–100)	99.7 (99.0–100)	0.99

Asymptomatic individuals Chelex extracted DNA (n = 465)

	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Kappa value
Pan-LAMP all species	90.7 (79.7–96.9)	100 (99.1–100)	100 (92.7–100)	98.8 (97.2–99.6)	0.95
Pan-LAMP <i>P.f.</i> *	97 (84.2–99.9)	100 (99.1–100)	100 (89.1–100)	99.8 (98.7–100)	0.97
Pan-LAMP <i>P.m.</i> **	76.9 (46.2–95)	100 (99.1–100)	100 (69.2–100)	99.3 (97.9–99.9)	0.89
Pf-LAMP <i>P.f.</i> ***	92.7 (80.1–98.5)	100 (99.1–100)	100 (90.7–100)	99.3 (98.0–99.9)	0.96

gold standard (real- time PCR corrected Cytochrome b nested PCR)

OPEN ACCESS Freely available online

PLOS ONE

Loop Mediated Isothermal Amplification (LAMP) Accurately Detects Malaria DNA from Filter Paper Blood Samples of Low Density Parasitaemias

Berit Aydin-Schmidt^{1,8,*}, Weiping Xu^{1,9}, Iveth J. González², Spencer D. Polley^{3,4}, David Bell⁵, Delér Shakely¹, Mwinyi I. Msellem⁷, Anders Björkman¹, Andreas Mårtensson^{1,6,9}

Table 3. Asymptomatic individuals with discordant results.

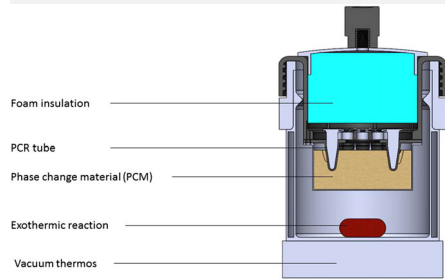
ID	Parasites/μL*	Pan LAMP	Pf LAMP	Nested PCR	Real-time PCR
1	3	+	+	-	Pf
2	3	+	+	-	Pf
3	10	+	+	Pm	Pf/Pm
4	<1	+	+	-	Pf
5	5	-	-	Pf/Pm	Pf/Pm
6	5	+	+	Pm	Pf/Pm
7	2	-	-	Pm	Pm
8	3	+	+	Pm	Pf/Pm
9	1	+	-	-	Pf
10	2	-	-	Pf	Pf
11	1	-	-	Pm	Pm
12	2	-	-	Pm	Pm



Parasitology

NINA-LAMP compared to microscopy, RDT, and nested PCR for the detection of imported malaria

Abu Naser Mohon ^{a,b}, Lydia Da-Yeong Lee ^a, Abebe Genetu Bayih ^b, Asongna Folefoc ^b, Dylan Guelig ^c, Robert A. Burton ^c, Paul LaBarre ^c, Wilson Chan ^b, Bonnie Meatherall ^d, Dylan R. Pillai ^{a,b,d,*}



Sensitivity and specificity of LAMP and BinaxNOW in comparison with microscopy and nested PCR.

Test		Nested PCR	
		Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
LAMP	Overall	100 (93.6–100)	98.6 (91.1–99.9)
	<i>P. falciparum</i>	97.6 (85.9–99.9)	100 (95.3–100)
	Non- <i>falciparum</i>	100 (86.3–100)	99.1 (94.3–100)
BinaxNOW	Overall	85.9 (75.2–92.7)	98.6 (91.1–99.9)
	<i>P. falciparum</i>	90.5 (76.5–96.9)	100 (95.3–100)
	Non- <i>falciparum</i>	71.0 (51.8–85.1)	99.1 (94.3–100)

P. falciparum lower limit of 5 parasites/μL for both Pan and Pf primer sets, for *P. vivax* it was 1 parasite/μL

After 40 minutes, turbidity was measured by naked eye by 3 independent readers.

OPEN Evaluation of the *illumigene* Malaria LAMP: A Robust Molecular Diagnostic Tool for Malaria Parasites

Received: 25 May 2016
Accepted: 22 October 2016
Published: 09 November 2016

Naomi W. Luchini^{1,2}, Maria Gayer^{1,2}, Mammadou Alpha Diallo¹, Ira F. Goldman¹,
Grapan Lejeune¹, Awa Imeta Dieme¹, Awa Ibrahima¹, Yaya Die Niaye¹, John W. Barnwell¹,
Venkatesh Kumar¹ & Danda Nalayi¹

De Koning et al. *Malar J* (2017) 16:118
DOI:10.1186/s13051-017-1065-8

Malaria Journal

RESEARCH

Open Access

Diagnostic performance of the loop-mediated isothermal amplification (LAMP) based *illumigene*® malaria assay in a non-endemic region

Anne-Sophie De Koning^{1,2}, Lieslotte Knops¹, Mattias Hofmans¹, Jan Jacobs¹, Lorian Van den Bossche² and Jan Philipp^{1,2}

Journal of
Clinical Microbiology®

PARASITOLOGY

Detection of *Plasmodium* Infection by the *illumigene* Malaria Assay Compared to Reference Microscopy and Real-Time PCR

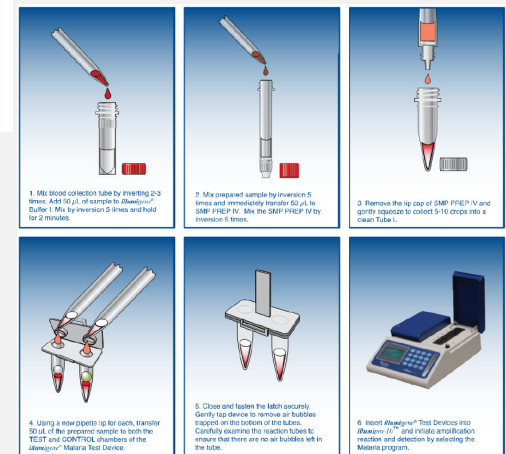
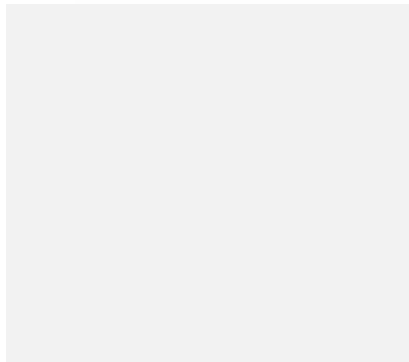
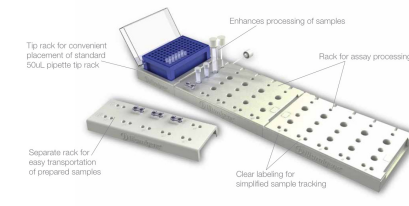
Cecile Ruyssen¹, Barbara Chow¹, Wilson W. Chan^{1,2}, Deirdre L. Church^{1,2},
Dylan R. Pillai^{1,2}

Contents lists available at ScienceDirect
Travel Medicine and Infectious Disease

journal homepage: www.elsevier.com/locate/ymit

Evaluation of automated loop-mediated amplification (LAMP) for routine malaria detection in blood samples of German travelers – A cross-sectional study

Hagen Frickmann^{1,2}, Rebecca Hinz¹, Sandra Rojak¹, Ina Bonow¹, Stefanie Rüger¹, Christine Wegner¹,
Iris Zeller¹, Ralf Matthias Hagen¹, Egbert Tannich¹



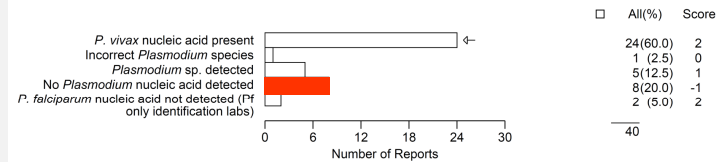
Commercial available, good sensitivity and specificity
Point of care test? No species identification, no quantification.

		Positive LAMP result	Negative LAMP result
Truly positive samples	235	3	
Truly negative samples	3	759	
Sensitivity	Specificity	Positive predictive value	Negative predictive value
98.7%	99.6%	98.7%	99.6%

Verification ETZ Illumigene Malaria

	In-house real-time PCR					
	Pf	Pv	Poc/w	Pm	Pk	neg
Illumigene						
Positief	7	4	1	2	3	
Neg		1				5

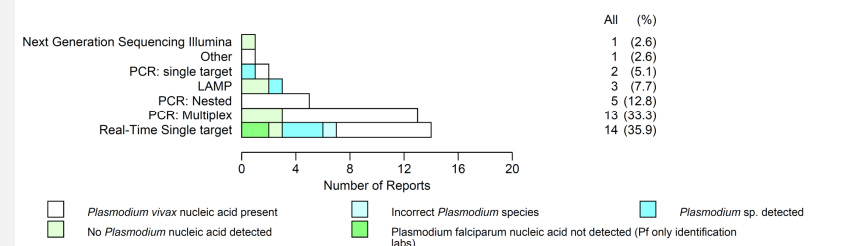
Specimen : 4262 *Plasmodium vivax*: 1,000 parasites/mL Overall Results



Verification ETZ Illumigene Malaria

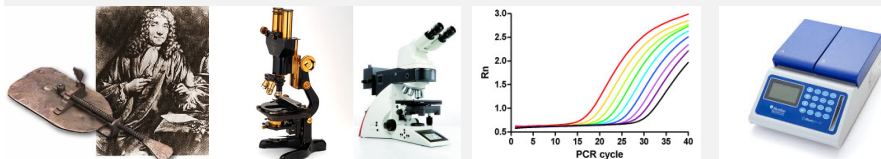
	In-house real-time PCR					
	Pf	Pv	Poc/w	Pm	Pk	neg
Illumigene						
Positief	7	4	1	2	3	
Neg		1				5

Specimen : 4262 *Plasmodium vivax*: 1,000 parasites/mL Detection Method 1



Molecular diagnostics malaria

- ✓ Evolution of PCR
 - Some technical aspects
 - Quantification
 - Sensitivity!
- ✓ Loop-mediated isothermal amplification (LAMP)
- Conclusions
- Future perspectives

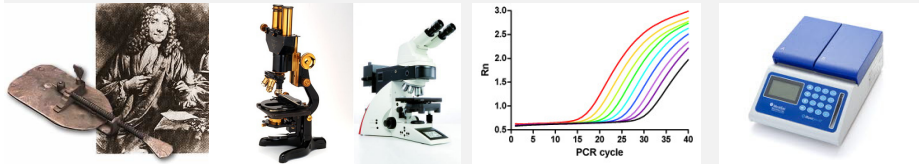


Conclusions

- DNA-based diagnostics highly sensitive and specific
- Know your assay
 - What does it detect
 - What does it miss
 - Regular updates
- LAMP
 - High NPV
 - No species differentiation (Illumigene)
 - No quantification

Molecular diagnostics malaria

- ✓ Evolution of PCR
 - Some technical aspects
 - Quantification
 - Sensitivity!
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- ✓ Conclusions
- Future perspectives



Novel Mutation in Cytochrome B of *Plasmodium falciparum* in One of Two Atovaquone-Proguanil Treatment Failures in Travelers Returning From Same Site in Nigeria

Mateusz M. Plucinski,^{1,2} Curtis S. Huber,¹ Sheila Akinyi,¹ Willard Dalton,³ Mary Eschete,³ Katharine Grady,¹ Luciana Silva-Flannery,¹ Blaine A. Mathison,¹ Venkatachalam Udhayakumar,¹ Paul M. Arguin,¹ and John W. Barnwell¹

Failure of atovaquone-proguanil malaria chemoprophylaxis in a traveler to Ghana



Andrea K. Boggild^{a,b,c,*}, Rachel Lau^c, Denis Reynaud^d, Kevin C. Kain^{a,b,e}, Marvin Gerson^f

Molecular surveillance of artemisinin resistance *falciparum* malaria among migrant goldmine workers in Myanmar



Myat Htut Nyunt^{1,2†}, Bo Wang^{1,3†}, Khin Myo Aye², Kyin Hla Aye², Jin-Hee Han¹, Seong-Kyun Lee¹, Kay Thwe Han², Ye Htut² and Eun-Taek Han^{1*}

Ta et al. *Malaria Journal* 2014, **13**:68
http://www.malariajournal.com/content/13/1/68

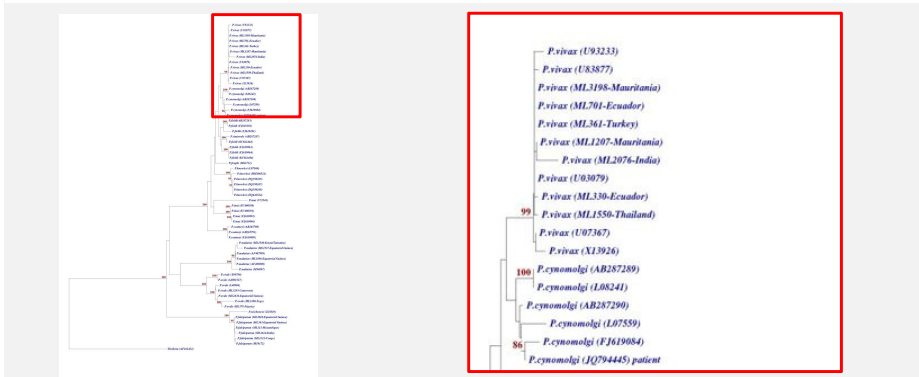


CASE REPORT

Open Access

First case of a naturally acquired human infection with *Plasmodium cynomolgi*

Thuy H Ta¹, Shamilah Hisam², Marta Lanza¹, Adela I Jiram², NorParina Ismail² and José M Rubio^{1*}



Ta et al. *Malaria Journal* 2014, **13**:68
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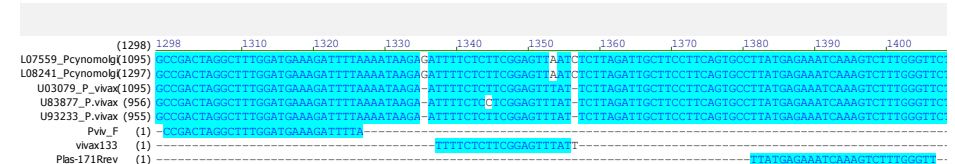


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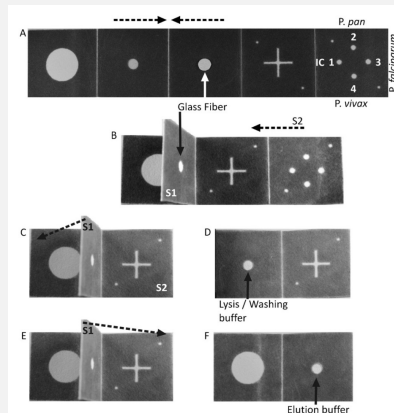
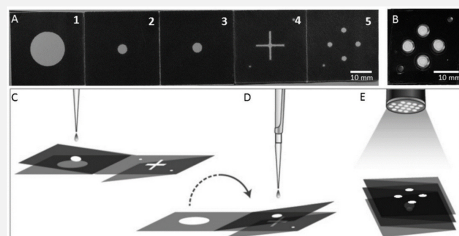


Malaria Diagnostics

International Edition: DOI: 10.1002/anie.201606060
 German Edition: DOI: 10.1002/ange.201606060

Paper-Origami-Based Multiplexed Malaria Diagnostics from Whole Blood

Gaolian Xu[†], Debbie Nolder[†], Julien Reboud, Mary C. Oguike, Donnelly A. van Schalkwyk, Colin J. Sutherland, and Jonathan M. Cooper^{*†}



Future of malaria diagnostics

Future?

- Antigen test 15 minutes



- DNA isolation 1-6 samples 15-20 minutes



- Reduce Your Run Times to Less Than 40 Minutes



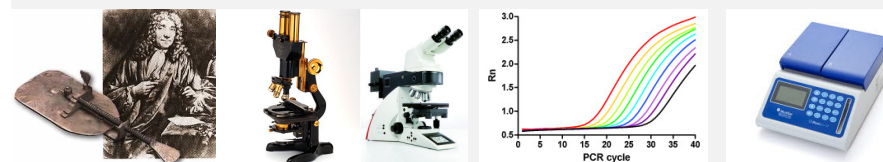
LU Center of Infectious Diseases
 MC Department of Parasitology

Future of malaria diagnostics

- Microscopy
- RDT and microscopy
- LAMP and microscopy
 - LAMP negative
 - LAMP positive and microscopy
- Fast PCR
 - It's a matter of logistics

Molecular diagnostics malaria

- ✓ Evolution of PCR
 - Some technical aspects
 - Quantification
 - Sensitivity!
- ✓ Loop-mediated isothermal amplification (LAMP)
- ✓ Conclusions
- ✓ Future perspectives



Molecular diagnostics malaria

Thanks for listening

Questions?

Jaco J. Verweij
j.verweij@etz.nl

