



The illumigene Malaria assays: new, promising screening assays for the diagnosis of malaria

Tom van Gool MD, PhD,
Section Clinical Parasitology,
Department Medical Microbiology,
Academic Medical Center, Amsterdam,
Netherlands

On behalf of the Dutch and Belgian *illumigene* malaria study group

Conflict of interest

None

Malaria



- ▶ Devastating disease in tropical countries with estimated 450.000 deaths worldwide per year
- ▶ In Europe: returning travellers and immigrants
- ▶ Most frequently imported: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, *P. knowlesi* less frequently, but important causes of morbidity!
- ▶ Malaria diagnosis is practiced in every patient with fever of unknown origin returning from a malaria endemic country.
- ▶ Most of these patients (up to 90%) prove not to have malaria. *Exclusion of malaria represents most work in malaria diagnosis...!*

Diagnostic methods for malaria in use in western laboratory setting

Thick smear



Thin smear



Antigen tests

=



BinaxNow (HRPII and aldolase)

screening

QBC

=



screening

PCR

=



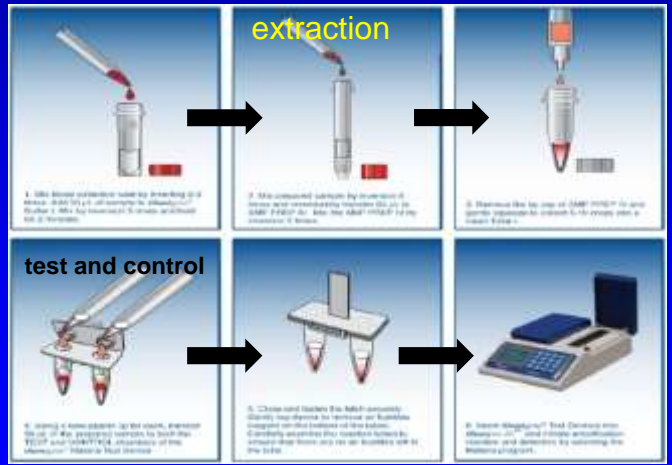
mainly for reference - work

New tests!

illumigene Malaria and illumigene Malaria PLUS

Meridian Bioscience, Inc.

new, fast and practical PCR methods based on loop mediated *isothermal* amplification (LAMP) methodology



Procedure illumigene Malaria*

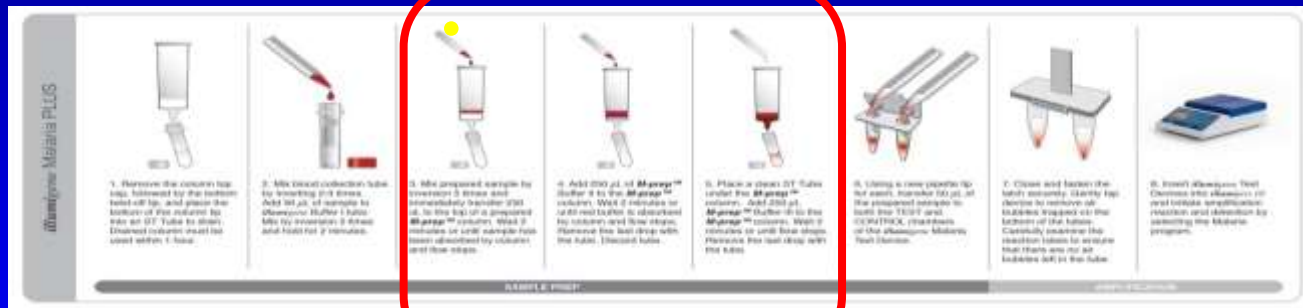
- Easy to handle
- 5 minutes hands on time*
- Result within 40 min
- Use in any standard lab.

- Claims: sensitivity of 0.006 - 2 parasite / ul and high specificity

Only genus- level: no species determination!

In contrast to iM, illumigene Malaria PLUS assay

more advanced DNA extraction system, 10 min. gravity-driven gel filtration preparation method, to further increase sensitivity..



Aims – questions - of current study

- ▶ How good are the *illumigene* Malaria - and *illumigene* Malaria PLUS assays as **screening tests** for malaria in returning travellers and immigrants in NL and Belgium

Special interest in:

- 1) correct diagnosis of **all (5) different malaria species**
- 2) correct diagnosis of **malaria-negative cases**
- 3) **ease and speed** of handling
- 4) performance **compared with RDT BinaxNow Malaria**

Design, patients and participating centers

Prospective study: April 2016 - March 2017

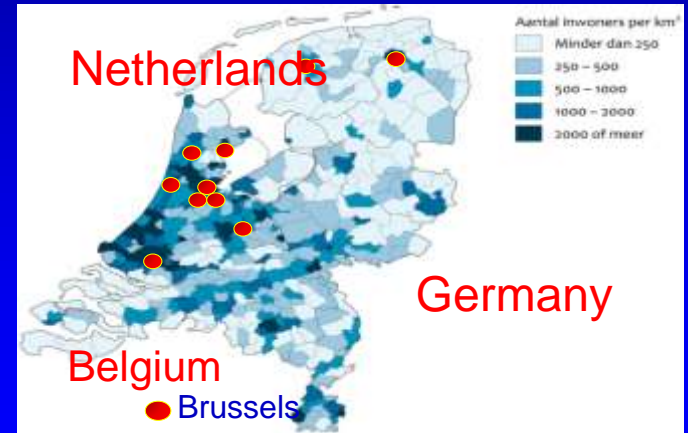
Study population: travellers and immigrants with fever of unknown origin returning or originating from endemic areas for malaria

11 Medical clinical laboratories

10 in Netherlands, one in Belgium

4 affiliated to Academic Hospital Centres

7 affiliated to other large Medical Centres



Samples sent in, routine diagnostic methods

- ▶ all positive samples in the study contained *asexual stages* of malaria parasites in stained blood films
- ▶ Only first samples included, no control samples after start treatment.
- ▶ Standard examination all laboratories: thick and thin smears (Giemsa, Fields, Diff quick). BinaxNow frequently used as screening tool.

Referral laboratory (AMC):

- ▶ *illumigene* Malaria and *illumigene* Malaria PLUS assays
- ▶ PCR for every sample (Shokoples et al. 2009)
- ▶ Sequencing when needed (Rougemont et al. 2004)
- ▶ BinaxNow Malaria, when not performed in participating center

A sample was regarded positive :

Construct Gold standard for positivity: *asexual stages of malaria parasites observed with microscopy, with **species determination** confirmed by PCR.*

and regarded negative.....:

Construct Gold standard for negativity: *absence of asexual and sexual stages of malaria with microscopy, with confirmation of negativity by PCR.*

Results

Included 273 patients (273 samples)

147 positive according to Gold standard

126 negative according to Gold standard

Sensitivity of *illumigene* malaria and *illumigene* malaria PLUS assays

147 patients with active malaria (asexual stages present in bloodsamples)*											
Positive patients according to Gold Standard of Positivity*			Results methods under investigation								
			<i>illumigene</i> ® Malaria	<i>illumigene</i> ® Malaria PLUS	Binax Now				Reactive bands: HRP II and/ or aldolase		
No. of patients	Malaria (sub)species	Parasitaemia parasites/ul range	no. patients with correct diagnosis of malaria (sensitivity %)	no. patients with correct diagnosis of malaria (sensitivity %)	No. positive tests, irrespective of type of band (sensitivity %)	No. tests with correct band(s) for species present (sensitivity %)	Negative	HRP II only	HRP II and aldolase	Aldolase only	
102	<i>P. falciparum</i>	27-990.000	102 (100)	102 (100)	102 (100)	101 (99)	0	33	68	1**	
28	<i>P. vivax</i>	132-73.650	28 (100)	28 (100)	21 (75)	21 (75)	7	0	0	21	
7	<i>P. ovale</i> (W 5 +C 2)	564-32.200	7 (100)	7 (100)	2 (29)	2 (29)	5	0	0	2	
4	<i>P. malariae</i>	40-90.000	4 (100)	4 (100)	1 (25)	1 (25)	3	0	0	1	
1	<i>P. knowlesi</i>	270.000	1 (100)	1 (100)	1 (100)	1 (100)	0	0	0	1	
3	MI: <i>P. falciparum</i> and <i>P. malariae</i>	1890-4087	3 (100)	3 (100)	3 (100)	1 (33)	0	0	1	2	
2	MI: <i>P. falciparum</i> and <i>P. ovale</i>	0,2-0,3	2 (100)	2 (100)	2 (100)	0 (0)	0	2	0	0	
Total: 147			147 (100 %)	147 (100 %)	132 (90 %)	127 (86 %)	15	35	69	28	

Legend: * Only first sample before treatment (no follow up samples after start of treatment) used.

** Sample of patient with proven HRP II gene deletion in *P. falciparum* isolate.

† *P. knowlesi* morphologically strongly resembles *P. malariae*: definitive determination only possible with PCR.

W= *P. ovale wallikeri* (no. 5), C= *P. ovale curtisi* (no. 2)

red = incorrect result reactive band

Specificity of illumigene malaria and malaria PLUS assays

126 patients **without** active malaria (**no asexual stages** present in bloodsamples)*

Negative patients according to Gold standard of negativity*	Results methods under investigation						
	<i>illumigene</i> ® Malaria	<i>illumigene</i> ® Malaria PLUS	Binax Now	Reactive bands (HRP II and/ or			
				Negative	HRP II only	HRP II and aldolase	Aldolase only
	no. patients with correct diagnosis of negativity for malaria (% specificity)	no. patients with correct diagnosis of negativity for malaria (% specificity)	no. patients with correct diagnosis of negativity for malaria (% specificity)				
126	126 (100)	126 (100)	125 (99)	125	1 **	0	0
Total: 126	126 (100%)	126 (100%)	125 (99%)	125	1	0	0

Legend:

Red= incorrect result

* Only first samples used before treatment (no follow up samples after start of treatment).

** False postivity based on travel history (> 1 year after last visit malaria endemic area), negative microscopy and QBC and negative PCR

Other (European) experiences with *illumigene* Malaria:

Apart of current study still few prospective studies....



2016 Apport de l'illumigene® Malaria dans le diagnostic du paludisme d'importation

Nicolas Argy^{1,2}, Christine Bonnal¹, Jean Baptiste Aillaud¹, Floriane Ferreira¹, Djamel Haouchine¹, Sandrine Houzé^{1,2}.

1. Laboratoire de Parasitologie, APHP, Hôpital Bichat Claude – Bernard, Paris; 2. Université Paris Descartes, Paris, France.

PP43



Tableau 1 : Performance de Illumigene® Malaria par rapport aux autres techniques diagnostiques dans le cadre du diagnostic

Espèces	Microscopie		BinaxNOW® Malaria *		Illumigene® Malaria	
	Nég.	Pos.	Nég.	Pos.	Nég.	Pos.
Négatifs (N=85)	85		84	1	85 (100%)	
<i>P. falciparum</i> (N=44) (<8p/µl – 423 000p/µl)	1	43	1	43		44 (100%)
<i>P. ovale</i> (N=11) (68p/µl – 24 300p/µl)		11	8	3		11 (100%)
<i>P. vivax</i> (N=3) (900p/µl – 9000p/µl)		3	0	3		3 (100%)
<i>P. malariae</i> (N=2) (180p/µl – 2250p/µl)		2	1	1		2 (100%)

*: HRP2 pour *P. falciparum*; aldolase pour les autres espèces

85 negative cases

44 x *P. falciparum*

16 x non. *P. falciparum*

“Excellent screening test for malaria, better performance as RDT...”

Discussion et Conclusion

- Le test Illumigene® Malaria a présenté d'excellentes performances dans le contexte d'un diagnostic initial de paludisme permettant d'exclure un accès palustre pour la totalité des prélèvements négatifs inclus et de confirmer le diagnostic d'accès palustre quelle que soit l'espèce responsable de l'accès, et pour des parasitemies inframicroscopiques ou élevées (≥ 10%). Ces performances sont supérieures à celles du TDR mis en œuvre en routine au laboratoire.

BinaxNOW results in non- P. falciparum cases

we have to accept its limitations...!

	BinaxNOW performance	
	Number of cases	Sensitivity
Current study* 2016	<i>P. vivax</i> n=28 <i>P. malariae</i> n=4 <i>P. ovale</i> n=7 <i>P. knowlesi</i> n=1	63%
Bigaillon et al. 2005** J Clin Microbiol	<i>P. vivax</i> n=9 <i>P. malariae</i> n=1 <i>P. ovale</i> n=12	59%
Cho (Chi-Hyun) et al. 2011* Trop Med Int Health*	<i>P. vivax</i> n=84	62%
Durand et al. 2005*** Clin Microbiol and Inf	<i>P. vivax</i> n=4 <i>P. malariae</i> n=1 <i>P. ovale</i> n=14	67%
Farcas et al. 2003**** Am J Trop Med Hyg	<i>P. vivax</i> n=90 <i>P. malariae</i> n=5 <i>P. ovale</i> n=9	84%
Foster et al. 2014**** Malar J	<i>P. knowlesi</i> n=28	29%
Mohon et al. Dis, 2016**** Diagn Microbiol Infect	<i>P. vivax</i> n=25 <i>P. malariae</i> n=1 <i>P. ovale</i> n=5	71%
Staufer et al. 2009*** Clin Infect Dis	<i>P. vivax</i> n=8 <i>P. malariae</i> n=6 <i>P. ovale</i> n=5	86%
Wiese et al. 2006** Scand J Infect Dis	<i>P. vivax</i> n=11 <i>P. malariae</i> n=3 <i>P. ovale</i> n=2	41%
Wongsrichanalai et al. 2003** Am J Trop Med Hyg	<i>P. vivax</i> n=63 <i>P. malariae</i> n=5	87%

◀ current study

Repeatedly
sub-optimal
sensitivity with
aldolase for non-Pf

* vs. PCR + Microscopy; ** vs. microscopy; *** vs. microscopy + QBC **** PCR, NA: Not available

Sensitivity based on 10 studies: range from 29% to 87%

Screening of malaria with *illumigene* Malaria.....: *how to handle the results ?*

➔ Positive illumigene: microscopic determination of species and calculation of parasitaemia in *P. falciparum*

➔ Negative illumigene: no further action or...

short study of Giemsa or Diff Quick stained thin film:
easy to perform, 5 min study time detects all parasites ≥ 200 p/ul.*
(*1000x, ≈ 200 fields , study among 108 malaria patients, 82 x Pf, 26 x non-Pf)

Conclusions

- ▶ *illumigene* Malaria and *illumigene* Malaria PLUS are excellent **screening assays** for diagnosis of malaria
- ▶ In routine practice *illumigene* Malaria has sufficient sensitivity. (likely ≤ 1 parasite / ul), no need to use “Plus” version...
- ▶ BinaxNow results for non - *P. falciparum* malaria are unreliable.
- ▶ Positive *illumigene* Malaria should immediately be followed by microscopic examination of stained blood films.



Acknowledgements

ERASMUS MC / Havenziekenhuis

J.J. van Hellemond
R. Koelewijn
P. van Genderen

Antonius Ziekenhuis

B.M. de Jongh
D. Ballegoy

Martini Ziekenhuis / CERTE

R.F.M Oude Elferink
A.V.M. Moller
G. de Jong

Izore, Centrum Infectieziekten

Friesland
A. Almoujahid
J. Weel

Comicro

J.H. Oudbier
E.J.C. Koenis

Pôle Hospitalier Universitaire de Bruxelles (Belgium)

B. Mahadeb
P. Nguyen Vo Thanh
O. Vandenberg

Noordwest Ziekenhuisgroep

J.W.T Cohenstuart
B. Schifferling-de Bruyn

VUMC

T. Hekker
R. Pique

OLVG

B. Werdmuller
A. van Dam
J. de Bruijn

Streeklab voor de Volksgezondheid Haarlem

Th. Mank

AMC

A. Bart
N. Verhaar
E. Wentink
M. van Vugt

Marc Molina
Richard Hughes
Mieke Vermander
Bas Hersmus



End