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

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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

QBC

PCR

= BinaxNow (HRPII and aldolase) screening

= mainly for reference - work
**illuminigene Malaria** and **illuminigene Malaria PLUS**

*Meridian Bioscience, Inc.*

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

- 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-negatieve* 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

<table>
<thead>
<tr>
<th>Positive patients according to Gold Standard of Positivity*</th>
<th>Results methods under investigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>Malaria (sub)species</td>
</tr>
<tr>
<td>-----------------</td>
<td>----------------------</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>102</td>
<td><em>P. falciparum</em></td>
</tr>
<tr>
<td>28</td>
<td><em>P. vivax</em></td>
</tr>
<tr>
<td>7</td>
<td><em>P. ovale</em> (W 5+C 2)</td>
</tr>
<tr>
<td>4</td>
<td><em>P. malariae</em></td>
</tr>
<tr>
<td>1</td>
<td><em>P. knowlesi</em></td>
</tr>
<tr>
<td>3</td>
<td>MI: <em>P. falciparum</em> and <em>P. malariae</em></td>
</tr>
<tr>
<td>2</td>
<td>MI: <em>P. falciparum</em> and <em>P. ovale</em></td>
</tr>
<tr>
<td><strong>Total:</strong> 147</td>
<td></td>
</tr>
</tbody>
</table>

Legend:
* Only first sample before treatment (no follow up samples after start of treatment) used.
** Sample of patient with proven HRPII 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)
Specificity of illumigene malaria and malaria PLUS assays

<table>
<thead>
<tr>
<th>Negative patients according to Gold standard of negativity*</th>
<th>illumigene® Malaria</th>
<th>illumigene® Malaria PLUS</th>
<th>Binax Now</th>
</tr>
</thead>
<tbody>
<tr>
<td>126 patients without active malaria (no asexual stages present in blood samples)*</td>
<td>no. patients with correct diagnosis of negativity for malaria (% specificity)</td>
<td>no. patients with correct diagnosis of negativity for malaria (% specificity)</td>
<td>no. patients with correct diagnosis of negativity for malaria (% specificity)</td>
</tr>
<tr>
<td>126</td>
<td>126 (100%)</td>
<td>126 (100%)</td>
<td>125 (99%)</td>
</tr>
<tr>
<td>Total: 126</td>
<td>126 (100%)</td>
<td>126 (100%)</td>
<td>125 (99%)</td>
</tr>
</tbody>
</table>

Legend:
* Only first samples used before treatment (no follow up samples after start of treatment).
** False positivity 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 Argy¹,², Christine Bonnal¹, Jean Baptiste Allaud¹, Floriane Ferreira¹, Djamal Haouchine¹, Sandrine Houzé¹,².

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

<table>
<thead>
<tr>
<th>Espèces</th>
<th>Microscopie</th>
<th>BinaxNOW® Malaria</th>
<th>Illumigene ©Malaria</th>
</tr>
</thead>
</table>
| Négatifs (N=85)  | 85   |     | 84   |     | 85   | 1    | 85 (100%)
| *P. falciparum* (N=44) (<80p/μL – 423 000p/μL) | 1    | 43  | 1    | 43  | 44   | 1    | 44 (100%)
| *P. ovale* (N=11) (68p/μL – 24 300p/μL) | 11   | 8   | 3    |     | 11   | 3    | 11 (100%)
| *P. vivax* (N=3) (900p/μL – 9000p/μL) | 3    | 0   | 3    |     | 3    | 0    | 3 (100%)
| *P. malariae* (N=2) (180p/μL – 2250p/μL) | 2    | 1   | 2    | 1   | 2    | 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...”
BinaxNOW results in non- P. falciparum cases
we have to accept its limitations…!

<table>
<thead>
<tr>
<th>BinaxNOW performance</th>
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<tbody>
<tr>
<td><strong>Number of cases</strong></td>
</tr>
<tr>
<td><strong>P. vivax n=28</strong></td>
</tr>
<tr>
<td><strong>P. ovale n=7</strong></td>
</tr>
<tr>
<td><strong>63%</strong></td>
</tr>
<tr>
<td><strong>P. vivax n=9</strong></td>
</tr>
<tr>
<td><strong>P. ovale n=12</strong></td>
</tr>
<tr>
<td><strong>P. vivax n=84</strong></td>
</tr>
<tr>
<td><strong>62%</strong></td>
</tr>
<tr>
<td><strong>P. vivax n=4</strong></td>
</tr>
<tr>
<td><strong>P. ovale n=14</strong></td>
</tr>
<tr>
<td><strong>P. vivax n=90</strong></td>
</tr>
<tr>
<td><strong>P. ovale n=9</strong></td>
</tr>
<tr>
<td><strong>P. knowlesi n=25</strong></td>
</tr>
<tr>
<td><strong>P. malariae n=1</strong></td>
</tr>
<tr>
<td><strong>P. ovale n=5</strong></td>
</tr>
<tr>
<td><strong>P. vivax n=63</strong></td>
</tr>
<tr>
<td><strong>87%</strong></td>
</tr>
</tbody>
</table>

* 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.
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