*illumi*gene Malaria assay: a new tool in the diagnosis of imported malaria

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INTRODUCTION

➢ Malaria is a potentially fatal, tropical vector-borne parasite. Five species of *Plasmodium* are pathogenic in humans.
➢ Clinical signs of influenza-like illness are non-specific. To ensure appropriate management and specific treatment, biological diagnosis must be carried out rapidly within 2 hours of blood collection.
➢ The standard diagnosis is based on the microscopic examination of thin and thick stained films, which allows identification of the species, estimate of parasite load and determination of parasite stage. However, many laboratories in non-endemic countries do not have microscopic expertise due to the low numbers of positive samples they receive.
➢ Rapid diagnostic tests (RDTs) can be a complement to the microscopic diagnosis of malaria for non-experienced staff, particularly for the diagnosis of *P. falciparum* but may be sub-optimal for other species and issues may arise due to parasite load.
➢PCR techniques due to their sensitivity and specificity are required as a reference method for difficult diagnostics, but they require a significant amount of equipment before they can be implemented and delays in implementation is not conducive to serving the emergency setting.
➢ A rapid molecular biology method was developed by Meridian Bioscience to overcome many of these issues in the routine and emergency setting.

OBJECTIVES

✓ Evaluate the performances of ***illumi****gene*® Malaria in the diagnosis of malaria in non-endemic settings
✓ Compare ***illumi****gene*® Malaria with the BinaxNOW® Malaria rapid diagnostic test and microscopy in the diagnosis of malaria in the laboratory..

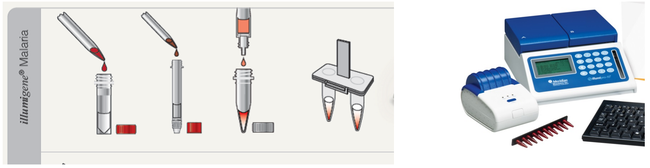

Figure 1 : Implementation of the illumigene Malaria Test and its dedicated equipment

REFERENCE

Lucchi NW, Gaye M, Diallo MA, Goldman IF, Ljolje D, Deme AB, Badiane A, Ndiaye YD, Barnwell JW, Udhayakumar V, Ndiaye D. Evaluation of the Illumigene Malaria LAMP: A Robust Molecular Diagnostic Tool for Malaria Parasites. Sci Rep. 2016; 6: 36808.

METHODS

➢ Prospective whole blood samples from suspected malaria patients returning from a malaria endemic area.

➢ Prospective whole blood samples taken from patients treated for malaria, as part of the monitoring of therapeutic efficacy

➢Microscopy on thin and thick films is considered the reference method.
- ❖ Specimens were considered negative if no parasites were observed after reading 100 microscopic fields on the thick film and positive if at least one asexual form *Plasmodium* was observed during the reading of the 100 fields of the thick film.
- ❖ The parasitemia was determined by counting the number of parasites observed in relation to the number of leukocytes, taking into account that each patient had an average number of 8000 leukocytes/µl of blood.

➢PCR (Fast Track Diagnostics) performed for all non-*Pf* (*P. falciparum*) samples and in case of any discrepancy between the tests.

➢RDT : BinaxNOW® Malaria which detects HRP2 and aldolase starting from 15µl of whole blood.

➢ ***illumi****gene*® Malaria : An isothermal DNA amplification technique (LAMP) which targets the mitochondrial DNA of all the Plasmodium species. The test was carried out in accordance with the manufacturer's instructions from 40µl of whole blood (Fig. 1). The reaction is carried out in the ***illumi***pro-10 supplied by Meridian Bioscience. The qualitative result is obtained in 40 mintues by turbidimetric reading (Fig. 1).

RESULTS

➢145 specimens received for initial diagnosis were included, 85 of which were negative by microscopy and RDT, and 60 were reported as positive, 44 were *P. falciparum* (73.4%), 11 were *P. ovale* (18.3%), 3 were *P. vivax* (5%) and 2 were *P. malariae* (3.3%). The parasitemia ranged from less than 8 parasites/µl (negative thick film and positive with PCR) to 423,000 p/µl
- ❖ The sensitivity of RDT was 83.6% for all studied samples, 97.8% (43/44) for *P. falciparum* for detection of HRP2 and 43.8% (7/16) for the other species detected by aldolase.
- ❖ The results with ***illumi****gene* Malaria were in line with expected diagnoses: 100% negative for true negative samples and 100% positive for true positive samples. The main results are presented in Table 1.

➢ 18 samples included in the monitoring of therapeutic efficacy of antimalarial drugs in 6 patients, including 5 presenting with *P. falciparum* and 1 presenting with *P. vivax,* all of which demonstrated adequate clinical and parasitological response. The results are presented in Table 2.

During the follow-up of *P. falciparum* access, positive results with ***illumi****gene* Malaria are observed on day 7 of an effective treatment for all included patients and on day 28 for 3 of the 5 included patients. In the case of monitoring the *P. vivax* case, the results are consistent between the different techniques.

Table 1 : Performance of *illumi*gene® Malaria compared with other diagnostic techniques for diagnosis

Species		Microscopy		BinaxNOW® Malaria *		Illumigene ®Malaria	
		Neg.	Pos.	Neg.	Pos.	Nég.	Pos.
Negatives (n=85)		85		85		**85 (100%)**	
P. falciparum (n=44)	(<8p/µl – 423 000p/µl)	1	43	1	43		**44 (100%)**
P. ovale (n=11)	(68p/µl – 24 300p/µl)		11	8	3		**11 (100%)**
P. vivax (n=3)	(900p/µl – 9000p/µl)		3	0	3		**3 (100%)**
P. malariae (n=2)	(180p/µl – 2250p/µl)		2	1	1		**2 (100%)**

*: HRP2 for *P. falciparum*; aldolase for all species

Table 2 : Performance de *illumi*gene® Malaria compared with other diagnostic techniques in the therapeutic efficacy monitoring

Species	Microscopy		BinaxNOW® Malaria		Illumigene ®Malaria	
	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
P. falciparum						
- J3 (n=5) (8p/µl – 1800p/µl)	1	4	0	5		**5**
- J7 (n= 5) (* whose 1 with few gametocytes)	5*	0	0	5		**5**
- J28 (n=5)	5	0	2	3	**2**	**3**
P. vivax (n=1)						
- J3	1		1		1	
- J7	1		1		1	
- J28	1		1		1	

*: HRP2 for *P. falciparum*; aldolase for *P. vivax*

CONCLUSIONS

➢ The ***illumi****gene* Malaria test demonstrated excellent performance in the context of an initial diagnosis of malaria, to exclude malaria infection for all negative samples and to confirm the diagnosis of malarial infection, regardless of species responsible for infection, and for inframicroscopic and high parasitemia (10%). These performances are superior to those of RDTs routinely performed in the laboratory.
➢ This molecular biology approach offers the advantage of not requiring any special training for its implementation nor for the use of its dedicated equipment. The absence of amplicon production and the completion of the entire reaction in a closed tube avoids the risks of contamination.
➢ The persistence of a positive result due to the detection of plasmodial DNA during the treatment of *P. falciparum* infection, has already been demonstrated with PCR methods and could be due in some cases to the presence of gametocytes which can persist for at least 3 weeks despite the antimalarial treatment which does not seem to eliminate them.
➢ Overall, the ***illumi****gene* Malaria test excludes the diagnosis of malaria in line with the 100% Negative Predictive Value (NPV) observed in this limited study. The inclusion in this study of samples received as part of the monitoring of therapeutic efficacy induces a Positive Predictive Value (PPV) of 87.7% which implies interpreting a positive result according to the clinical and epidemiological context if this test is performed on all samples received in the laboratory researching *Plasmodium*.