

Our experience with LAMP (Loop mediated isothermal AMPLification) for the detection of malaria at a university hospital

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BACKGROUND: Half of the world's population is at risk of malaria infection. The WHO objective is to decrease its incidence worldwide, the number of deaths decreased significantly between 2000-2015 (48%). Conventional methods of diagnosis require expertise in microscopy, molecular biology is not subjective. The aim of this study is to evaluate *illumigene*® Malaria - Meridian, a new test in the Meridian LAMP menu, as a routine malaria diagnostic tool, within the WHO recommended 2 hours of blood collection ⁽¹⁾.

METHODS: Detection of malaria in laboratory

-Antigen immunoassay by immunochromatography (Palutop + 4 optima®-All Diag) detecting HPR2 (identification of *P. falciparum*), LDH (*P.vivax*), Pan LDH (genus *Plasmodium*)

-Thin and thick smears (WHO recommendations)

-Plasmodial DNA by LAMP technique (Loop mediated isothermal AMPLification, *illumigene*® Malaria - Meridian)

Sensitivities⁽²⁾ of the techniques:

- RDT: 100 parasites / µl or a parasitemia of ~0.002%
- Thin smear: 100 to 200 parasites / µl
- Thick smear: 10 to 20 parasites / µl
- LAMP technique: 2 parasites / µl (*Plasmodium falciparum*)

Samples: 94 malaria requests between May and September 2016 were made in the laboratory, 60 of which were tested by LAMP.

REFERENCES :

(1)-OMS. World Malaria report 2015. Available at: https://apps.who.int/iris/bitstream/10665/200018/1/9789241565158_eng.pdf

(2)-P.Aubry, B-A.Gaüzère. Test de diagnostic rapide par immunochromatographie en zones tropicales actualités 2015. Méd.Trop.

RESULTS: results distribution

| | Malaria positives | Malaria negatives | Total |
|-----------------------------------------------|---------------------------------------|------------------------|-------|
| Palutop+4 optima®(+)/Thin(+) Thick (+) smears | 34 (16 diagnosis & -18 follow-ups) | 0 | 34 |
| Palutop+4 optima®(-)/Thin(-) Thick (-) smears | 1 (LAMP Positive) | 58 (LAMP Negatives) | 59 |
| Palutop+4 optima®(+)/Thin(-) Thick (-) smears | 1 (LAMP Positive) | 0 | 1 |
| Total | 36 | 58 | 94 |

All diagnosis positives are *P.falciparum*

RESULTS : Plasmodial DNA detection tests show an earlier diagnosis in 5.5% of the cases. 100% of negatives with the conventional techniques are confirmed with the amplification technique and in 1,7% of the cases, *illumigene*® permits the diagnosis while the microscopic techniques were not sensitive enough.

Positive LAMP:

1- Positive LAMP, positive with 1 band of very low intensity on Palutop + 4 optima® in *P.falciparum* and negative thin, thick smears.

• A 51 year old woman from Cameroon, hospitalized in intensive care unit for generalized convulsive attack and coma. A second blood sample at a febrile peak shows a trophozoite of *P. falciparum* on thin smear. The diagnosis is that of a convulsive condition on an atypical form of probable neurological malaria. The patient's development was favorable under artesunate and anticonvulsant.

• Positive LAMP on 1st sample confirmed the positive circulating antigen RDT.

2- Positive LAMP, negative Palutop + 4 optima®, negative thin and thick smears.

• A 27-year-old patient from Guinea. Migrated early 2014 from Africa to Europe. Arrived in France April 2016. Presents to the emergency department with malaise, hyperthermia and chills. The biological balance shows: -CRP 24 (N <6mg / l), normal NFS, normal liver function (transaminases).

He is hospitalized for a review of polyadenopathies with no infectious etiology found.

There was no anti-malaria treatment implemented.

The patient was seen again in early October: he is better and has regained weight (3 kg).

Negative LAMP:

1- A child back from Central Africa 10 days (from a stay of one month ½) whose mother was hospitalized for simple malaria access to *P.falciparum* a week ago, Malarone chemoprophylaxis taken in an incomplete way: the negative LAMP result confirmed the thin and thick smears result. A pharyngitis of viral origin was subsequently diagnosed.

2- A 17-year-old male from Guinea, returned to France since 6 weeks and sudden collapse with headache and coma. The LAMP results confirming the thick smear permits exclusion of malaria infection.

3- Case of diarrhea with vomiting and alteration of the general state on return from Africa (Togo), it was a salmonellosis.

- **CONCLUSION:** The LAMP technique improves the sensitivity of the diagnosis but no difference between active or treated malaria.
- *illumigene*® Malaria detects all species of *Plasmodium* including those not diagnosed by Palutop + 4 optima® (*P.ovale*, *malariae*, *knowlesi*).
- In the laboratory, it reinforced exclusion from the diagnosis of malaria. In those two cases where the *illumigene*® Malaria test was positive, plasmodium parasites were not found in 1 case by the other techniques however the management of the patient remained unchanged (no anti-malaria treatment for chronic malaria migrant).
- The *illumigene*® Malaria testing is decided case-by-case and is performed only if patient returns from an endemic area, shows a negative Palutop + 4 optima® test or in case of discordance between the rapid antigen test and smears results. *illumigene*® Malaria is used in diagnosis and not for follow-ups.
- In our experience, *illumigene*® Malaria is a major benefit especially when there is clinical evidence of neurological disorders.
- In the future, a test allowing discrimination between species as *P. falciparum* would certainly change the current strategy and would place the LAMP technique in first line.

Proposed decisional algorithm

