Region Hovedstaden
Det nye Rigshospital

On-Target Malaria

Refining a Malaria in-house PCR

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INTRODUCTION

There are now several antigen- and DNA-based methods for rapid detection of *Plasmodium spp*. with varying specificity and sensitivity. Due to the need of substantial experience in microscopy for malaria diagnosis, especially in a non-endemic country, an automated method for species differentiation is needed. This is especially of interest in non-falciparum infections, due too their relatively low parasitemia, as well as in mixed infection which easily can be overseen in a microscopic examination.

The aim of this project is to refine, verify and implement species-specific Plasmodium PCR as a routine diagnostic test at Department of Clinical Microbiology, Rigshospitalet, Denmark.

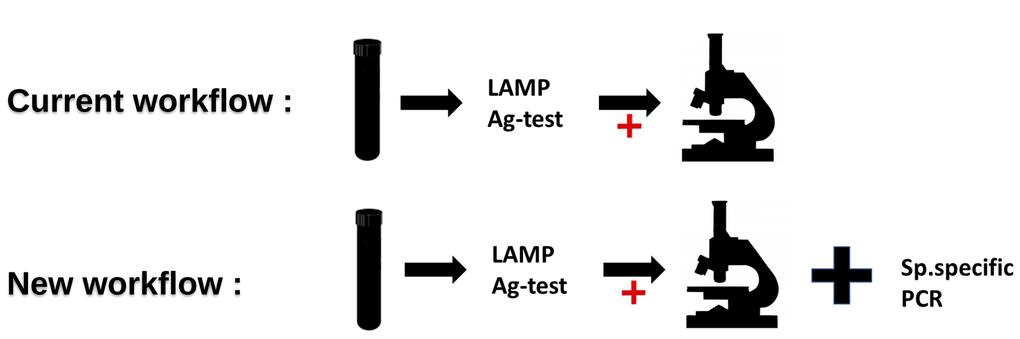

Figure 1. Current workflow compared to future workflow including sp. specific PCR run on all positive Plasmodium samples.

METHODS

31 whole-blood specimens from confirmed malaria patients, and 13 specimens of plasmodium positive dried lypholized blood from NEQAS (National External Quality Assessment Scheme) where colleted. Specimens represent 5 different *Plasmodium species: P.falciparum, P,vivax, P.ovale wallikeri / curtisi, P. malarie and P. knowlesi,* altogether confirmed by microscopy as well as antigen test and/or loop mediated isothermal amplification, LAMP, all of which are used in our current workflow.

Each sample was analyzed by in-house PCR, runed on the BDmax™ platform.
BDmax™ is an automated cassette-based PCR platform, ideal to run low numbers of samples. Extraction, real-time PCR and interpretation of the results are fully integrated in the process. We are using the BD-max (DNA-1) extraction kit, BD-max Master mix (B3) with specific Plasmodium primers and probes.

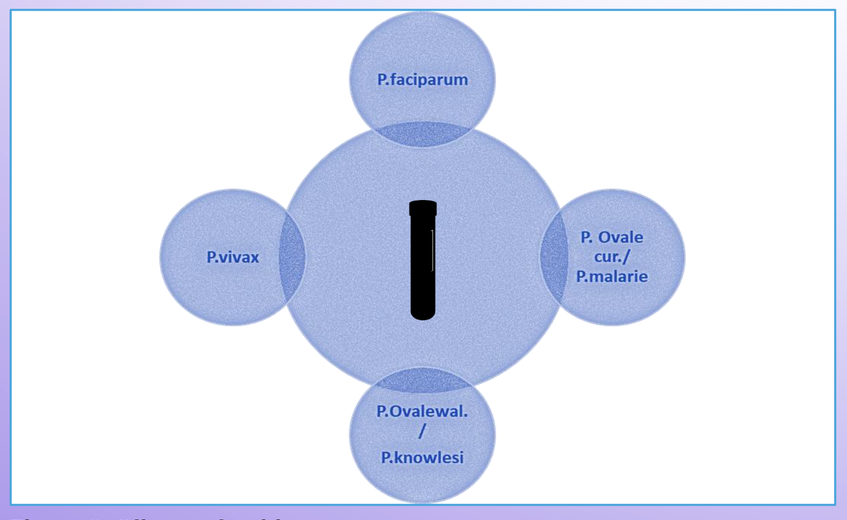

Figure 2. Oligonucleotides
Sample setup on 4 different primer/probes combinations

Each sample of 4 x 150µl full-blood was run on 4 cassettes with 4 different klick-in's containing different mixture of pre-synthetized primer/probes
When run on different channels, the
outcome is a differentiation between 5 *Plasmodium spp*. of interest.
The turnaround time for the PCR procedure, including sample preparation, is approximately 2 hours.

RESULTS

After changing the initial primer/probe setup, with all *Plasmodium spp.* in one klick-in, and fine tuning the platform program, 33 positive Plasmodium spp. samples where tested with only 1 of 33 (3%) resulting in false negative result. Positive results are also seen when tested on samples with a parasitic load as low as
< 0,1%.

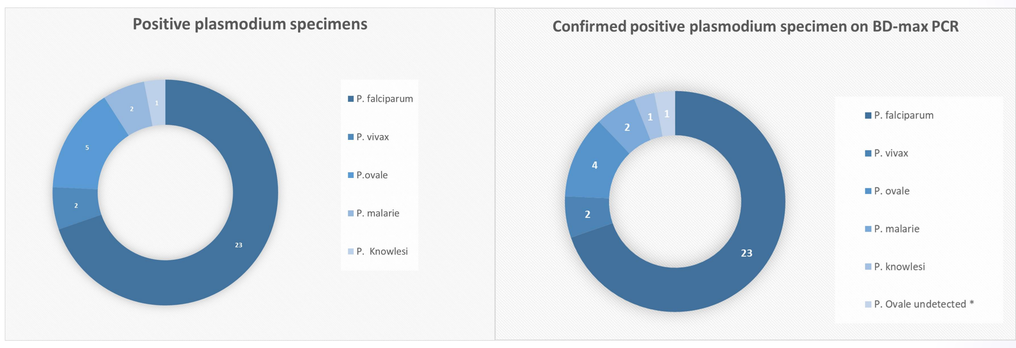

Figure 3. Positive Plasmodium samples confirmed on LAMP, Ag-test and microscopy, compared to the same samples run on the species-specific BD-max in-house PCR.

Inatial results from mixed Plasmodium spp. samples with different concentrations show warrying results. As seen in table 1.

Positive results are seen for P.falciparum and P. Ovale regardless of the different mix combination. P. Vivax and P.knowlesi are not detected in any of the mixed combinations.

Mixed infection combinations	P.Falciparum	P.Ovale	P.Vivax	P.Knowlesi	P.Malarie
P.Falciparum+ P.Ovale	+	+			
P.Knowlesi + P.Malarie				-	+
P.Falc. + P.Ovale + P.Vivax + P.Knowlesi + P. Malarie	+	+	-	-	-

Table 1. Mixed infections

CONCLUSION

Inatally: Although the verification and implementation of the malaria species specific PCR is not fully assessed and finalized, the outcome looks positive.
Implementation of the PCR in our workflow will improve our overall malaria diagnostics and serve as a complimentary addition to microscopic findings.
The negative aspects would be the need for a higher blood volume, though every sample is run on 4 cassettes, which means that a minimum blood volume of 4 X 150µl is needed. There is also a small economic increase per sample run.

Future: We still need too finetune our primer/probes concentrations and look at how we fully can optimize the BD-max™ platform considering the negative outcome in trials with mixed *Plasmodium spp*.