A novel approach for *Plasmodium* spp. detection: A direct multiplex qPCR technology.

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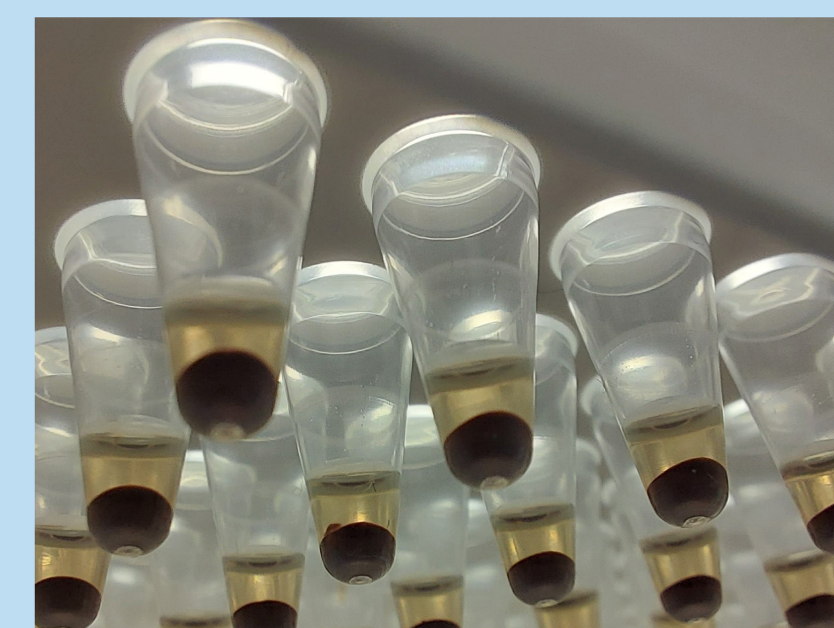
QIAprep&™ for direct, rapid and reliable *Plasmodium* spp. detection in surveillance and epidemiological research.

In human blood, *Plasmodium* spp. are typically detected via microscopy – the gold standard for *Plasmodium* detection – as well as rapid-diagnostic tests (RDTs). New methods based on molecular biological principles are used for detection and species differentiation in epidemiological settings with lower parasitemias, but require a separate nucleic acid extraction step.

We present a direct qPCR method, QIAprep&, for detecting the presence of *Plasmodium* spp. in a variety of sample types while saving time and costs. Compatible sample types are whole blood collected in various types of collection tubes, plus dried blood spots (DBS) and extracted DNA. When using whole blood, a large amount of blood can be directly added to the reaction (up to 20% per reaction).

QIAprep& is characterized by its rapid, simple and affordable workflow and open chemistry. The proposed solution is supported by the Pf/Non-Pf Detection Assay for distinguishing between *P. falciparum* vs. non-*P. falciparum* species and the Pv/Pm/Po/Pk Detection Assay for differentiating between *P. vivax, P. malariae, P. ovale,* and *P. knowlesi.* The chemistry is suitable for multiplex reactions and includes a human sampling control to ensure successful amplification. The method is compatible with routine lab equipment, such as common thermocyclers, and is highly cost-effective.

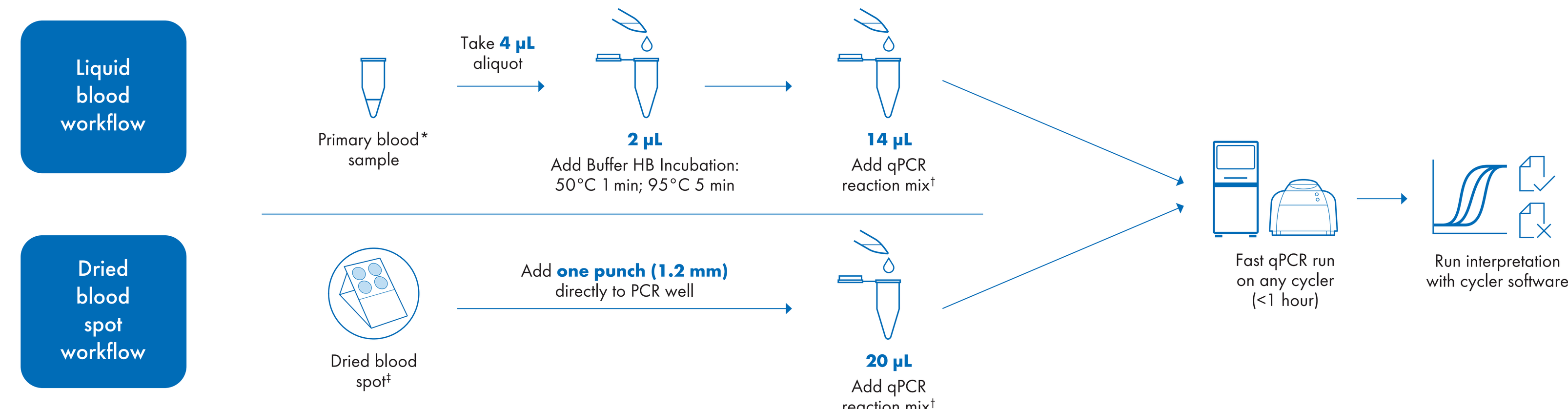
A mosquito on human skin.



Whole blood used directly in the PCR reaction after a short pretreatment.

QIAprep& workflows for detection of *Plasmodium* spp. in liquid whole blood samples and dried blood spots

Direct and sensitive qPCR method with streamlined workflows that require few pipetting steps and <1 hour cycling. Dried blood spots can be proceed in a direct (faster results in fewer steps) or elution workflow (more sensitive results). Whole blood can be processed quickly with the whole blood workflow or with higher sensitivity with the sedimentation workflow. Schematic overview of fast workflows is shown here.

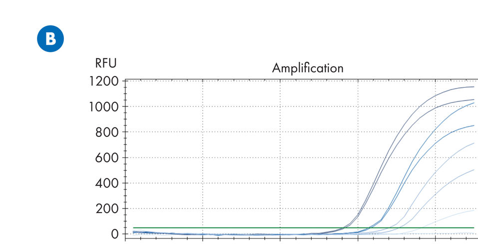


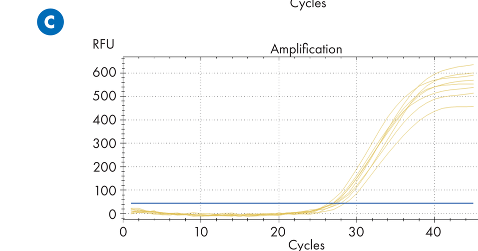
QIAprep& workflows for detection of *Plasmodium* spp. directly in blood. A direct workflow with 4 µL liquid blood sample or one 1.2 mm punch of dried blood spots. A short sample preparation step involves treating the liquid blood with buffer HB. Punches are directly transferred into a qPCR reaction. *Tested to-date: K_2EDTA, K_3EDTA, citrate and heparin blood, QIAcard® FTA Classic and QIAcard Bloodstain. †Containing primer/probes for the detection or differentiation of *Plasmodium* spp. ‡Tested to-date: QIAcard FTA Classic and QIAcard Bloodstain.

Comparison of QIAprep& Plasmodium Kit to classical DNA extraction

A Hit rates on DBS samples. The sensitivity of QIAprep& on DBS is either comparable (DBS direct workflow) or better (DBS elution workflow) than the classic extraction workflow.

			Hit rate QIAprep&						Hit rate Classical DNA extraction followed by PCR	
					Bloodstain card		Classic card			
Sample	Dilution	Parasites per µL	Whole blood	Sedimentation	DBS direct	DBS elution	DBS direct	DBS elution	Whole blood	DBS
P. falciparum culture	1:1000	79.4	100%	100%	100%	100%	100%	100%	100%	100%
	1:10,000	7.9	100%	100%	100%	100%	100%	100%	100%	100%
	1:100,000	0.8	75%	100%	100%	100%	50%	100%	100%	50%
	1:1,000,000	0.08	0%	88%	0%	88%	0%	100%	100%	25%
Sample A	1:1000	163.8	100%	100%	100%	100%	100%	100%	100%	100%
	1:10,000	16.3	100%	100%	100%	100%	100%	100%	100%	100%
	1:100,000	1.6	88%	100%	100%	100%	83%	100%	100%	100%
	1:1,000,000	0.16	50%	100%	33%	88%	50%	100%	100%	25%
Sample B	1:1000	159.9	100%	100%	100%	100%	100%	100%	100%	100%
	1:10,000	16.0	100%	100%	100%	100%	100%	100%	100%	100%
	1:100,000	1.6	100%	100%	100%	100%	88%	100%	100%	75%
	1:1,000,000	0.16	0%	100%	50%	75%	38%	100%	100%	0%

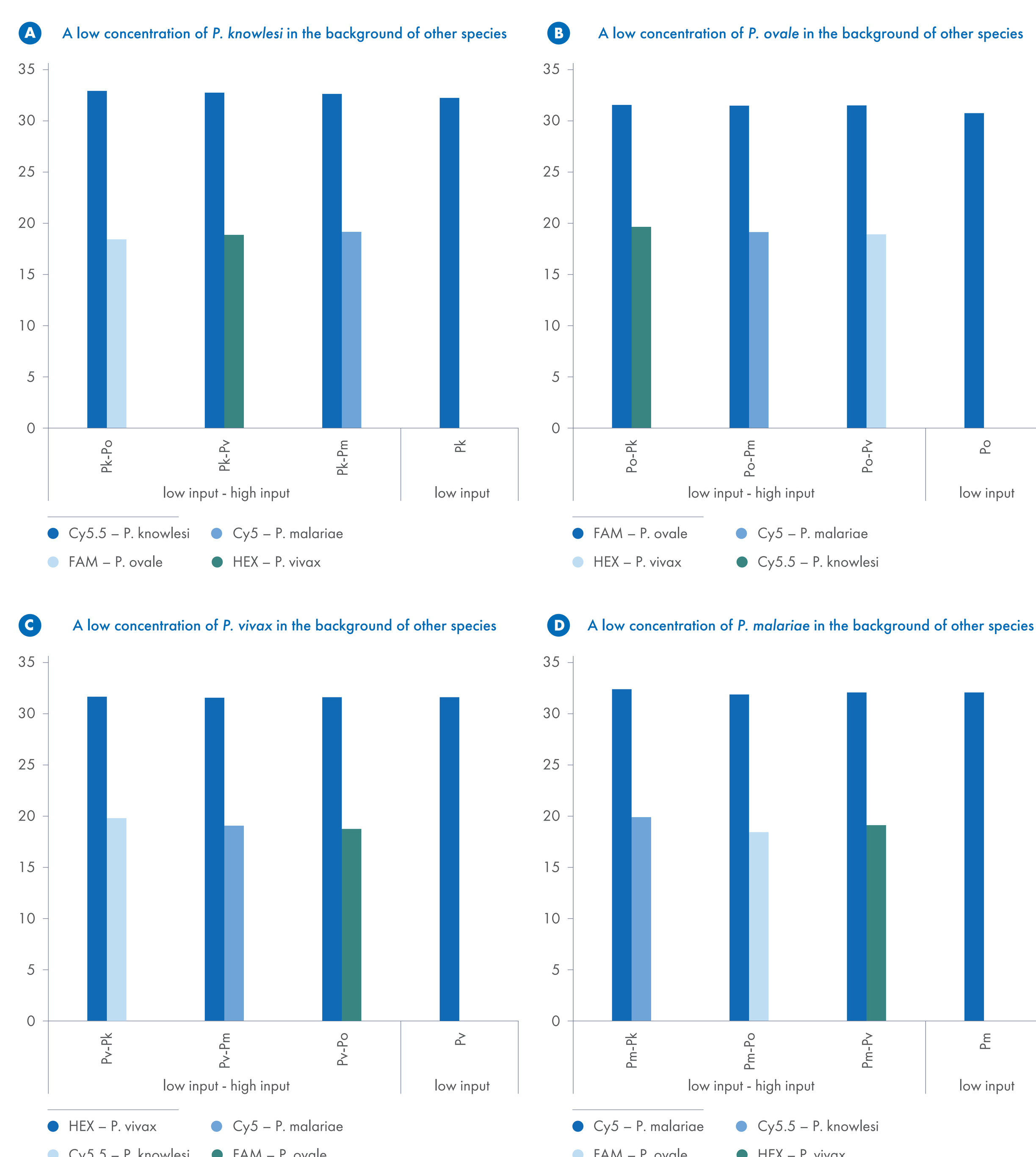




Sensitivity of QIAprep& workflows. *P. falciparum* culture and two human samples were diluted with *Plasmodium*-negative blood and processed with the different workflows. To determine parasitemia, DNA was isolated from the same samples using the QIAamp DNA Blood Mini Kit and quantified via digital PCR. Hit rates were determined for four workflows (DBS direct, DBS elution, whole blood and sedimentation) using Pf and non-Pf QIAprep& assays. **A** Hit rates for the liquid blood and DBS workflows are indicated (N=8 for liquid blood workflow with *P. falciparum* culture, N=4 for WHO standard, N=5 for DBS workflow with *P. falciparum* culture, run on Bio-Rad® CFX96™; N=5 for DBS workflow with WHO standard, run on Bio-Rad CFX384). Representative amplification curves from liquid blood workflow with WHO standard sample are shown for **B** the Pan-Plasmodium target in FAM™ channel and **C** for the RNaseP human control target in HEX™ channel.

Performance of second-line Pv/Pm/Po/Pk Detection Assay – mixed samples screening

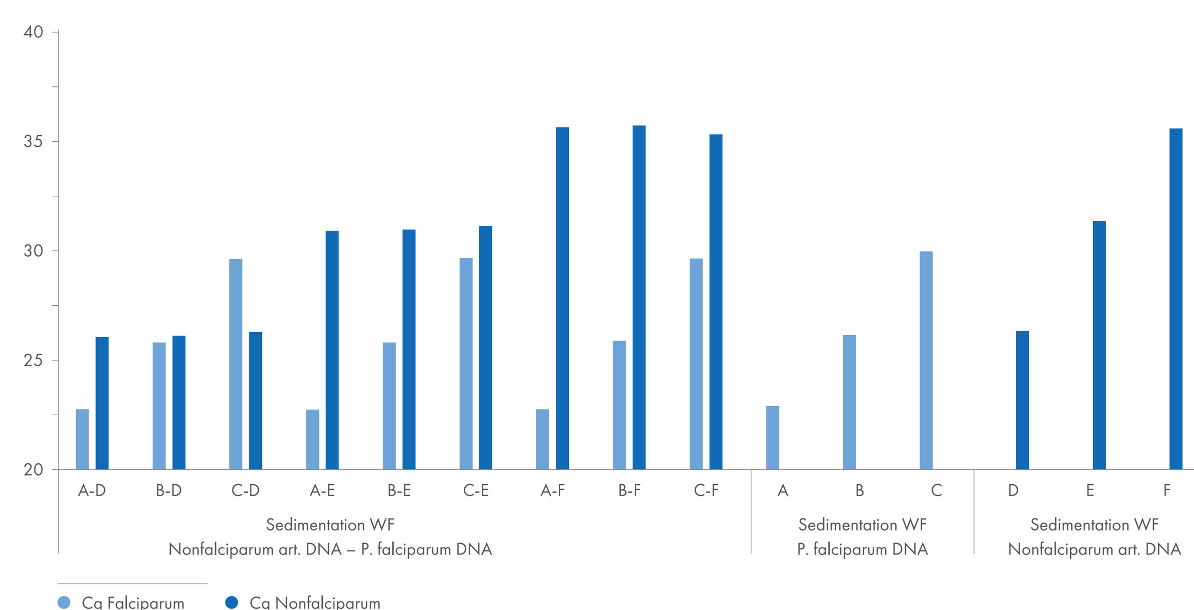
P. knowlesi, P.ovale, P. malariae and *P. knowlesi* in the background of other species. The presence of a mixed infection does not influence the Ct value of the lower concentrated *Plasmodium* species.



Different mixed infections were simulated by mixing artificial DNA for the different *Plasmodium* species. Different concentrations were used (low and high). For comparison, the low concentration in unmixed samples are on the right. All samples were processed with the sedimentation workflow with negative blood in the background. Pm = *P. malariae,* Po = *P. ovale,* Pv = *P. vivax,* Pk = *P. knowlesi.*

Robust performance of first-line Pf/non-Pf assay in mixed samples screening

Results show that none of the simulated mixed infections showed a decrease in sensitivity in comparison to the non-mixed samples.



Several dilutions of *falciparum* DNA and non-*falciparum* synthetic DNA were mixed to represent mixed infections at different parasitemias (high *falciparum* parasitemia, intermediate *falciparum* parasitemia, low *falciparum* parasitemia). For non-*falciparum*, the concentrations were: intermediate, low or very low. Next, all concentrations between *falciparum* and non-*falciparum* were combined. All samples were processed with the sedimentation workflow with negative blood in the background.

Summary of QIAprep& workflows

- *Plasmodium* detection with high sensitivity of 1 parasite/µL
- Time savings: A direct solution with a short turnaround time of ~1 hour
- Detection of *Plasmodium* DNA directly from dried blood spots or whole blood
- Fast, direct and cost-effective RT-qPCR approach
- Multiplex qPCR assays enable species differentiation (*P. falciparum, P. vivax, P. malariae, P. ovale* and *P. knowlesi*)
- Open chemistry, allowing the use of custom assays
- Compatibility with most thermocyclers
- A significant reduction in plasticware and equipment needed

Reference:

1. Rougemont M, et al. Detection of four *Plasmodium* species in blood from humans by 18S rRNA gene subunit-based and species-specific real-time PCR assays. *J Clin Microbiol.* 2004;42(12):5636-43.

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Sample to Insight