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RAPID ARTEMISININ-RESISTANCE DETECTION USING A CMOS LAB-ON-CHIP PLATFORM

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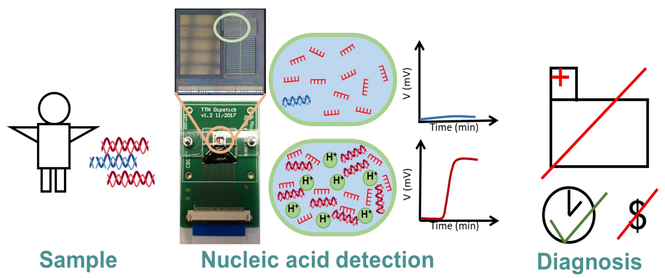
INTRODUCTION

- Malaria is one of the most impactful **INFECTIOUS** diseases affecting 219 million people and reporting 435,000 deaths in 2017 [1].
- Current treatments are based on artemisinin-based combination therapies (ACTs), but the emergence of drug resistance is compromising their effectiveness.
- Several single nucleotide polymorphism (SNPs) within the gene *kelch 13* of *Plasmodium falciparum* are associated to the development of artemisinin-resistant malaria [2].

Detecting rapid and specifically the presence of the mutant (MT) allele from a blood sample will enable early rapid diagnostics at the point-of-care (PoC), and will preserve the effectiveness of current therapies.

OBJECTIVES

- Specific, sensitive, rapid, **ISOTHERMAL amplification** [3] method capable of quantitatively detecting SNPs [4] → **allele-specific pH-LAMP.**
- Low-cost, portable, scalable, real-time signal transduction without the need of any post-amplification analysis [5,6] → **CMOS-based ISFET biosensing platform.**



EXPERIMENTAL METHODS

- Two reactions were performed using the MT specific primer set [4,7]:
  - Specific reaction: mutant synthetic DNA + pH-LAMP$_{MT}$
  - Unspecific reaction: wild type synthetic DNA + pH-LAMP$_{MT}$

- ISFET sensors were fabricated in **UNMODIFIED CMOS** technology using the top passivation layer (Si_3N_4) for pH sensing.
- Variations in pH were sensed by an array of **4096 SENSORS** (total silicon area of 0.57 mm^2) and converted into an electronic (voltage) signal which was recorded (0.3 fps) and interfaced to a PC [6].
- A microfluidic reaction chamber was assembled on top of the sensing platform.

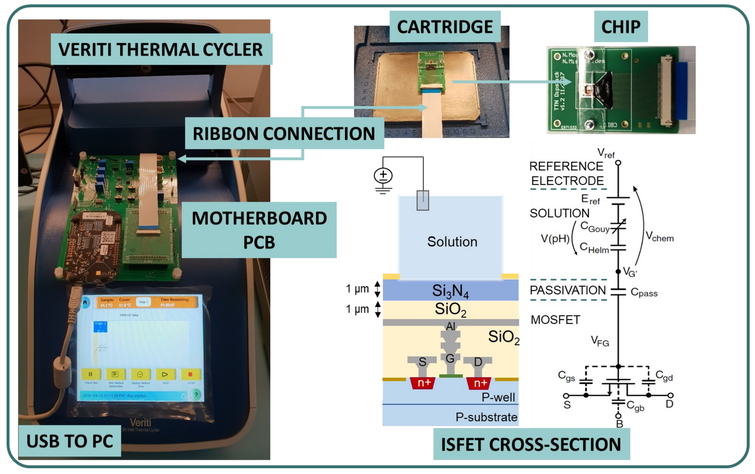


Figure 2. Set up of the biosensing platform and diagram of allele-specific pH-LAMP. Biosensing platform showing the microchip interfaced to a PCB with a microcontroller for data readout and the Veriti Thermal Cycler for temperature control. The reaction will only amplify the sample containing the MT allele.

RESULTS - ISFET platform vs qPCR

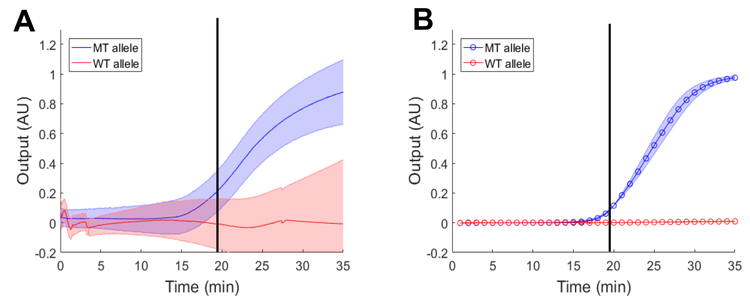


Figure 3. Allele-specific pH-LAMP reaction with (A) ISFET biosensing platform, showing the mean output of active pixels with standard deviation as shaded error bar, and (B) with qPCR instrument. Non-specific reaction containing synthetic DNA harbouring the WT allele (WT allele) and specific reaction containing synthetic DNA harbouring the MT allele (MT allele).

Table 1. TTP and pH measurements of allele-specific pH-LAMP.

Reaction	qPCR (Light Cycler 96)		ISFET platform	
	TTP (min ± SD)	ΔpH (pH units)	TTP (min ± SD)	ΔpH (pH units)
MT allele	18.65 ± 0.29	1.32	20.82 ± 1.05	1.40
WT allele	-	0.24	-	0.16

CONCLUSIONS

- High allele **specificity** (ONLY MT allele amplified)
- High speed (less than 25 min)
- **Real-time** detection
- High sensitivity

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