A point-of-care field trial applying ATR spectroscopy to diagnose asymptomatic carriers of malaria

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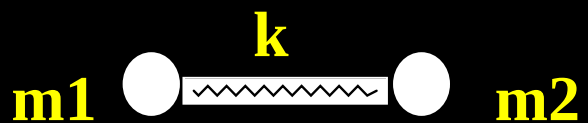
Outline of Talk

MONASH UNIVERSITY
Centre for Biospectroscopy

1. Detection of malaria (laboratory studies)
2. Detection of malaria (field studies using venous blood samples)
    a. Thailand (approaching 319 patients)
    b. Laos (525 patients)-Data analysed
    c. PNG (235 patients)-Data to be analysed
3. Detection of malaria (field study using fingerprick blood sample and RBC lysis)
4. Future Directions

Classical picture

$$\bar{\nu} = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}}$$

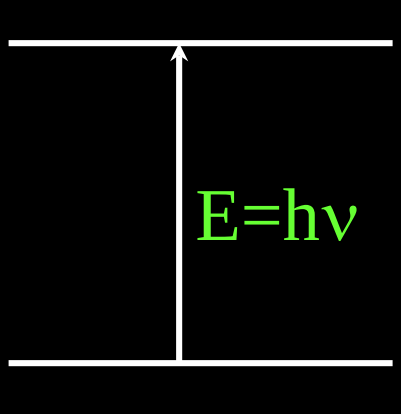


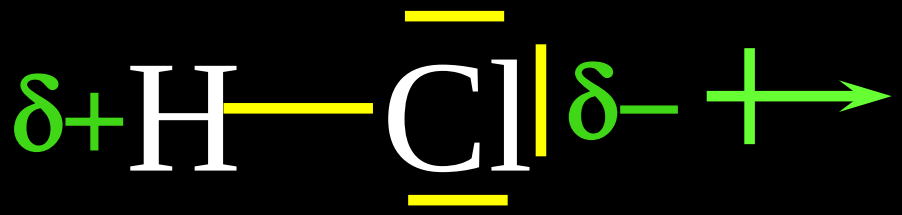
stretching
contracting

$$\mu = \frac{m1m2}{m1+m2}$$

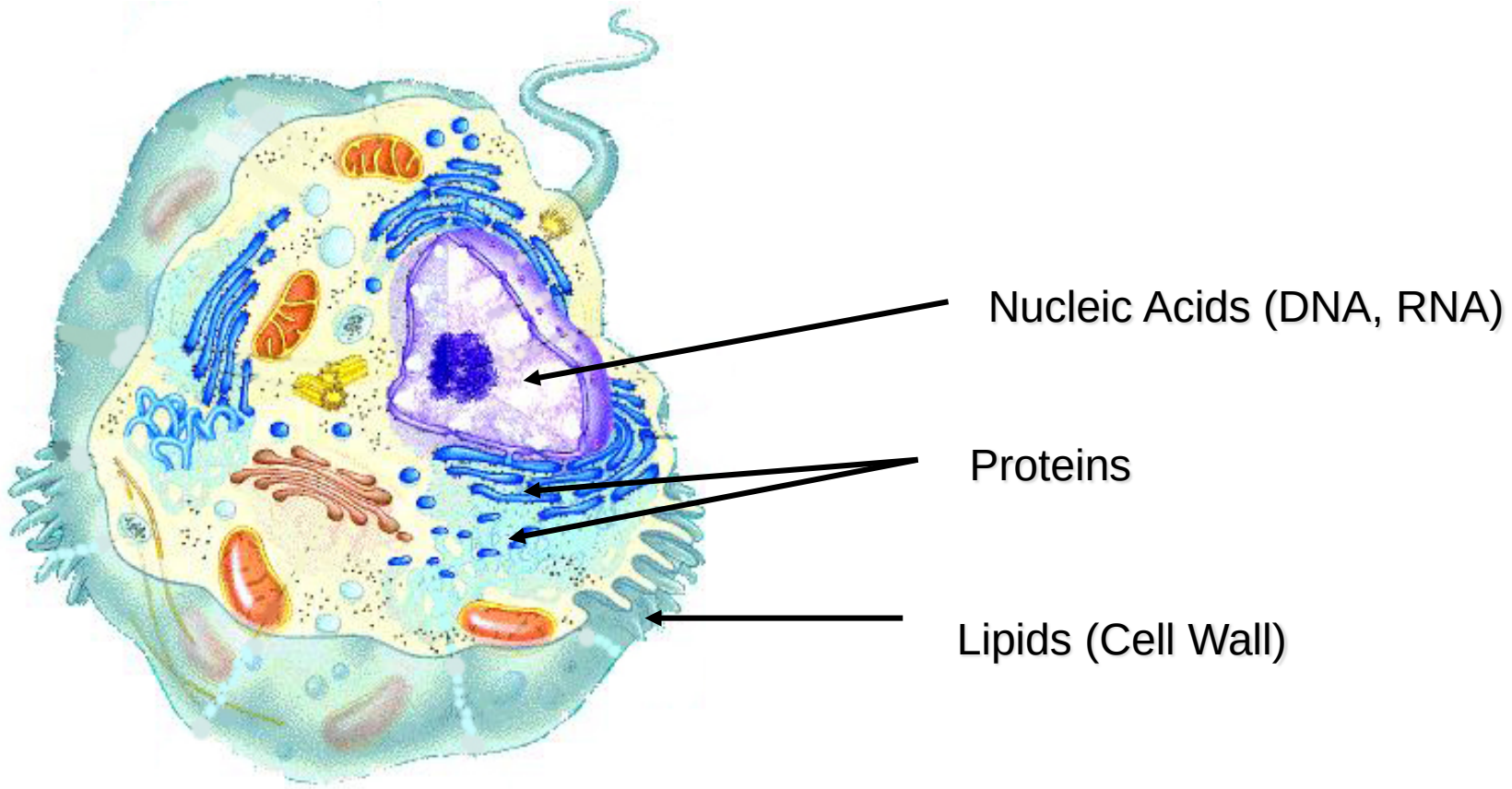
Quantum picture

Requires a change in dipole moment to be IR active



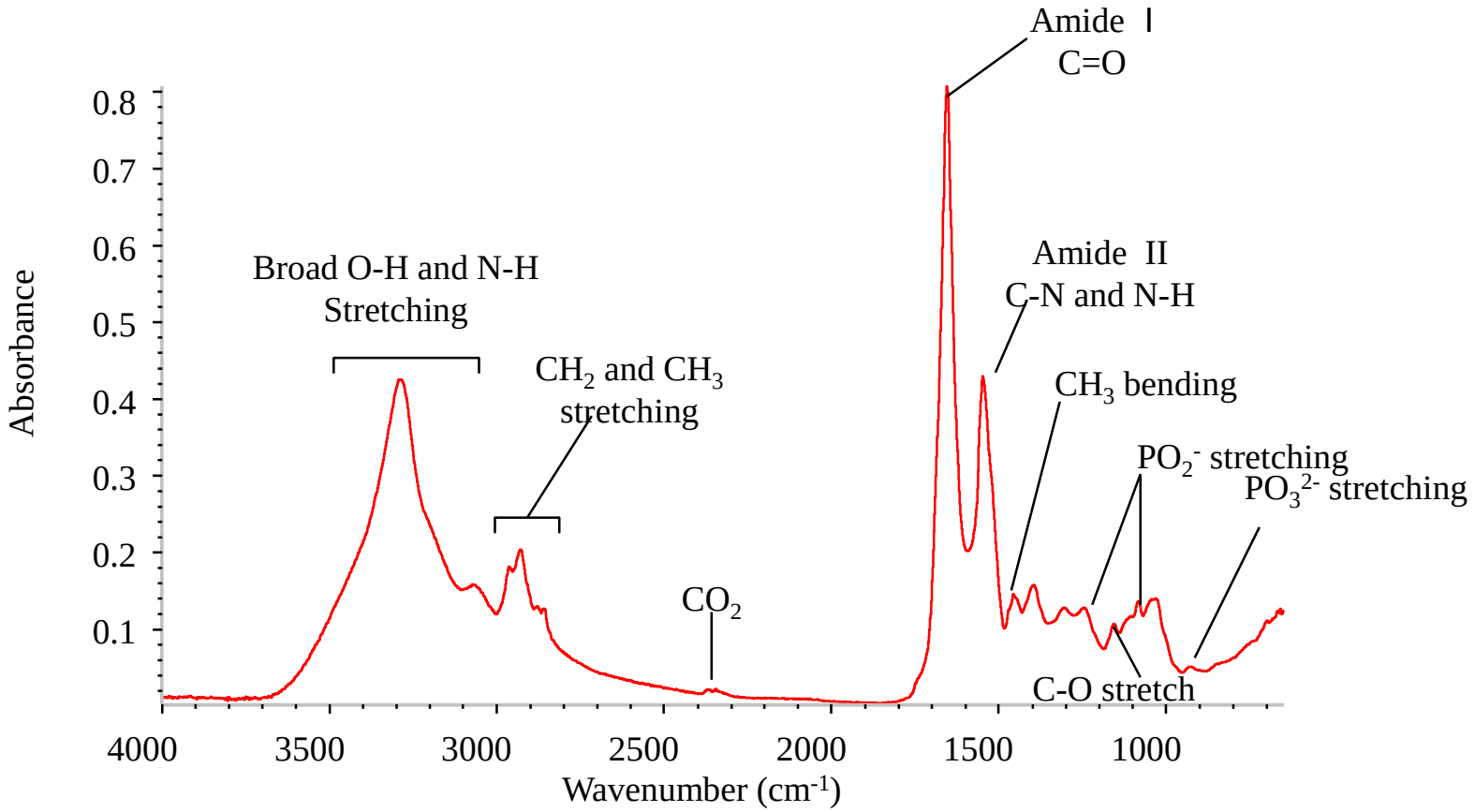


IR Marker



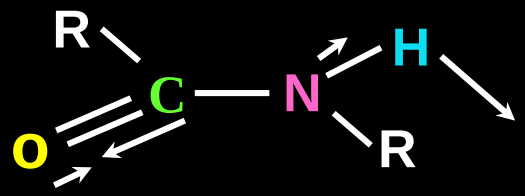
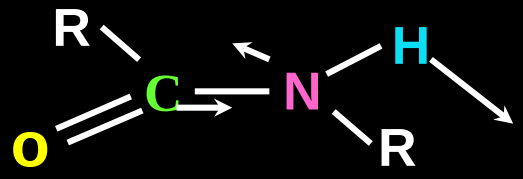
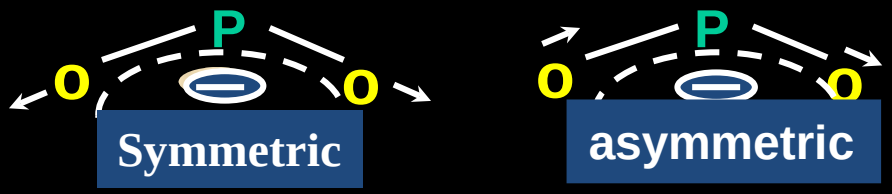
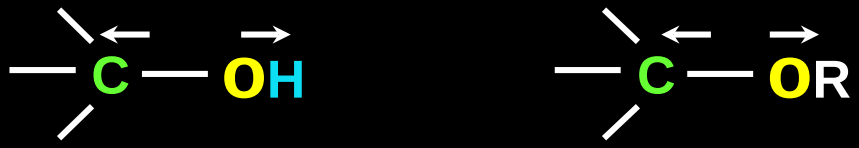
Each of these major classes of cellular components have **distinct IR markers**

Typical IR Spectrum of a biological sample

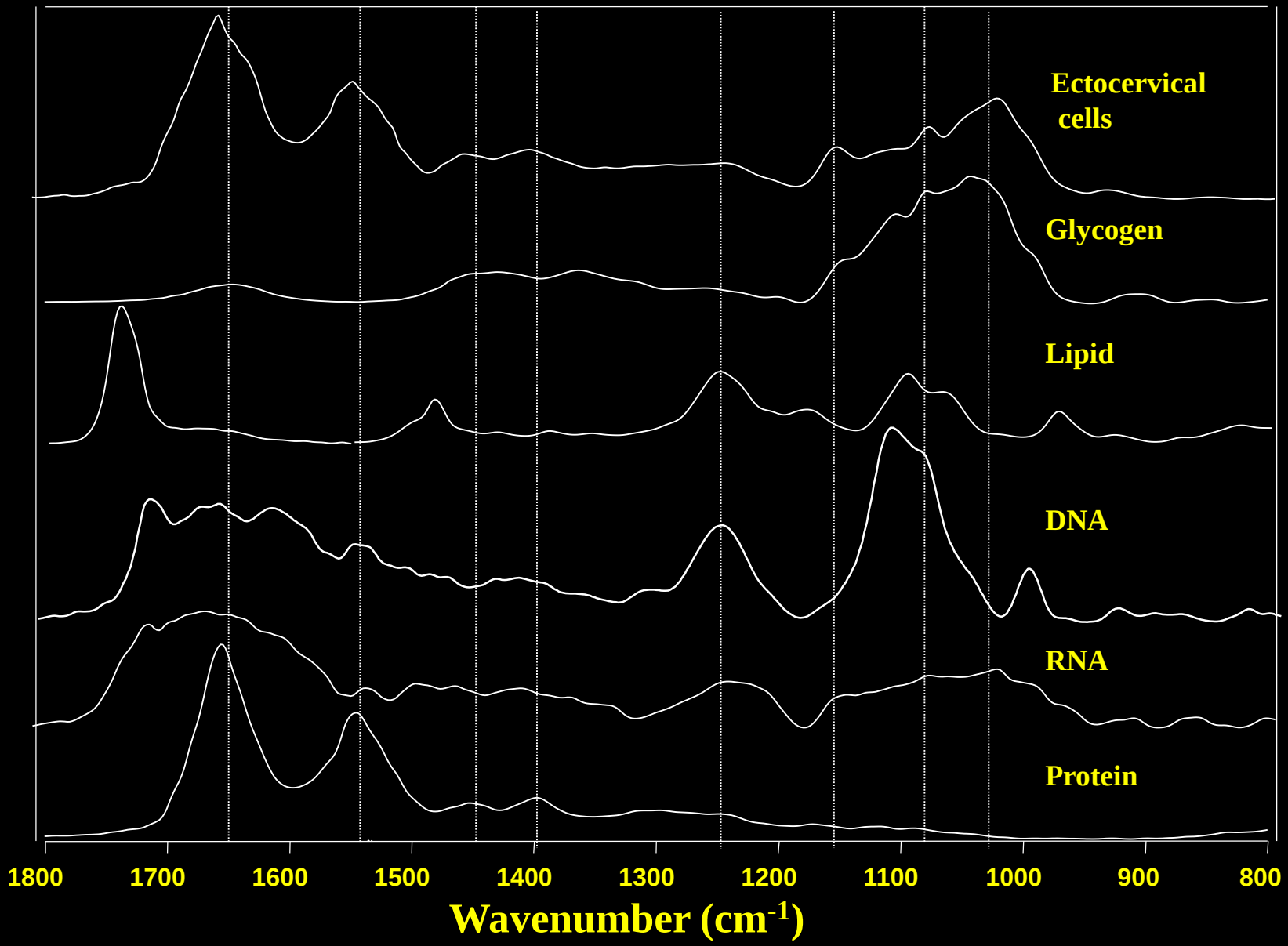


Amide I: 1690 – 1600
Amide II: 1575 – 1480
Lipid $=CH_2$: 3100 – 3000
Lipid $-CH_2$, $-CH_3$: 3000 – 2850
Nucleic Acid $-PO_2$: 1225, 1084

Vibrational modes of Biomolecules

Name of Mode	Mode	Wavenumber
Amide I		1650 cm^{-1}
Amide II		1544 cm^{-1}
Phosphate stretches		1080 cm^{-1} and 1244 cm^{-1}
Carbohydrate Stretches		1058 cm^{-1} and 1024 cm^{-1}

FTIR Analysis of Macromolecules



Types of malaria parasites and vectors

- *P. vivax* is less virulent form
- *P. falciparum* accounts for 80% of all human malarial infections and 90% of malarial deaths throughout the world
- Transmitted by *Anopheles* mosquito

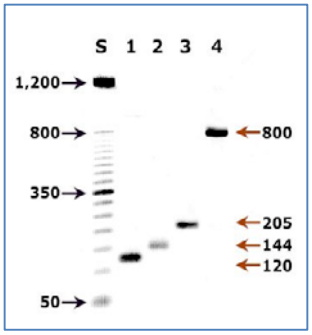
Current routine diagnostic tests available

Dipsticks, RDT strips and cassettes

- Cheap
- Can detect 70-80 parasites/µL
- Results are usually obtained within 5 – 15 minutes
- Can detect some other species of parasite but NOT early stages
- Can't quantitate parasitemia

Optical microscopy

- Cheap
- Can detect 40 parasites/µL
- Requires skilled microscopist



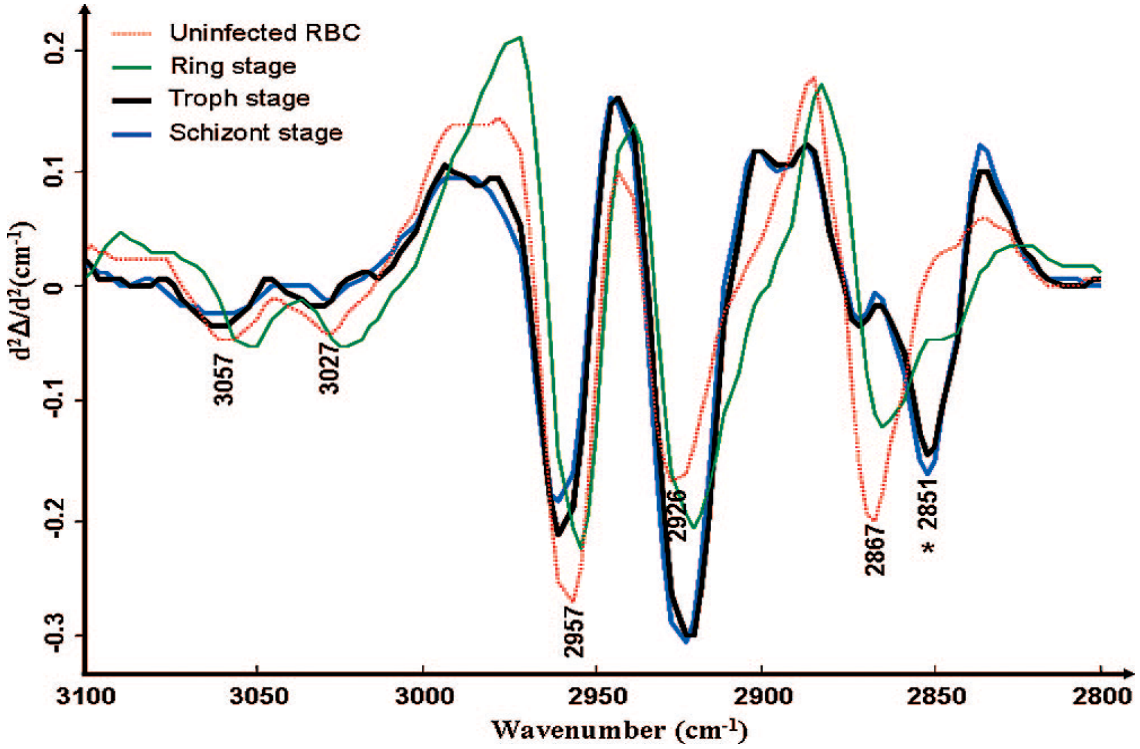
PCR assay

- Expensive
- High sensitivity

1 parasites/µL

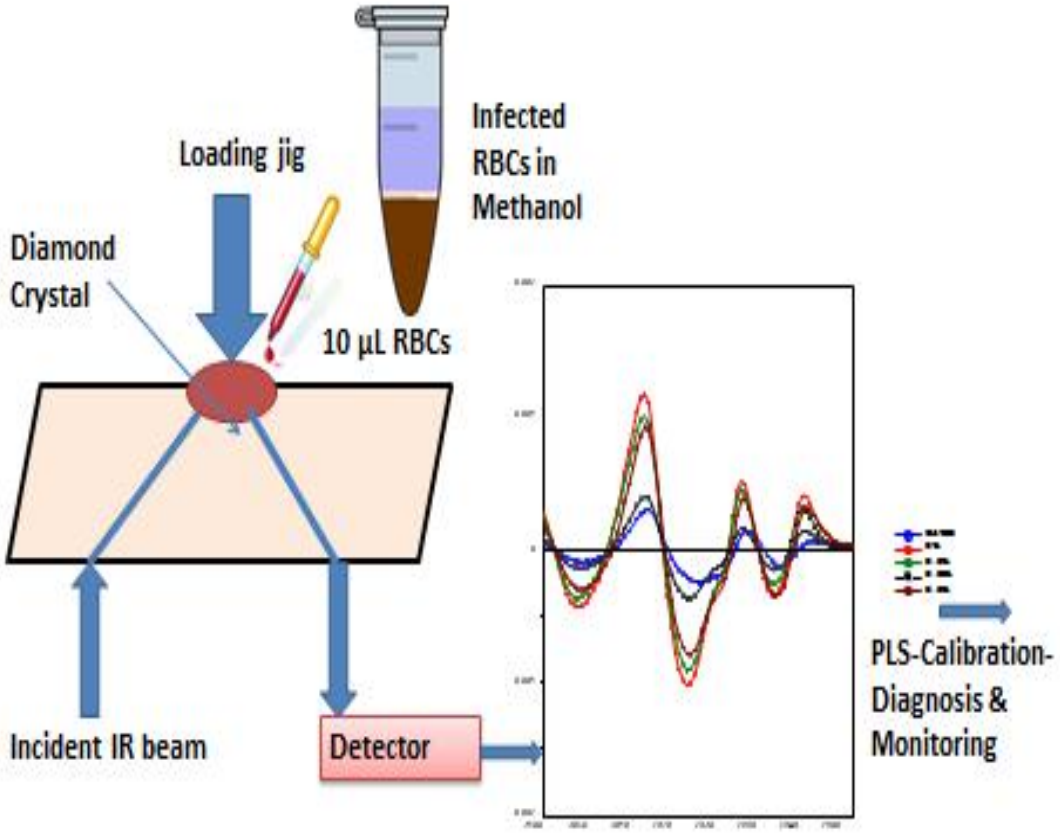
- Requires skilled lab tech
- 2-3 hours for a result

Synchrotron FTIR reveals unique lipid signature for malaria



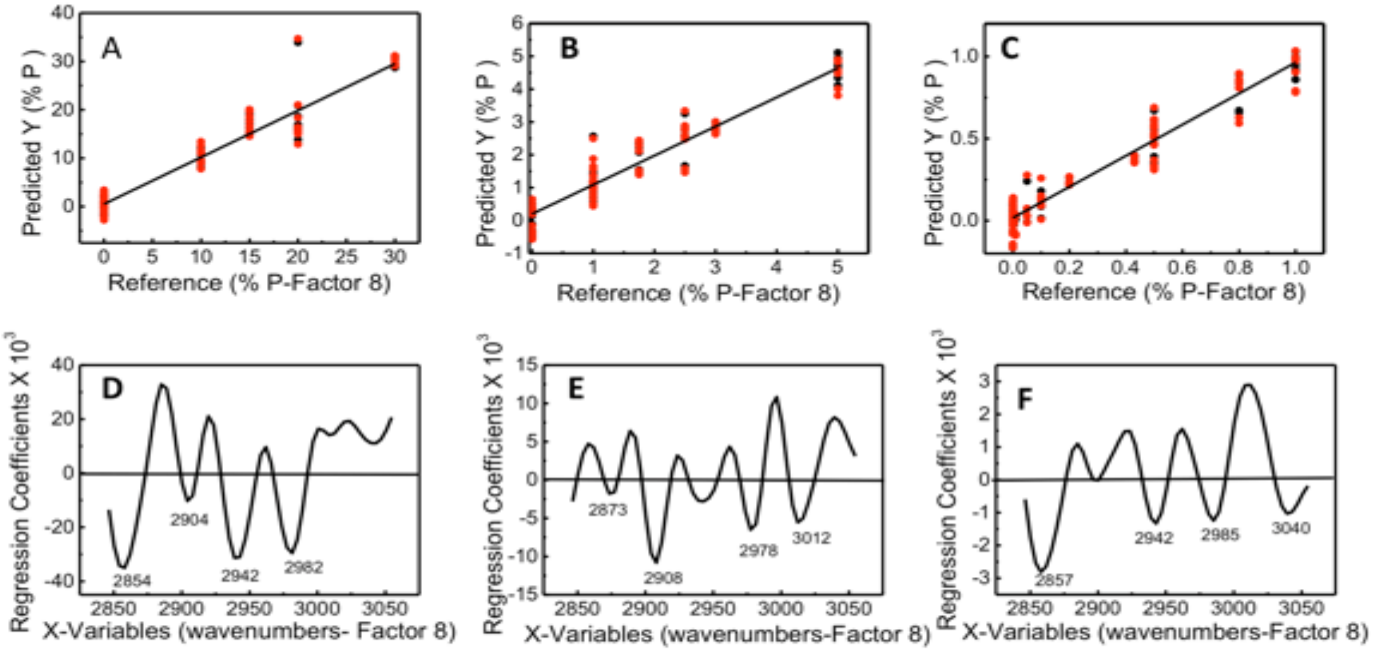
Average second-derivative spectra for infected RBCs (ring, trophozoite, and schizont stage parasites) and uninfected RBCs (control) of the C-H stretching region. (The asterisk indicates the appearance of a unique lipid band that characterises the parasite at all stages).

Attenuated Total Reflection Spectroscopy



ATR-FTIR with Single reflection diamond ATR accessory

PLS Calibration and validation plots for
A) 5-15 %, B)1-5 % and C) 0-1%, along with corresponding regression plots



Pilot Field Study in Thailand

Goal 1

To determine sensitivity and specificity of our existing technology in a field setting

Goal 2

Comparison of our technology versus PCR
and thin/ thick film microscopy.

Machines to be supplied by Agilent (non-commercial sponsor)
Diagnostic Trial Sponsored by Monash University

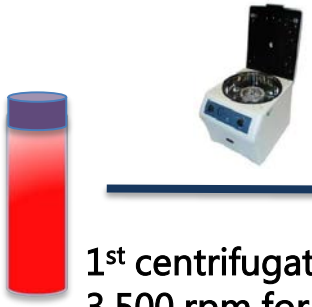
Professor Patcharee Jearanaikoon
University of Kohn Kahn

Monash-KKU Malaria Diagnostic Trial 2015 (MK MDT 2015)

Processing from site

Drawing blood from venue blood

Collecting blood into EDTA



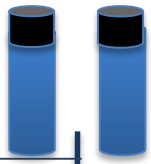
1st centrifugation at 3,500 rpm for 10 minutes (blood component separation)

Plasma
WBC
RBC

Plasma collection

Discarding white blood cells

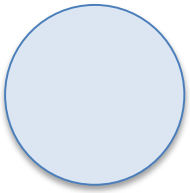
2nd centrifugation at 3,500 rpm for 10 minutes (red blood cell packing)



Collecting red blood cells into 2 methanol tubes (tube A and B)

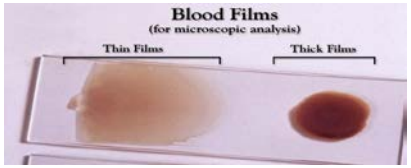
Whole blood collection and rapid strip test

Drop on filter paper



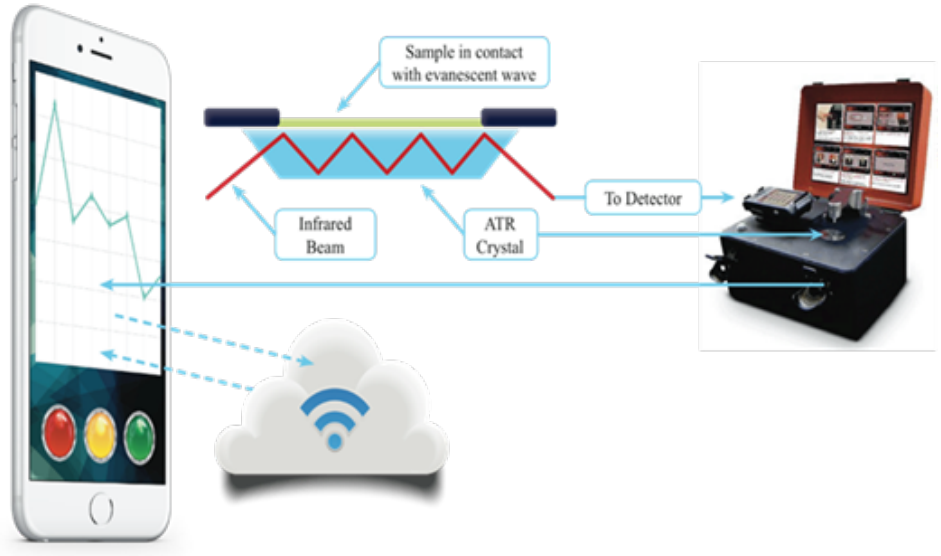
Thin film

Thick film



Data loaded on Google drive in spread sheet

Malaria sample collection in Thailand

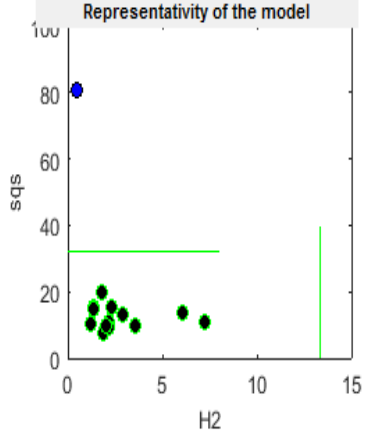


LOCATION	Cal Positive	Cal Negative	Val Positive	Val Negative	TOTAL POSITIVE	TOTAL NEGATIVE	SUM
UB	87	52	31	15	118	67	**185**
KK	0	54	0	37	0	91	**91**
KS	20	3	1	0	21	3	**24**
PP	5	2	7	5	12	7	**19**
SUM	112	111	39	57	151	168	**319**

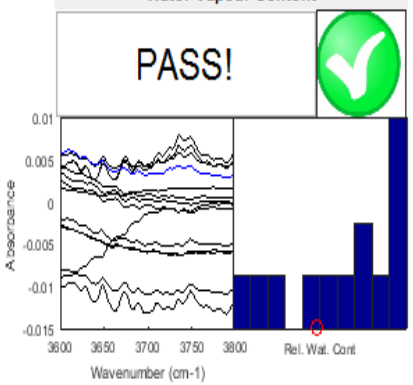
LOAD SPECTRA

QUALITY CONTROLS

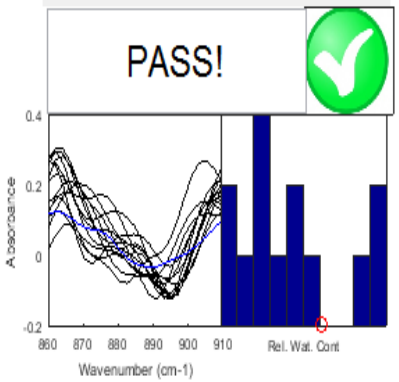
Representativity of the model



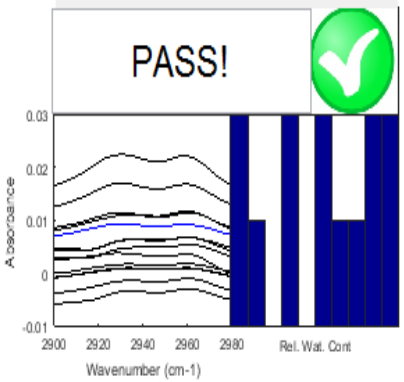
Water Vapour Content



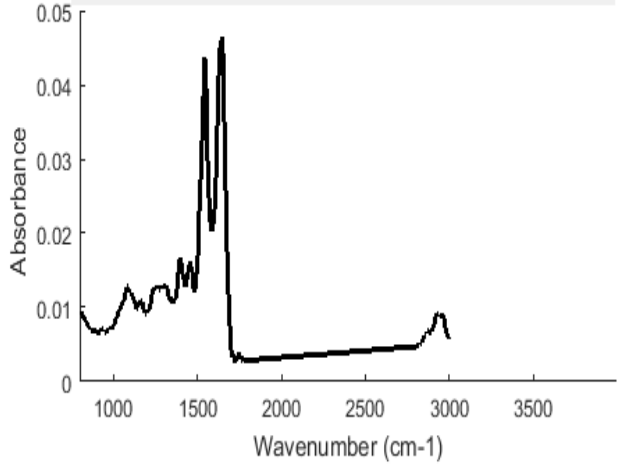
EtOH Content



Gel Content



Spectrum



GOOD! YOU CAN UPLOAD THE SPECTRA

SAMPLE PARAMETERS

Add Sample to the Dataset

Hemoglobine 12.6

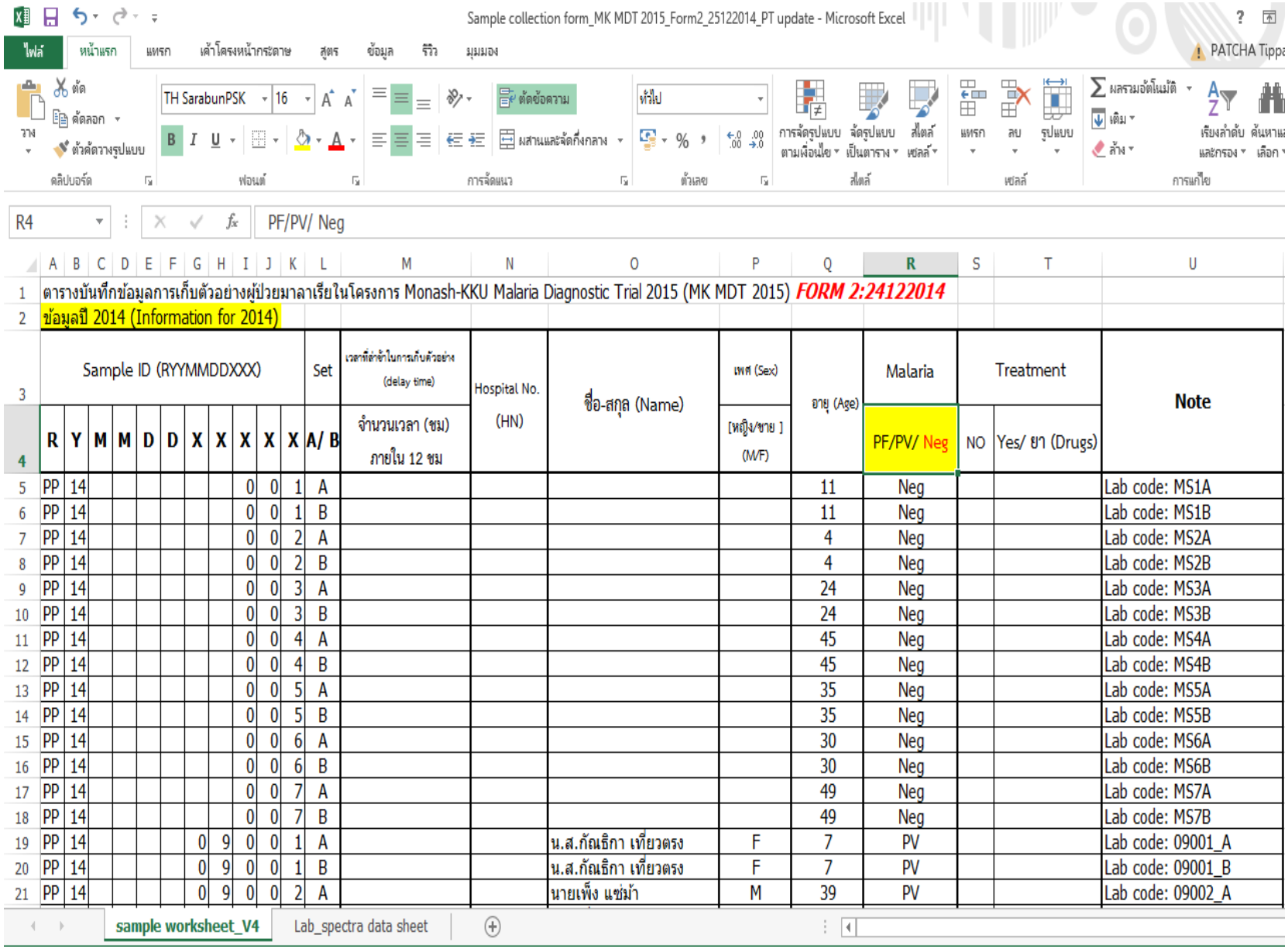
Glucose 4.6

Sample Name PNG02_B007_R1_W001.0

Date 31-Mar-2017

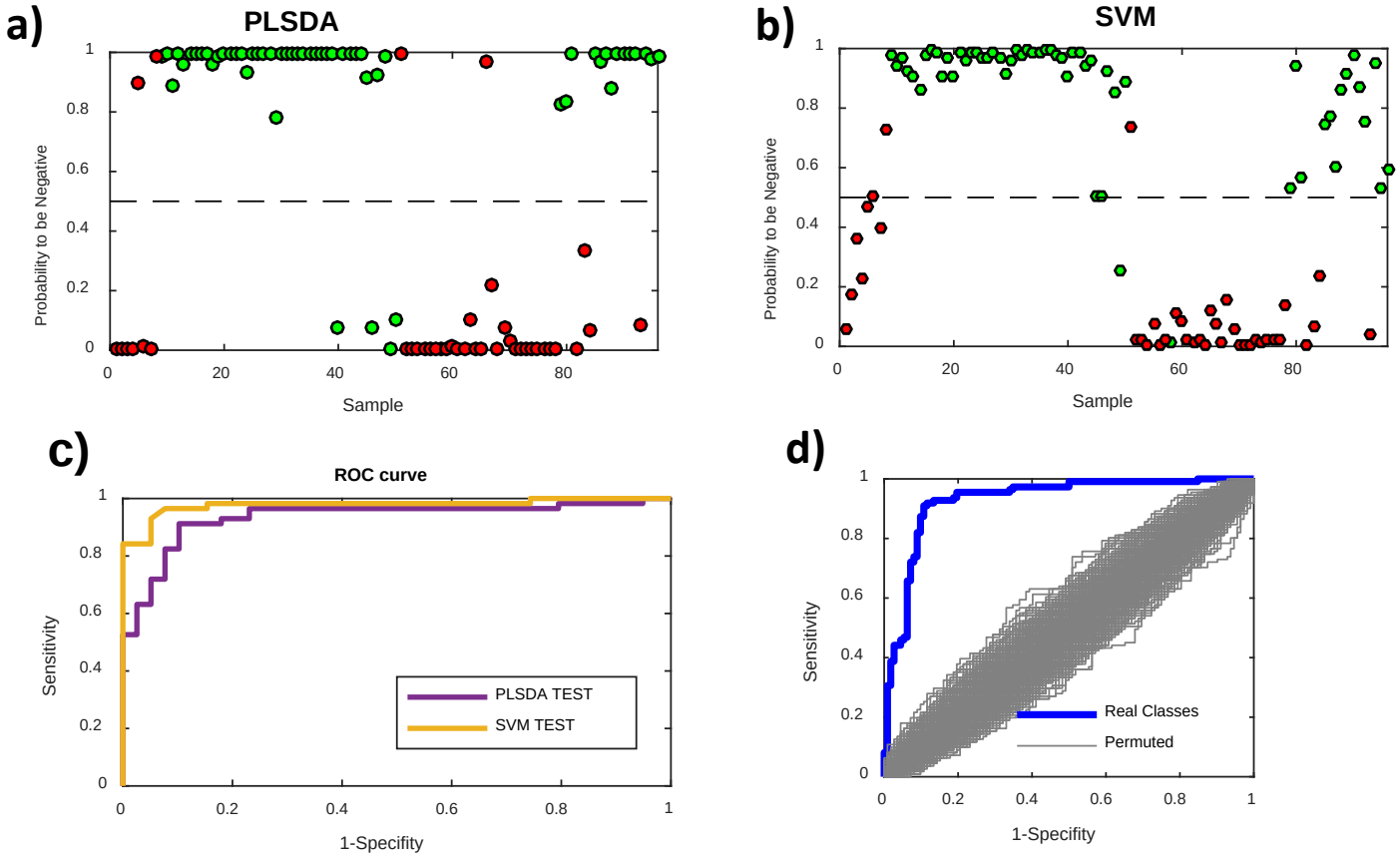
Instrument Inst 1

Data record



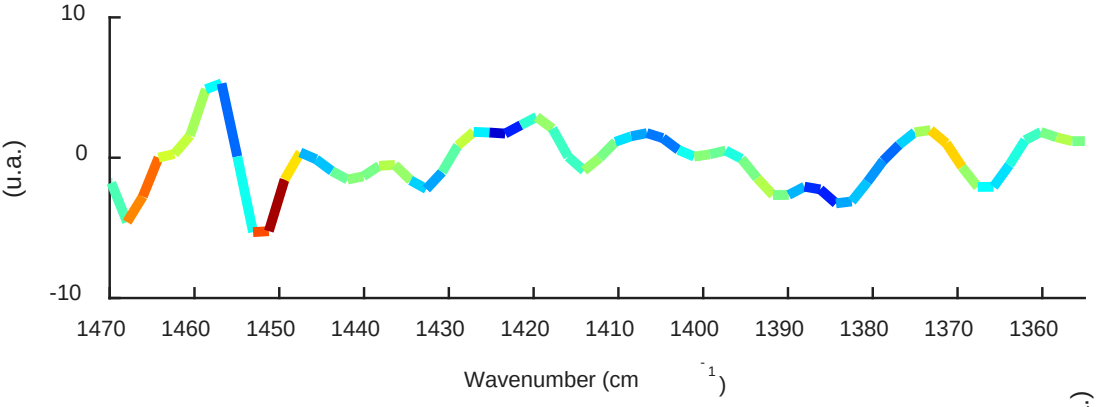
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	ตารางบันทึกข้อมูลการเก็บตัวอย่างผู้ป่วยมาลาเรียในโครงการ Monash-KKU Malaria Diagnostic Trial 2015 (MK MDT 2015) *FORM 2:24122014*																				
2	ข้อมูลปี 2014 (Information for 2014)																				
3	Sample ID (RYYMMDDXXX)											Set	เวลาที่ล่าช้าในการเก็บตัวอย่าง (delay time)	Hospital No.	ชื่อ-สกุล (Name)	เพศ (Sex)	อายุ (Age)	Malaria	Treatment		Note
4	R	Y	M	M	D	D	X	X	X	X	X	A/ B	จำนวนเวลา (ชม) ภายใน 12 ชม	(HN)		[หญิง/ชาย] (M/F)		PF/PV/ Neg	NO	Yes/ ยา (Drugs)	
5	PP	14							0	0	1	A					11	Neg			Lab code: MS1A
6	PP	14							0	0	1	B					11	Neg			Lab code: MS1B
7	PP	14							0	0	2	A					4	Neg			Lab code: MS2A
8	PP	14							0	0	2	B					4	Neg			Lab code: MS2B
9	PP	14							0	0	3	A					24	Neg			Lab code: MS3A
10	PP	14							0	0	3	B					24	Neg			Lab code: MS3B
11	PP	14							0	0	4	A					45	Neg			Lab code: MS4A
12	PP	14							0	0	4	B					45	Neg			Lab code: MS4B
13	PP	14							0	0	5	A					35	Neg			Lab code: MS5A
14	PP	14							0	0	5	B					35	Neg			Lab code: MS5B
15	PP	14							0	0	6	A					30	Neg			Lab code: MS6A
16	PP	14							0	0	6	B					30	Neg			Lab code: MS6B
17	PP	14							0	0	7	A					49	Neg			Lab code: MS7A
18	PP	14							0	0	7	B					49	Neg			Lab code: MS7B
19	PP	14					0	9	0	0	1	A			น.ส.กัณธิกา เที่ยวตรง	F	7	PV			Lab code: 09001_A
20	PP	14					0	9	0	0	1	B			น.ส.กัณธิกา เที่ยวตรง	F	7	PV			Lab code: 09001_B
21	PP	14					0	9	0	0	2	A			นายเพ็ง แซ่ม้า	M	39	PV			Lab code: 09002_A

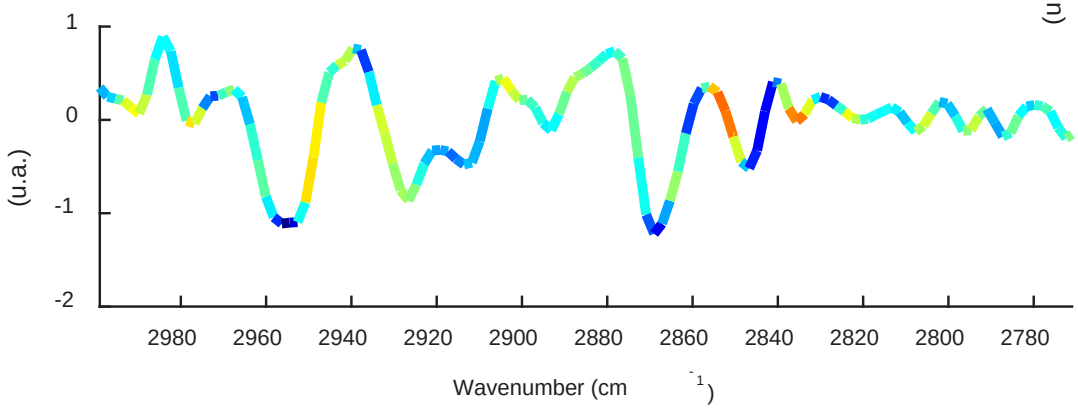
RESULTS

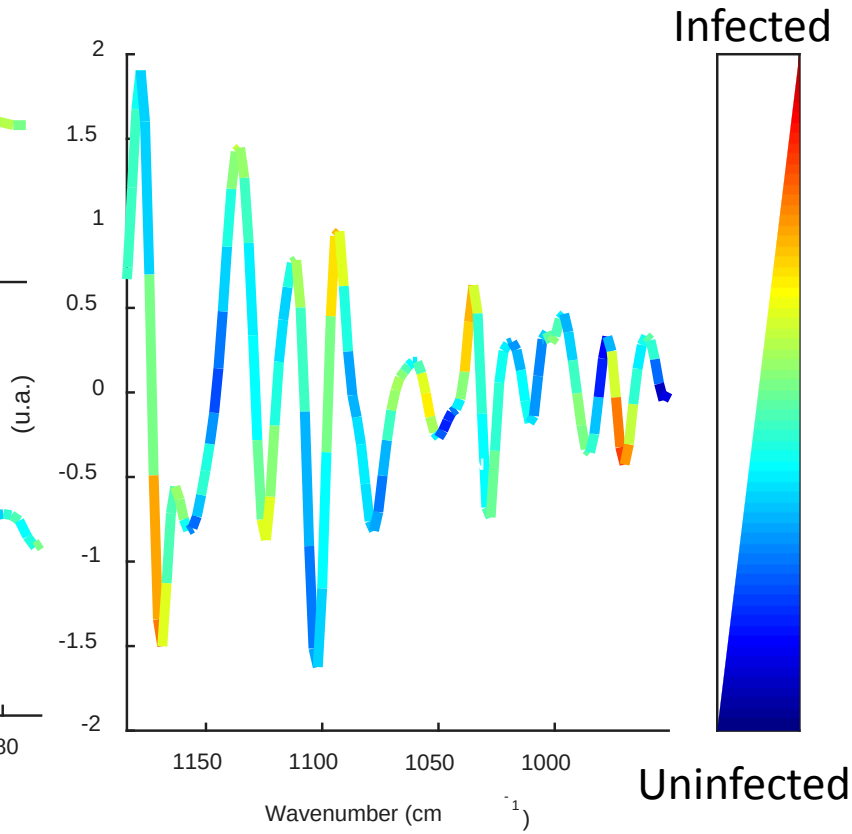


MODEL	PLSDA	SVM
SPEC (%)	91.22	96.5
SENS (%)	90.01	92.3
ERROR (%)	9.37	5.2
AUROC	0.934	0.9775

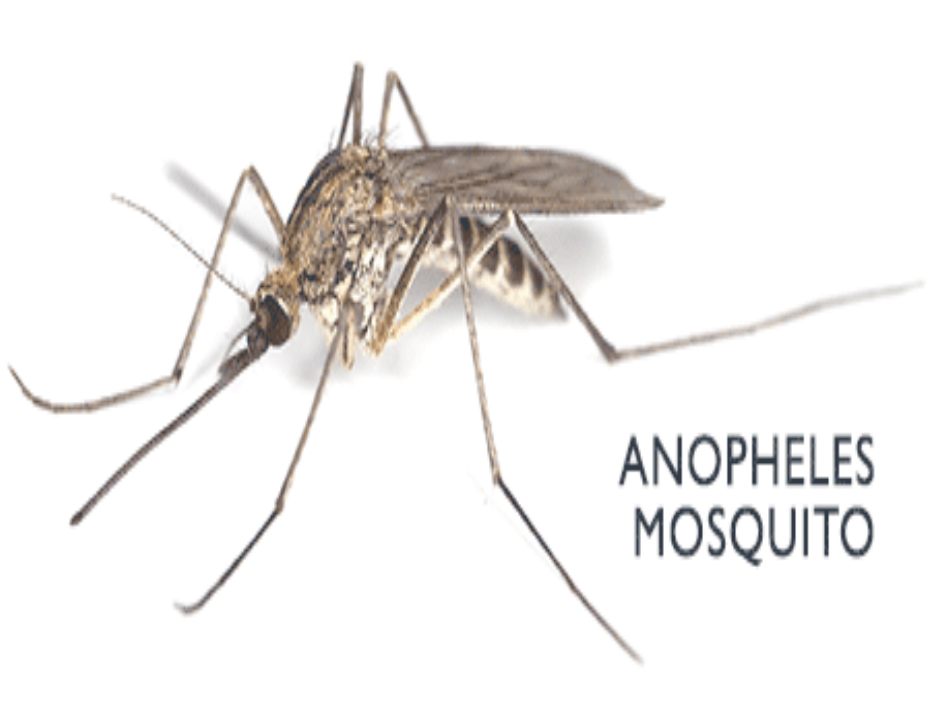
Spectral classification (is not a black box)







Blood, sweat and tears: Spectroscopy in the PNG


ANOPHELES
MOSQUITO

Malaria in the PNG is on the rise

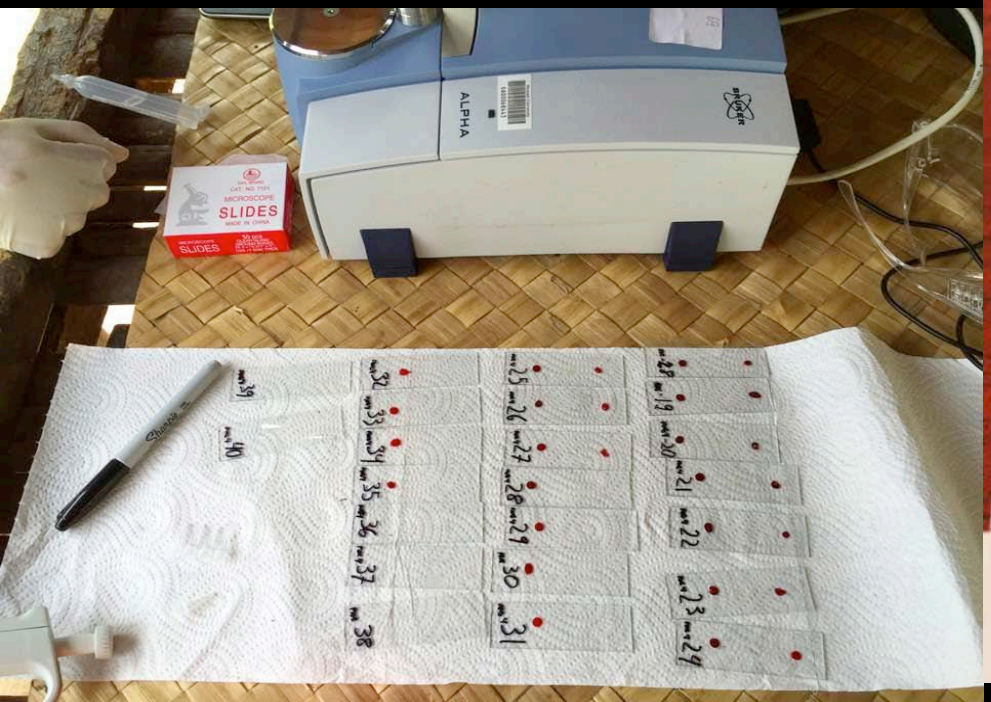
- Largest disease burden in PNG
- Morbidity: leading cause of all outpatient visits
- Mortality: third leading cause of hospital admissions and deaths
- Endemic in every province, including those that were once malaria-free
- Underlying cause of high levels of anemia
- Contributes to high levels of maternal and infant mortality
- Social and economic costs to PNG's development


Welcome to Tufi
International


bodichek
ALCOHOL SWAB
bodichek
ALCOHOL SWAB

235 patients x 8 tests

-wet whole blood
-wet serum
-dry whole blood
-dry serum
-dry RBCs
-RDTs
-PCR
-glucose (Diabetes)
-total Hb (Anaemia)



PATIENT CLINICAL SUMMARY — VILLAGE: Tufi — DATE: 16/3/17

Pt ID	Name	Home Village	Age / Sex	Ht	Wt	BGL	Hb	Clinical Presentation	Treatment
1	J	Tufi	22yr M.			5.2	12.0	T. 36.1°C Monash Malaria Test CareStart Malaria tve No.1.	Pt. thought he may have Malaria.
4	F	Eastern Heaven Highla.	47yo. M.			5.5	12.6	T. 35.7°C Monash Malaria Test	Eye problem, explained revisit when eye doctor here.
7	C	Sefoa Tufi	26yo. F.			4.6	12.6	T. 36.6°C Monash Malaria Test	Right knee. Fell and hurt it → Tubigrip.
12		Tufi	34yo. M.			6.1	13.3	T. 36.9°C Monash Malaria Test. Voltarenrub	Swollen left calf, started on 14/3/17. Elevation of limb and Ibuprofen 2×TID.
17.	D		24yo F.			4.3	12.5	T. N/A Monash Malaria Test.	No other complaints (Son Jeffrey - YAWS)
16.	J	Tufi	32yo M.			5.2	15.5	T. 35.8°C. Monash Malaria Test	No other complaints.
19	Pl	Tufi	16yo. F.			4.6	10.6	T. 37.0°C Stomach/gut pain. 3 days Monash Malaria Test	Rehydration salts. Diarrhoea tve blood, vomiting. Headache. Missed period.
	Cl	Tufi	3yo. F.		8kg			T. 37.0°C Sweating profusely. Grandparents think. CareStart Malaria Test Negative.	she may have malaria. Bilateral perforated ear-drums.
24	Ec	Tufi	17yo. M.			4.7	16.3	T. 35.8°C Monash Malaria Test Past history of malaria.	Monash Test only
27.	Je	Tufi	57yo.			5.7	12.5	T. Monash Malaria Test only. Past history of Malaria.	Malaria Test only.

Field trial in Laos Southern Province

Champasak Province

Collaboration between:

Monash Centre for Biospectroscopy

Institute Pasteur du Laos (Dr Paul Brey)

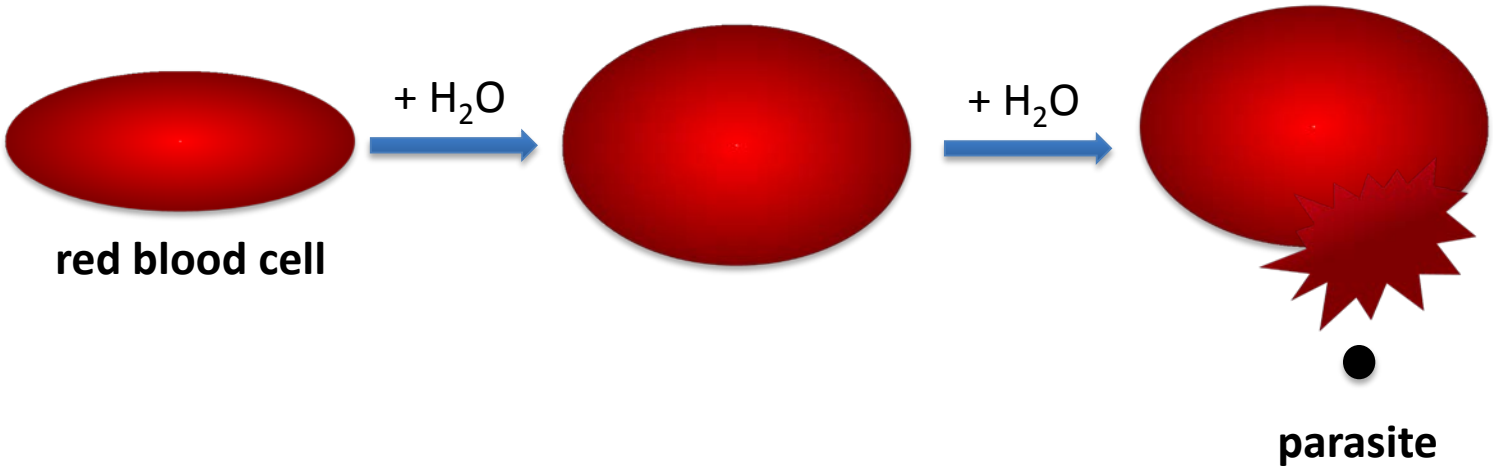
National Centre for Global Health and Medicine, Japan (Dr Moritoshi Iwagami)

595 patients tested with 7 RDT positives (3 P.f. and 4 P.v.)

Estimated 50 asymptomatic carriers (to be tested by PCR)

But we only got 20 in the end!!

Lyses of blood

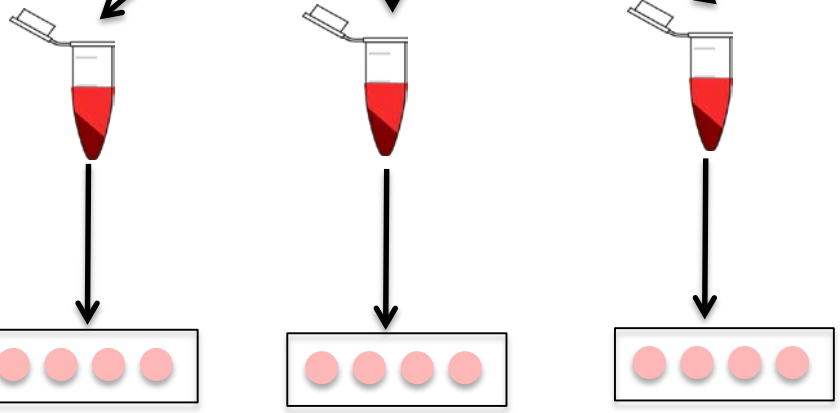


Workflow for lysed samples (dry)

Culturing of parasites at different concentrations

Spiking of whole blood with infected red blood cells to different concentrations:
0.001%, 0.08%, 0.3%, 0.4%, 1%, 2%

200 µL 200 µL 200 µL



+ 400 µL aqua dest
shaking
centrifugation
remove 450 µL supernatent

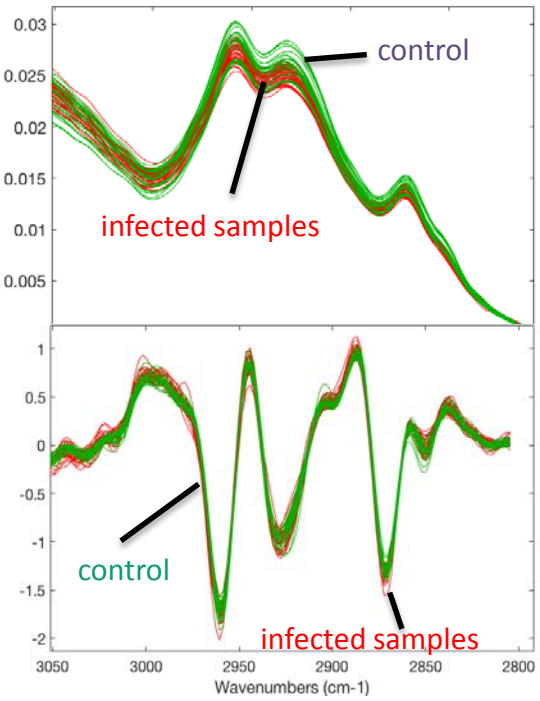
Lysed blood on glass slides (dry)

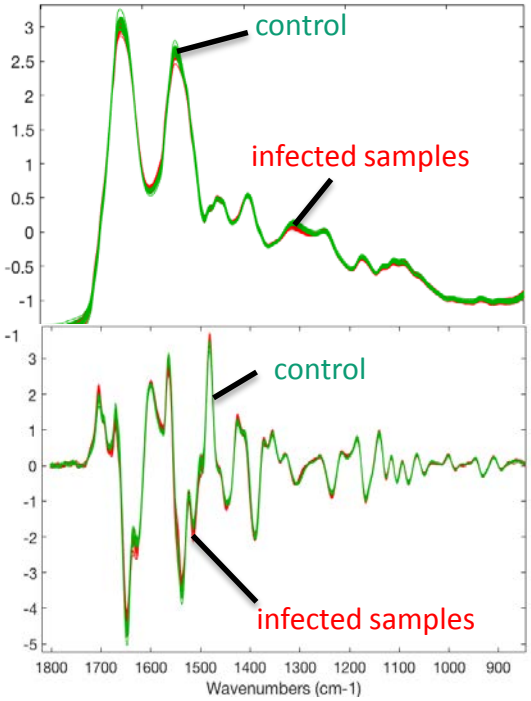
CH-stretching region

fingerprint region

ATR-FTIR spectra
(SNV normalized)

second derivative
(SNV normalized)



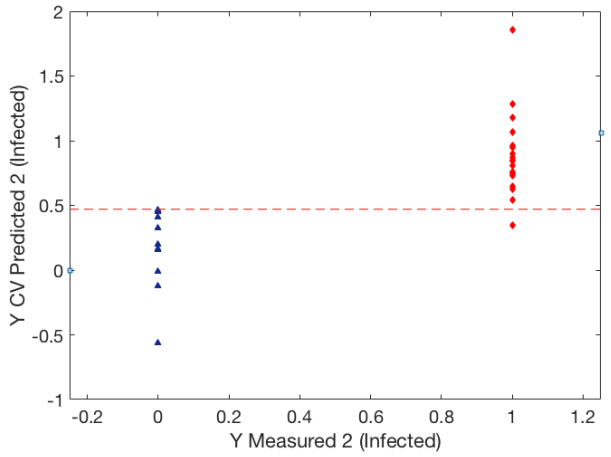


PLSDA on lysed samples (dry)

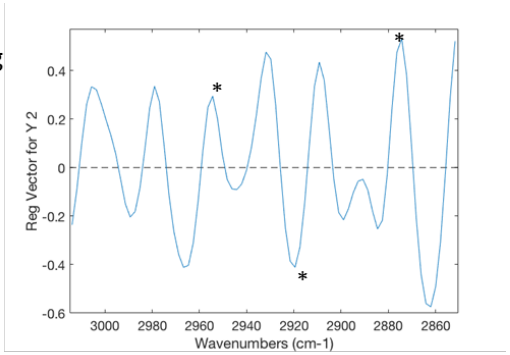
0.08%, 0.3% and 0.4% parasitemia

Second derivative (13pt smooth)
SNV
Mean center

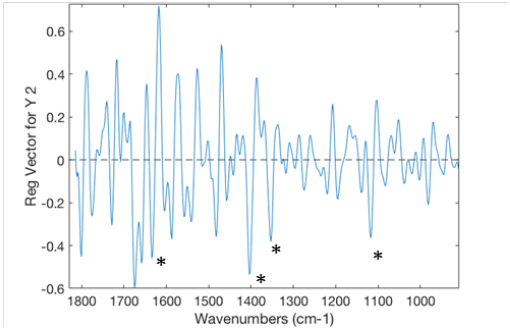
Sensitivity: 94.7%
Specificity: 90.9%
LV: 6
Error: 7.2%
Missclassified: 1
AUROC: 0.9809



CH-stretching region



fingerprint-region



Methods

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)

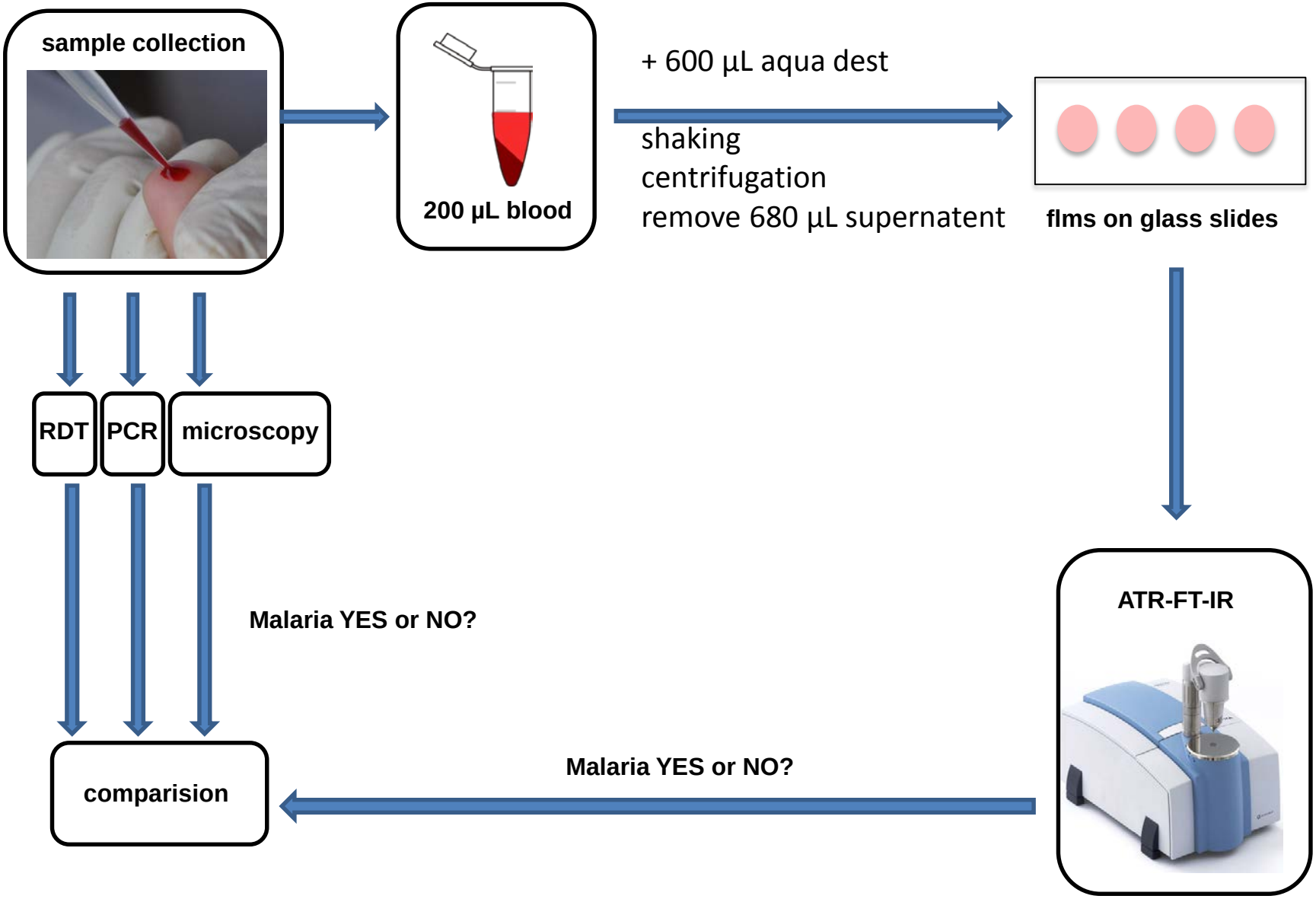
- Lysed blood samples
- Bruker Alpha ATR-FTIR spectrometer
- 64 scans
- 3 replicates
- Background before each new sample (64 scans)

Microscopy

Polymerase chain reaction (PCR)

Rapid diagnosis tests (RDT)

Workflow


sample collection
200 µL blood
+ 600 µL aqua dest
shaking
centrifugation
remove 680 µL supernatent
films on glass slides
RDT
PCR
microscopy
Malaria YES or NO?
ATR-FT-IR
comparision
Malaria YES or NO?

Villages: Phak kha
Pha lai thong
Pha lai bok
Nong hin
Thong xai
Thong Pha

Only 20 Asymptomatic carriers detected with qPCR…..

Not enough to build a robust model…

Sensitivity/specificity for asymptomatic carriers 81/82 %

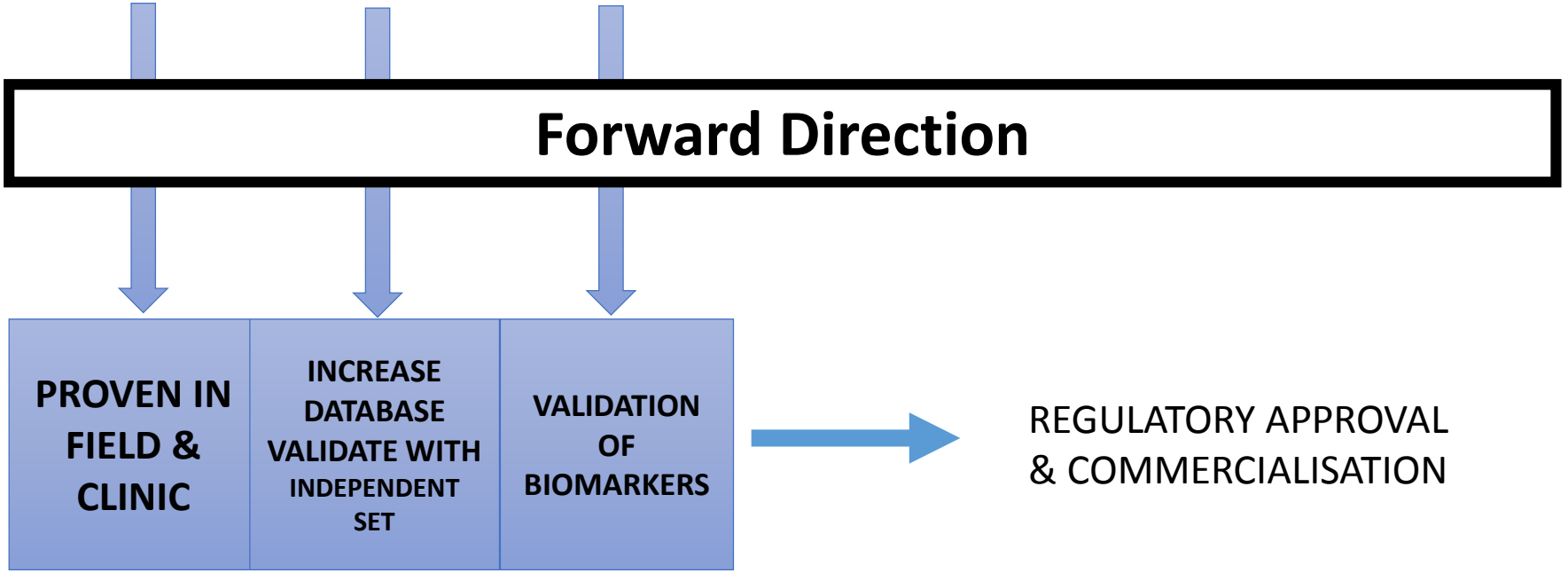
Model will dramaitcally improve if we could about 100 asymptomatic carriers

595 samples

7 RDT positives:
2 *P. falciparum*
5 *P. vivax*

Summary and Outlook

Sensitive Accurate Selective Fast (60 seconds) Cost 10 K No Reagent! Portable Easy to use



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