Development of Dried *Plasmodium falciparum* samples for quality control of malaria rapid diagnostics tests and as proficiency testing panels

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Rapid Diagnostic Tests

RDTs expected to play a critical role in adherence to WHO recommendations to test all suspected cases:

- Do not require an equipment
- Do not require electricity
- Relatively easy to train HCW to perform tests
- Most kits contain all items required for test
- ~20-25 minutes to perform.

Major challenges to RDT implementation

- *high storage temperatures may affect test performance*
- *high inter and intra-lot variation*
- *inadequate quality control at the point of care*

RDT Quality Control

Current methods for RDT QC include:

- comparing RDT results to smear microscopy
  - quality of microscopy
  - antigen detection vs. parasite detection
- Preparing known patient sample (fresh or frozen)
  - unknown characteristics
  - sample stability

Can dried *P. falciparum* infected blood be used as quality control samples for malaria RDTs?

Methods

Three Parasites Isolates

Nigeria XII, FC27/A3 and PH1@ 200 p/µl and 2000 p/µl

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Baseline testing on 10 RDT Brands

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Air-dry samples in tubes

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Store for up to 12 weeks @ 4°C, Room Temperature (~25°C) and 35°C

↓

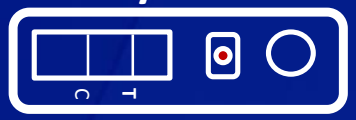
Rehydrate sample (3 parasites at each concentration and storage condition) and test on all 10 RDTs

↙ ↓ ↘

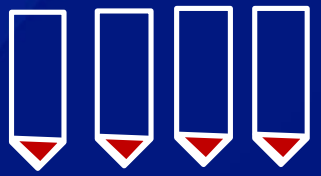
Week 1 Week 4 Week 12

Preparing Malaria Dried Tube Specimens (DTS)

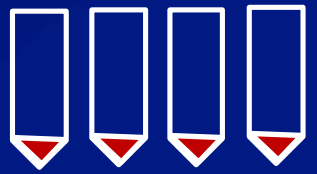
Test sample for Baseline reactivity on RDT



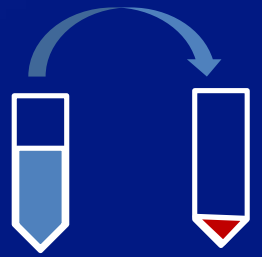
Make 50µL aliquots



Dry blood in BSC overnight



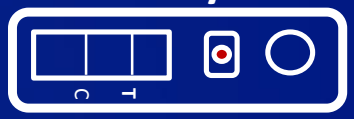
Rehydrate 1 vial after drying with 50 µL rehydration buffer (PBS-Tween20)



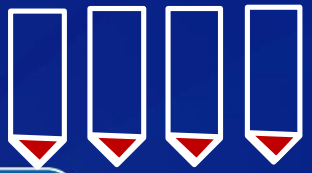
PBS/Tween20

Let rehydrated sample stand for ≥ 1 ½ hour

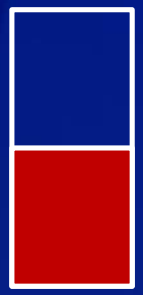
Test rehydrated sample for reactivity on RDT



Store DTS at 4°C



Obtain parasite positive blood with known parasite density



RDT Type and antigen specificity for the 10 RDTs used in this study

	RDT Type	Parasite Antigen Specificity	Number of Bands (including control)
RDT 1	Pf	HRP2/pLDH	2
RDT 2	Pf/Pan	HRP2/pLDH	3
RDT 3	Pf/Pv	HRP2/pLDH	3
RDT 4	Pf	HRP2	2
RDT 5	Pan	pLDH	2
RDT 6	Pan	pLDH/HRP2	3
RDT 7	Pf	HRP2	2
RDT 8	Pf/Pv	HRP2/pLDH	3
RDT 9	Pf	HRP2	2
RDT 10	Pf/Pan	HRP2/pLDH	3

RDT selection criteria:

1. Panel Detection Score (PDS) ≥90% in WHO/FIND RDT Evaluation Rounds 1 and 2
2. Availability for purchase

HRP2= Histidine rich protein 2
pLDH= Plasmodium lactate dehydrogenase
Pf= Plasmodium falciparum
Pv= Plasmodium vivax
Pan=All 4 plasmodium species

Results 1

The 10 RDT Brands grouped into 3 categories based on results:

1. RDT brands that detected all samples (n=4)
2. RDT brands that detected all but 1 sample (n=2)
3. RDT brands that missed multiple (53 of 506) samples (n=4)

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Results 1

Sensitivity of DTS detection influenced by the inter-related factors:

Target antigen (HRP2 or pLDH)

Parasite Density

Parasite isolate

How stable is DTS

Stability testing of Dried *P. falciparum* 3D7 stored at 4°C

	3D7 PD	RDT 5		RDT 2			RDT 10		
		C	T	C	Pan	Pf	C	Pan	Pf
Baseline	1000p/µl	++++	++++	++++	++++	++++	++++	++	++++
Week 12		++++	++	++++	++	++++	++++	+	+++
Week 21		++++	++	++++	++	++++	++++	+	+++
Week 26		++++	++	++++	++	++++	++++	+	+++
Week 35		++++	++	++++	++	++++	ND		
Week 38		++++	++	++++	++	+++	ND		
Week 41		++++	++	++++	++	+++	ND		
		C	T	C	Pan	Pf	C	Pan	Pf
Baseline	2000p/µl	++++	++++	++++	++++	++++	++++	++++	++++
Week 12		++++	++++	++++	+++	++++	++++	++	++++
Week 21		++++	++++	++++	++	++++	++++	++	+++
Week 26		++++	++++	++++	++	+++	ND		
Week 35		++++	++++	++++	++	+++	ND		
Week 38		++++	++	++++	++	+++	ND		
Week 41		++++	++	++++	++	+++	ND		

Dried cultured-derived 3D7 stored at 4°C after air drying
RDT5 2-band pLDH
RDT2 3-band HRP2/pLDH
RDT10 3-band HRP2/pLDH
Number of "+" indicates relative band intensity

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Results 2

Stability testing of Dried *P. falciparum* 3D7 stored at 4°C

	3D7 PD	RDT 1		RDT 4	
		C	Pf	C	Pf
Week 73	1000p/µl	++++	++++	++++	++++
	2000p/µl	++++	++++	++++	++++

	3D7 PD	RDT 6	
		C	Pf
Week 95	1000p/µl	++++	++
	2000p/µl	++++	++

	3D7 PD	RDT 6	
		C	Pf
Week 109	1000p/µl	++++	++
	2000p/µl	++++	+++

Number of "+" indicates relative band intensity

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Can DTS be used to identify failing tests?

Failed Test simulation

Result 3
DTS can be used to identify failing tests

Time series experiments using punctured RDT pouches

	Punctured			Intact			Punctured			Intact		
	Room Temperature						37°C Humidified					
RDT 2	C	Pan	Pf	C	Pan	Pf	C	Pan	Pf	C	Pan	Pf
2 hrs Test 1	4+	2+	3+	4+	2+	3+	4+	1+	2+	4+	1+	2+
2 hrs Test 2	4+	2+	3+	4+	2+	3+	4+	1+	2+	4+	1+	2+
6 hrs Test 1	4+	2+	2+	4+	2+	2+	4+	1+	2+	4+	1+	2+
6 hrs Test 2	4+	2+	2+	4+	2+	2+	4+	1+	2+	4+	1+	2+
30 hrs Test 1	3+	1+	1+	4+	1+	2+	4+	1+	2+	4+	1+	2+
30 hrs Test 2	4+	1+	2+	4+	1+	2+	4+	1+	2+	4+	1+	1+
Week 2 Test 1	ND	ND	ND	4+	2+	2+	±	±	Neg	4+	1+	2+
Week 2 Test 2	ND	ND	ND	4+	2+	3+	±	±	Neg	4+	1+	2+
Week 4 Test 1	ND	ND	ND	4+	1+	2+	±	Neg	Neg	4+	1+	2+
Week 4 Test 2	ND	ND	ND	4+	1+	2+	±	Neg	Neg	4+	1+	2+

	Punctured		Intact		Punctured		Intact	
	Room Temperature				37°C Humidified			
RDT 5	C	T	C	T	C	T	C	T
2 hrs Test 1	4+	2+	4+	2+	4+	2+	4+	2+
2 hrs Test 2	4+	2+	4+	2+	4+	2+	4+	2+
6 hrs Test 1	4+	2+	4+	2+	4+	2+	4+	2+
6 hrs Test 2	4+	4+	4+	2+	4+	2+	4+	2+
30 hrs Test 1	4+	2+	4+	2+	4+	2+	4+	2+
30 hrs Test 2	4+	2+	4+	2+	4+	1+	4+	1+
Week 2 Test 1	ND	ND	4+	2+	±	Neg	4+	2+
Week 2 Test 2	ND	ND	4+	2+	±	±	4+	2+
Week 4 Test 1	ND	ND	4+	2+	±	Neg	4+	2+
Week 4 Test 2	ND	ND	4+	2+	Neg	±	4+	2+

Sample used was dried 3D7 at 1000 parasite/µl. Punctured RDTPouches had two 8mm holes on each side of the pouch

37°C= humidified incubator set at 37°C

Number of "+" indicates relative band intensity

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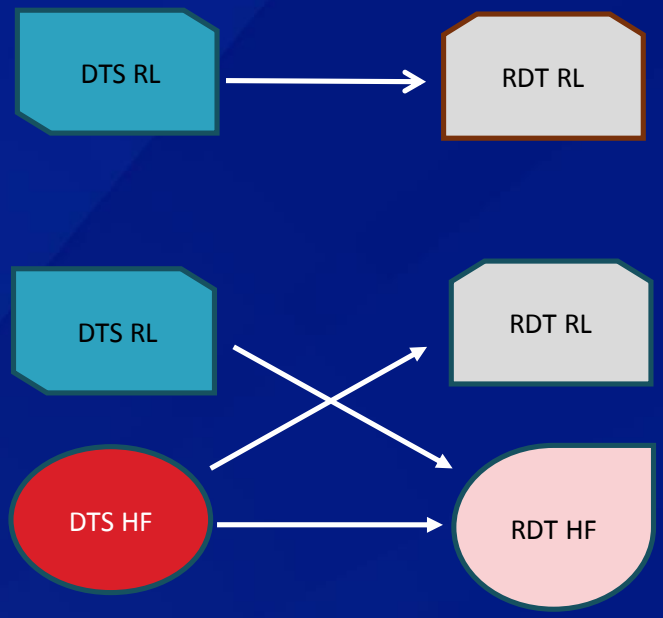
Can DTS be used under field conditions to monitor RDT performance and for Proficiency testing?

Testing Scheme



QC samples tested on weeks 0, 4, 8, 12, 16, 20 and 24

Ethiopia

DTS reactivity at Adama Regional Reference Laboratory

ARL DTS	ARL RDT Result						
	Wk0	Wk4	Wk8	Wk12	Wk16	Wk20	Wk24
0 p/µl	----	----	----	----	----	----	----
500 p/µl	++++	++++	++++	++++	++++	++++	++++
1000 p/µl	++++	++++	++++	++++	++++	++++	++++

DTS stored at 4^{O}C and RDTs stored under manufacturer recommended temperature

Results of reciprocal testing of HC and ARL-stored DTS and RDTs

Wanji DTS	ARL RDT		Wanji RDT		ARL DTS	Wanji RDT	
	Concordant (%)	Discordant (%)	Concordant (%)	Discordant		Concordant (%)	Discordant
0p/µl	7/7 (100%)	0	7/7 (100%)	0	0p/µl	7/7 (100%)	0
500p/µl	7/7 (100%)	0	7/7 (100%)	0	500p/µl	7/7 (100%)	0
1000p/µl	7/7 (100%)	0	7/7 (100%)	0	1000p/µl	7/7 (100%)	0

Walanchiti DTS	ARL RDT		Walanchiti RDT		ARL DTS	Walanchiti RDT	
0p/µl	7/7 (100%)	0	7/7 (100%)	0	0p/µl	7/7 (100%)	0
500p/µl	7/7 (100%)	0	7/7 (100%)	0	500p/µl	7/7 (100%)	0
1000p/µl	7/7 (100%)	0	7/7 (100%)	0	1000p/µl	7/7 (100%)	0

DTS for Proficiency Testing

Proficiency Panel (*weeks 12 and 24*)

Panel Sample A: 0 parasites/µl
Panel Sample B: 500 parasites/µl
Panel Sample C: 1000 parasites/µl
Panel Sample D: 500 parasites/µl

Checklist for observing health worker performance of DTS Proficiency Testing

	Yes = 1	No = 2	Comment
Correct volume of blood collected			
Correct volume of blood applied			
Blood applied to sample well			
Correct amount of buffer drops dispensed			
Buffer dispensed to buffer well			
Timer set for incubation			
Correct time set for incubation			
Results read at time specified by test			
Results interpreted correctly			

DTS for Proficiency Testing

Checklist for observing health worker performance of DTS Proficiency Testing

	Yes = 1	No = 2	Comment
Correct volume of blood collected	1		
Correct volume of blood applied	1		
Blood applied to sample well	1		
Correct amount of buffer drops dispensed	1		
Buffer dispensed to buffer well	1		
Timer set for incubation	1		
Correct time set for incubation	1		
Results read at time specified by test	1		
Results interpreted correctly		2	Faint Line missed

	Yes = 1	No = 2	Comment
Correct volume of blood collected		2	Excess Blood
Correct volume of blood applied		2	Excess Blood
Blood applied to sample well	1		
Correct amount of buffer drops dispensed	1		
Buffer dispensed to buffer well	1		
Timer set for incubation	1		
Correct time set for incubation	1		
Results read at time specified by test	1		
Results interpreted correctly	1		

Liberia

National Drug Quality Control Laboratory (NDQCL) DTS Reactivity

DTS stored at 4^{O}C and RDTs stored under manufacturer recommended temperature

NDQCL DTS on Health Facility and Reference Lab RDTs			
	% concordance		
Week	RL Results	SLP Results	SoS Results
0	100	100	100
4	100	100	100
8	100	***83****	100
12	100	100	100
16	100	100	***83****
20	100	100	100
24	100	100	100

** These samples were incompletely hydrated*

Duplicate tests for 0, 500 and 100 parasites/µL (Total of 6 tests/time point)

Results of reciprocal testing of Health facility and MQAL-stored DTS and RDTs

Duplicate tests for 0, 500 and 100 parasites/µL (Total of 6 tests/time point)

Slipway Health center		
Week	% concordance	
	RL RDTs	SLP RDTs
0	100	100
4	100	100
8	***83****	***83****
12	100	100
16	***67****	***50****
20	***33****	***33****
24	***33****	***33****

Star of The Sea community Health Center		
Week	% concordance	
	RL RDTs	SoS RDTs
0	100	100
4	100	100
8	***67****	***83****
12	100	100
16	**67***	**50***
20	**33***	**33***
24	**33***	**33***

** These samples were incompletely hydrated*

Summary and conclusions

1. Dried *P. falciparum* infected blood can be used as quality control samples for monitoring RDT performance
2. DTS can identify failing tests
3. DTS can be used to regulate QC sample parasite/antigen concentration
4. DTS is stable for 2yrs when stored at 4^{o}C . However, storage in ambient temperatures may affect sample integrity

Collaborators

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Thank You