

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

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*African Society for Laboratory Medicine 2014, Cape Town. December 1, 2014*



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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



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



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## Methods

Three Parasites Isolates  
Nigeria XII, FC27/A3 and PH1@ 200 p/ $\mu$ l and 2000 p/ $\mu$ l



Baseline testing on 10 RDT Brands



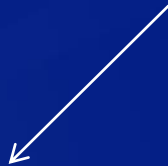
Air-dry samples in tubes



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



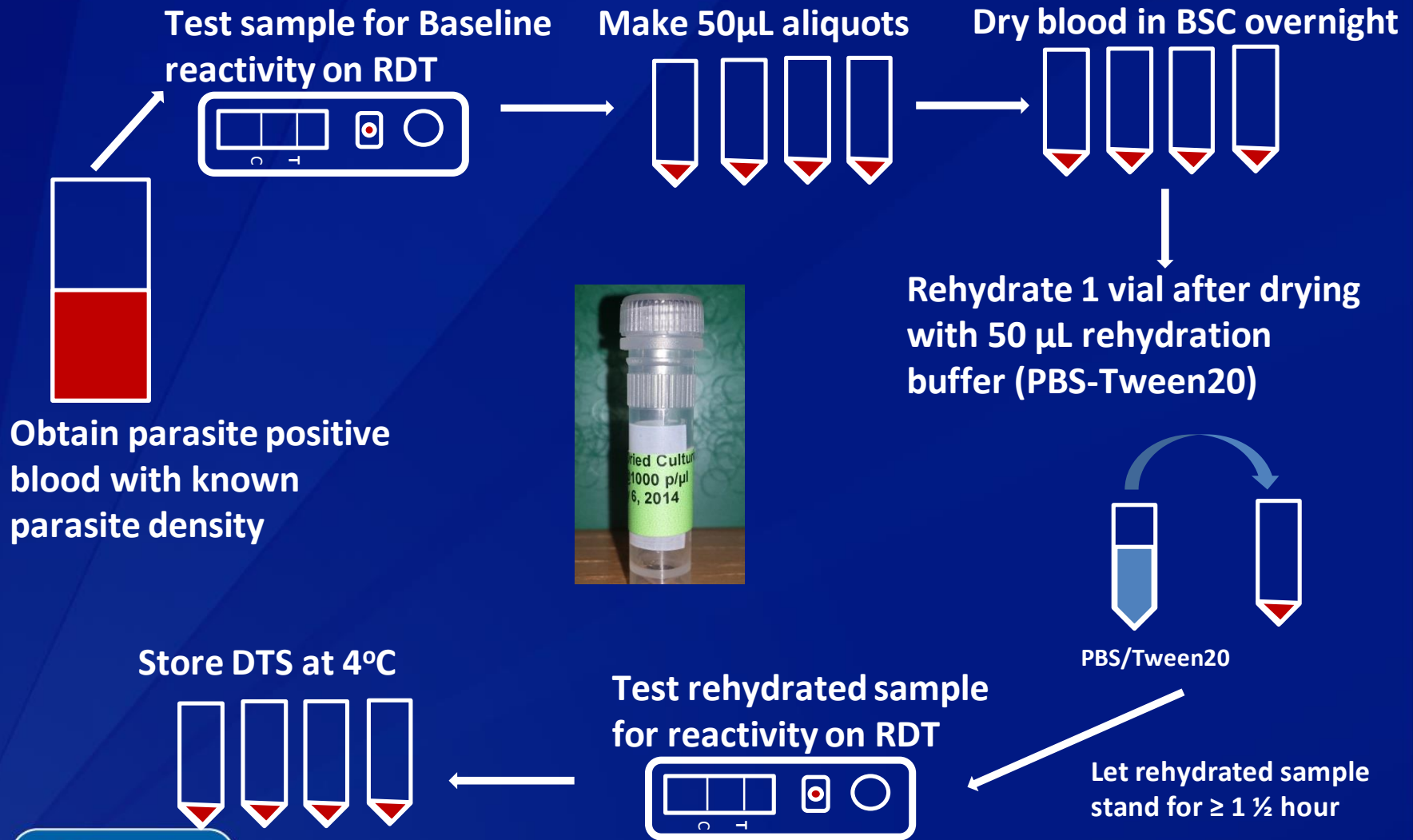
Week 4



Week 12



# Preparing Malaria Dried Tube Specimens (DTS)



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

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

## RDT selection criteria:

1. Panel Detection Score (PDS)  $\geq 90\%$  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*



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# 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)

*Aidoo M et al Malaria Journal 2012*



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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



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# How stable is DTS



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## 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

Aidoo M et al Malaria Journal 2012



## Results 2

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

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

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

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

Number of “+” indicates relative band intensity

*Aidoo M et al unpublished*



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# Can DTS be used to identify failing tests?



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# Failed Test simulation



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# 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 RDT Pouches 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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Aidoo M et al Malaria Journal 2012

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

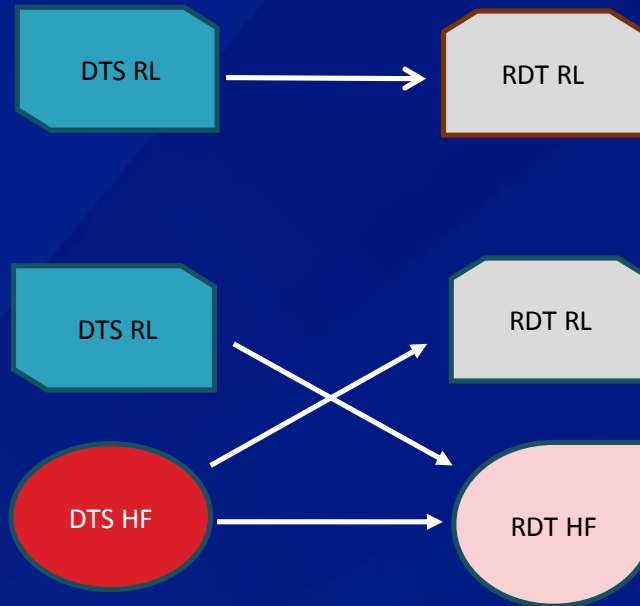


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# Testing Scheme



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



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# Ethiopia



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# 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°C and RDTs stored under manufacturer recommended temperature*



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# 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/ $\mu$ l

Panel Sample B: 500 parasites/ $\mu$ l

Panel Sample C: 1000 parasites/ $\mu$ l

Panel Sample D: 500 parasites/ $\mu$ l



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# 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



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# National Drug Quality Control Laboratory (NDQCL) DTS Reactivity

*DTS stored at 4°C and RDTs stored under manufacturer recommended temperature*

## NDQCL DTS on Health Facility and Reference Lab RDTs

Week	% concordance		
	RL Results	SLP Results	SoS Results
0	100	100	100
4	100	100	100
8	100	<b><u>83*</u></b>	100
12	100	100	100
16	100	100	<b><u>83*</u></b>
20	100	100	100
24	100	100	100

*\* These samples were incompletely hydrated*

Duplicate tests for 0, 500 and 100 parasites/ $\mu$ L (Total of 6 tests/time point)



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

Duplicate tests for 0, 500 and 100 parasites/ $\mu$ L (Total of 6 tests/time point)

Slipway Health center			Star of The Sea community Health Center		
Week	% concordance		Week	% concordance	
	RL RDTs	SLP RDTs		RL RDTs	SoS RDTs
0	100	100	0	100	100
4	100	100	4	100	100
8	<u>83*</u>	<u>83*</u>	8	<u>67*</u>	<u>83*</u>
12	100	100	12	100	100
16	<u>67*</u>	<u>50*</u>	16	<u>67*</u>	<u>50*</u>
20	<u>33*</u>	<u>33*</u>	20	<u>33*</u>	<u>33*</u>
24	<u>33*</u>	<u>33*</u>	24	<u>33*</u>	<u>33*</u>

*\* These samples were incompletely hydrated*



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# 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°C . However, storage in ambient temperatures may affect sample integrity



## Collaborators

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Malaria Control Program, Liberia  
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# Thank You



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