

Comparison of two real-time PCR assays for the detection of malaria from blood samples and feasibility study of an integral solution for malaria diagnostic in resource-limited settings.

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INTRODUCTION

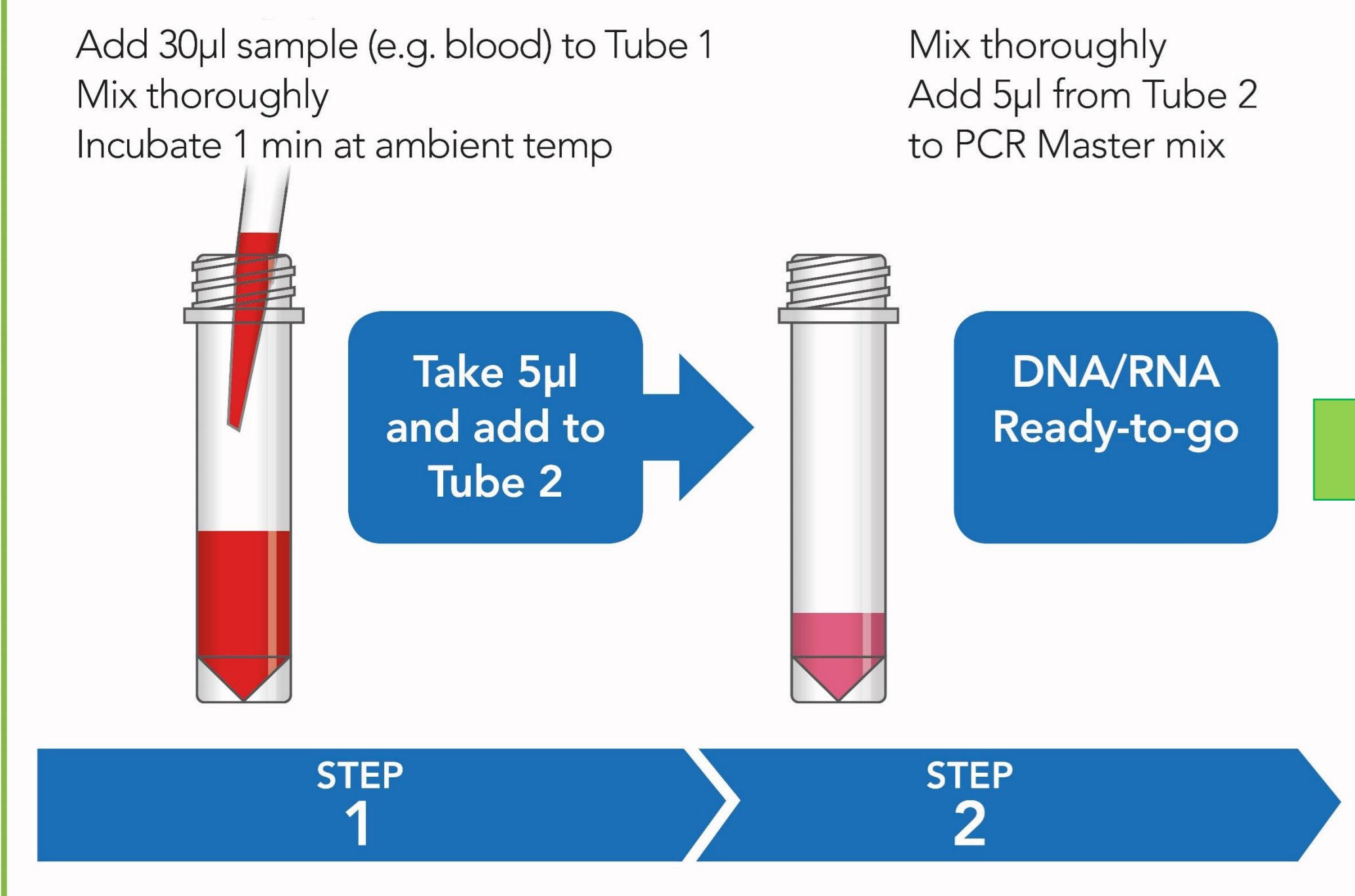
Malaria remains one of the major health concerns on deployments in subtropical and tropical endemic settings, making rapid diagnosis in case of suspected malaria is mandatory. Traditional microscopy of Giemsa-stained thin and thick blood smears remains the diagnostic golden standard. However in resource-limited settings, alternative, less investigator-dependent diagnostic approaches are desirable. Rapid diagnostic tests (RDTs) are a frequently chosen option but their sensitivity of RDTs is still a matter of concern as much as the presence of false negatives due to the presence of heterophilic IgM antibodies in cases of exceedingly high parasitemia [1, 2].

In this work we compare two molecular DNA-based assays as alternative approaches, with high sensitivity and specificity for malaria diagnostics, which can be standardized and require less experienced skills than microscopy.

The reference polymerase chain reaction (PCR) is the generic reaction for real time detection of plasmodium described and validated in 2004 by Rougemont et al. in "Detection of four Plasmodium species in blood from humans by 18S rRNA gene subunit-based and species-specific real-time PCR assays" [3]. The challenging PCR belongs to an integral end-to-end solution developed by ARCIS-Biotechnology, whose commercial malaria retro-transcription-real-time-polymerase chain reaction (RTqPCR) assay aims for greater sensitivity by targeting the parasitic mRNA.

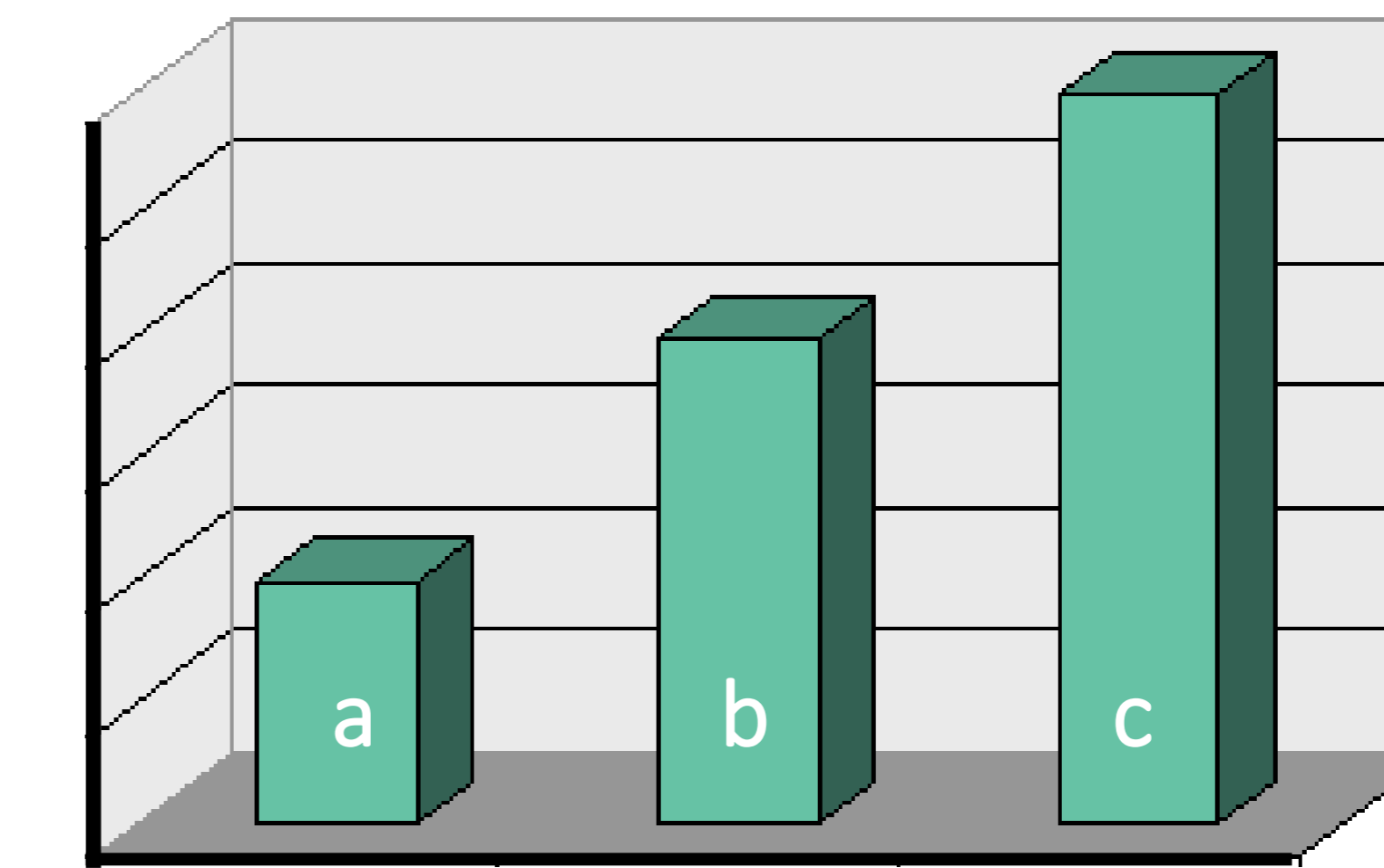
RESULTS

2-Step Process, 3 minutes



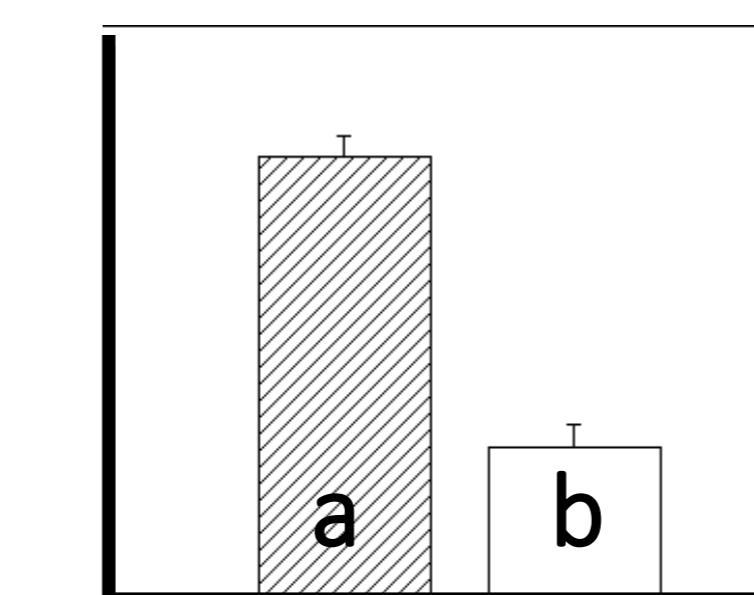
Protocol for ultra rapid sample prep process for extraction of plasmodium RNA and DNA from blood

SAMPLE	ICT	parasites/µL	Q/Rougemont (Gold Standard)				Q/Rougemont dilution factor in (7.5 CT)	A/Rougemont qPCR		ARCIS High sensitivity malaria RTqPCR Kit (MARK-2)		ARCIS High sensitivity malaria RTqPCR NZY boosted Kit (MARK-3)		Fraction of detection directly obtained from mRNA		Advantage in Ct	(vs Q/Rougemont)						
			Generic	Species				Generic	Generic	Generic	Generic	Generic	Generic	Q/Rougemont-MARK 2	Q/Rougemont-MARK 3								
1	+	0.3	23.97	23.38	30.88	38.19	38.16	38.10	18.24	18.05	18.11	14.95	12.89	13.84									
2	-	0	35.02	23/25	25.01	24.30	32.51	31.80	39	40.72	37.43	39.18	NA	22.93	14.50	20.45	1.37	7.32					
3	+	3	26.36		24.52		32.02		33.4	33.46		27.72	27.75		20.16	20.07		20.12					
4	-	0	33.95		27.96		35.46		UND	42.72		29.41	23.86		22.56	22.54		22.54					
5	+	0.2	25.98	26/28	26.95	27.62	34.45	35.12	37.13	36.96	39.82	20.44	20.43	23.18	16.14	16.26	17.86	16.64	21.95	4.44	9.76		
6	+	<1	29.95		27.95		35.45		39.54	39.77		19.78	19.86		14.17	14.79							
7	+	0	40.95		32.92		40.42		43.11	43.84		29.43	28.74		20.58	21.42							
8	-	0	32.03	29/32	31.57	32.48	39.07	39.98	39.26	38.56	41.67	23.73	23.86	29.12	17.7	17.82	21.60	12.55	20.07	3.36	4.20	10.88	11.04
9	+	<0.01	37.92		32.96		40.46		43.13	42.61		35.81	34.76		26.45	25.57							
10	+	<1	No PCR in Specimen		35.95		43.45		42.18	42.79		24.16	24.18		18.16	17.85							
11	-	0	40.84	33/35	34.1	34.95	41.60	42.45	44.28	44.64	43.55	35.04	36.25	31.48	26.96	26.21	22.73	12.07	20.82	3.47		12.22	
12	+	0	33.54		34.8		42.30		43.26	43.23		34.04	34.01		24.05	24.13							
13	+	0	38.49		37.43		44.93		43.58	43.58		25.65	25.77		20.84	20.22							
14	+	0	40.56	36/40	37.96	37.60	45.46	45.10	44.31	41.93	41.52	32.99	33.09	29.26	24.46	24.56	22.57	12.26	18.95	8.34		15.03	
15	+	0	38.6		37.4		44.90		UND	39.06		29.01	28.92		22.96	22.93							



Relative performance (% of probability for positive detection of plasmodium calculated over the data in the table) for malaria detection :

- a) Microscopy < 65%
- b) Rougemont std. qPCR < 84%
- c) ARCIS MK2 RTqPCR < 93%



% of probability to generate a false positive result for plasmodium loads over 39 Cts:

- a) Rougemont std. qPCR > 82%
- b) ARCIS MK3 RTqPCR < 35%

GENERAL CONCLUSION

The statistical analysis of the data obtained from the retrospective samples detailed in the table set the real gain in LOD obtained by application of RTqPCR instead of standard qPCR to be aligned with the transcription rates described in bibliography both for active plasmodium infection and latency periods (4, 5).

The overall gains in performance of the extraction, ease of use and cost-effectiveness of ARCIS integral solution tested vs the reference PCR here suggest that a new viable solution for molecular detection under tropical deployment conditions may become available shortly.