NOVEL PROTOCOL FOR PROCESSING CLINICAL SAMPLES USED FOR MALARIA RT-qPCR DIAGNOSTICS

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OBJECTIVES

Assessment of a novel two-step approach (Arcis DNA Blood Kit) for extraction and detection of stable RNA and DNA through reverse transcription by PCR (RT-qPCR) for malaria diagnostics. Sensitivity of the system was assessed as well as RNA stability over one month for samples kept at room temperature,

MATERIALS AND METHODS

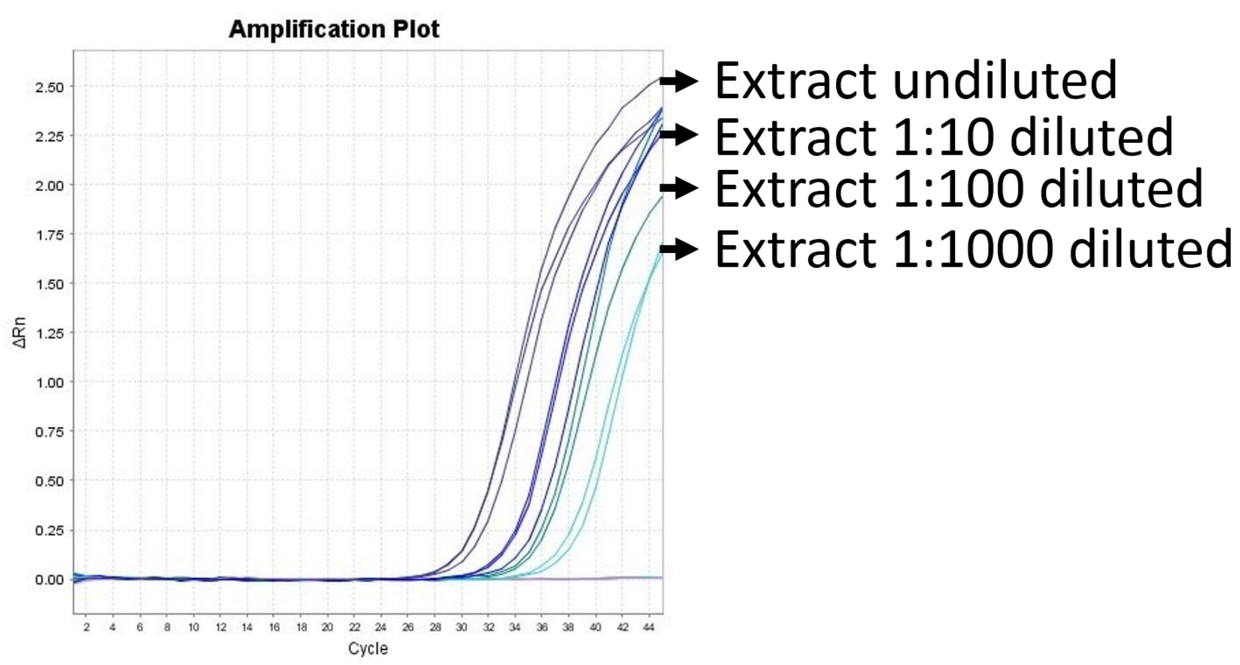
This new two-step approach for extraction and preservation of genetic material was evaluated on malaria infected whole blood samples. The ultra-fast extraction protocol takes less than 5 minutes. Samples (30µl) were placed in Arcis DNA Blood Kit Tube 1 and stored at room temp for a period of 26 days. Immediately prior to testing, the sample was added to Tube 2. A 10 minute reverse-transcription of all mRNA targets was performed followed by qPCR. The data from RT-qPCR were compared to data from standard qPCR from Qiagen extracts (350µl) with results obtained from the same specimens and demonstrated the protection of mRNA over the time period.

The RT-qPCR for *Plasmodium* spp was performed using the StepOne Real-Time PCR System (Applied Biosystems) with the following thermal profile: initial step of 10 min at 45°C for RT-qPCR followed by 10 min at 95°C and 45 cycles of 15 s at 95°C and 1 min at 60°C.

RESULTS

A: Clinical Sensitivity

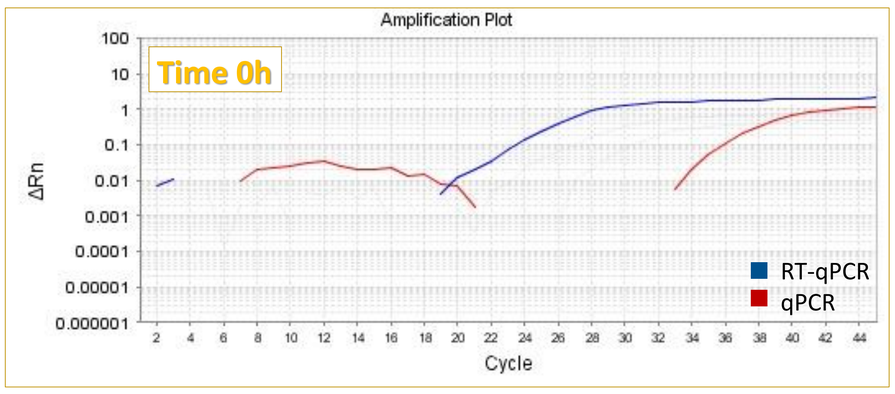
A high-load sample processed using this system, then serially diluted, showed detection down to 5 parasite/µl.

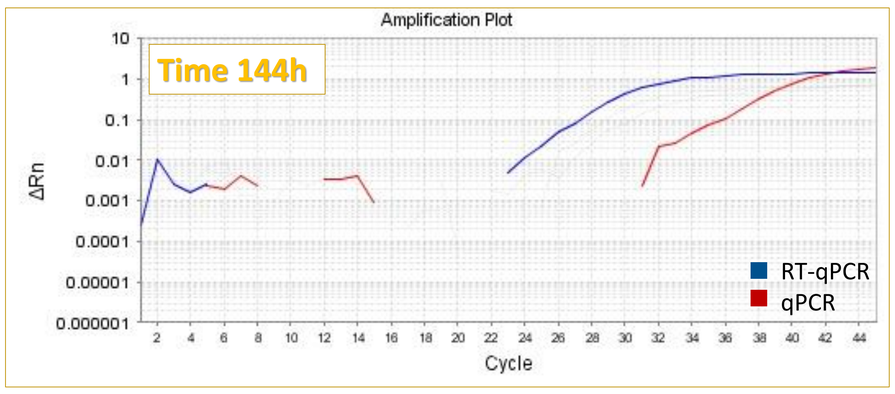


Extraction	Parasites/µl	Rep 1 (Ct)	Rep 2 (Ct)
Undiluted	5000	31.42	31.42
1:10	500	35.9	34.5
1:100	50	36.5	36.8
1:1000	5	39.3	38.7
1:10000	0.5	Not Detected	Not Detected

B: Stability

The results from the RT-qPCR showed a protective effect on mRNA molecules over a period of 26 days at room temperature. The novel RT-PCR approach showed greater sensitivity than the standard clinical qPCR assay.





CONCLUSION

The results obtained using Arcis DNA Blood Kit demonstrate stabilisation of RNA and DNA from blood cells following the simple 2 step protocol for at least 26 days at room temperature. Arcis DNA Blood Kit will allow the transfer of preserved genetic material without the need for isolation and eliminates the need for cold storage transfer which is not always practical in hospital laboratories.