Arcis Buccal Swab Nucleic Acid Extraction Protocol

Buccal cell usage has been shown by many to be a cost effective and safe method to isolate DNA for various biological experiments especially large epidemiological studies, genetic ID testing and forensic analysis. Non-invasive DNA collection methods are preferred over phlebotomy in order to increase study participation and compliance in research centres and for sick patients in hospital settings. Here we describe a protocol using the Arcis Sample Prep Kit - a ready to use, room temperature kit comprising two reagents enabling pre-analytical processing of samples. In 3 minutes, with no prior sample preparation, the kit allows you to go from swab to downstream nucleic acid investigations without the need for isolation or purification. The kit is suitable for untreated specimens and specimens that have been frozen. The DNA released is ready for immediate use in PCR or other molecular applications. The Arcis Sample Prep Kit is intended for in vitro identification of nucleic acids or diagnostic use.

The following protocol describes the extraction of DNA from buccal swabs collected from two donors (10 scrapes per cheek) and the subsequent analysis of the genetic material extracted by the Arcis Sample Prep Kit.

Storage conditions

Tubes are shipped and stored at room temperature. Samples which have been lysed in buffer 1 are stable at room temperature for 30 days.

Materials provided

Materials provided	Quantity	No. Reactions
Tube 1 Lysis Buffer	1	48
Tube 2 Wash Buffer	1	48

Samples

For best results rinse mouth with water immediately prior to sampling. Samples tested were from two donors (10 scrapes per cheek), swab was rubbed firmly against the cheek. The swab was then agitated in the lysis buffer for 60 seconds.

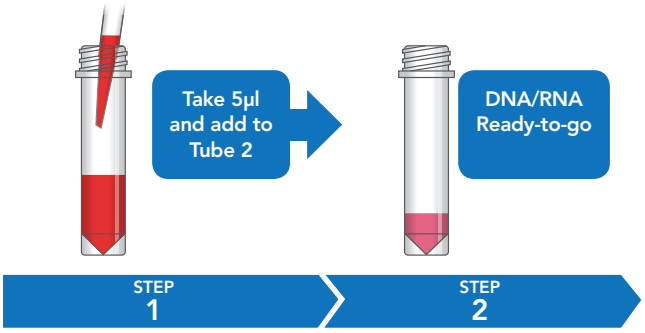
Method

- Post scrape swabs were dropped into the buffer 1 Lysis solution (150µl) and agitated briefly to release the nucleic acids by rotating 10 times and squeezing against the sidewalls of the tube. Swab is then snapped at breakpoint before leaving the swab in the tube. Tubes were then vortexed.
- The sample was then incubated for 1 minute at room temperature. After this 5µl was transferred to Tube 2 (20µl) and mixed thoroughly.
- 5µl of the resultant solution was then added to the PCR master mix for a final reaction volume of 25µl

PCR conditions

Initial denaturation 95°C 10 min, denaturation 95°C 15 sec, annealing 60°C 60 sec, 45 cycles on LC480 II Lightcycler. Fluorescence readings acquired in the VIC channel (540-580nm) at the annealing step.

hRNase P PCR Mix	Vol µl
Master Mix 2x	12.5
F Primer (50µM)	0.4
R Primer (50µM)	0.4
Probe (1µM)	0.5
Water	6.2
Template (tube 2)	5

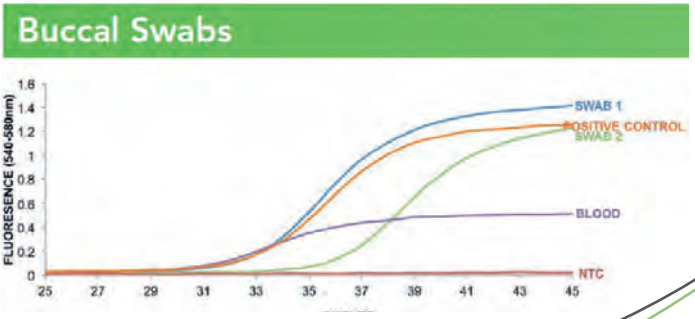


Results (Ct values averaged)

	Donor 1	Donor 2
Positive control	32.59	32.49
Swab 1	31.94	32.88
Swab 2	35.5	35.62
30µl blood	30.53	30.37
NTC	No amp	No amp

Conclusion

The protocol can successfully extract amplifiable DNA from buccal swabs. For swab 1 the Ct values for the hRNaseP assay are between 31.94 and 32.88, the Ct values for swab 2 are later showing the heterogeneity between samples. All swab PCR curves were prominent and easily detected.



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Arcis Sample Prep Kit Extraction Protocol

Extraction of DNA from Dried Blood Spots (DBS) is not only important in forensics and genetic identity testing, it is becoming increasingly important within areas such as neonatal DBS storage in Bio-banking, epigenetics and disease testing. The challenges in recovering genetic material from dried blood are often due to the degradation that occurs during processing and extraction.

The Arcis Sample Prep Kit is a ready to use, room temperature kit comprising two reagents enabling pre-analytical processing of blood samples. In 3 minutes, with no prior sample preparation, the kit allows you to go from blood to downstream nucleic acid investigations without the need for isolation or purification. The Arcis Sample Prep Kit will release nucleic acids from fresh or frozen or dried whole blood samples. The kit is suitable for untreated specimens and specimens that have been stored in EDTA and heparin-containing solutions. The DNA released is ready for immediate use in PCR or other molecular applications. The Arcis Sample Prep Kit is intended for in vitro identification of dried blood or diagnostic use.

The following protocol describes the extraction of DNA from air dried samples and the subsequent analysis of the genetic material extracted by the Arcis Sample Prep Kit.

Storage conditions

Tubes are shipped and stored at room temperature. Samples which have been lysed in buffer 1 are stable at room temperature for 30 days.

Materials provided

Materials provided	Quantity	No. Reactions
Tube 1 Lysis Buffer	1	48
Tube 2 Wash Buffer	1	48

Samples

Samples tested were from multiple samples of 30µl of whole blood dropped onto polycarbonate plastic and spread with a pipette tip. Samples were then air dried.

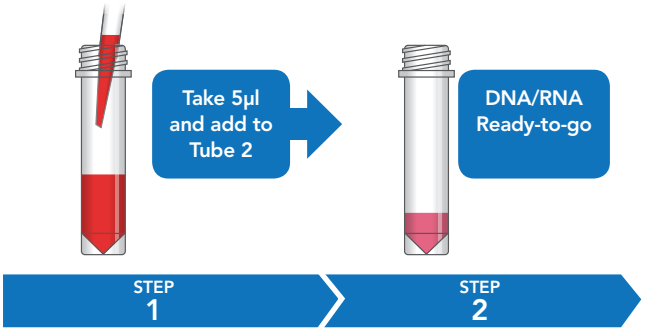
Method

- The dried blood sample types were then equally divided and collected by swabbing with cotton tipped swabs pre-wetted with either water or 10mM Tris-EDTA (TE) buffer.
- The tip of the swab was then placed in Arcis Solution 1 – Lysis buffer (150µl) and agitated briefly to release the nucleic acids.
- The sample was then incubated for 1 minute at room temperature. After this 5µl was transferred to Tube 2 (1:4 ratio) and mixed thoroughly.
- 5µl of the resultant solution was then added to the PCR master mix for a final reaction volume of 25µl

PCR conditions

Initial denaturation 95°C 10 min, denaturation 95°C 15 sec, annealing 60°C 60 sec 45 cycles on LC480 II Lightcycler. Fluorescence readings acquired in the VIC channel (540-580nm) at the annealing step.

hRNase P PCR Mix	Vol µl
Master Mix 2x	12.5
F Primer (50µM)	0.4
R Primer (50µM)	0.4
Probe (1µM)	0.5
Water	6.2
Template (tube 2)	5



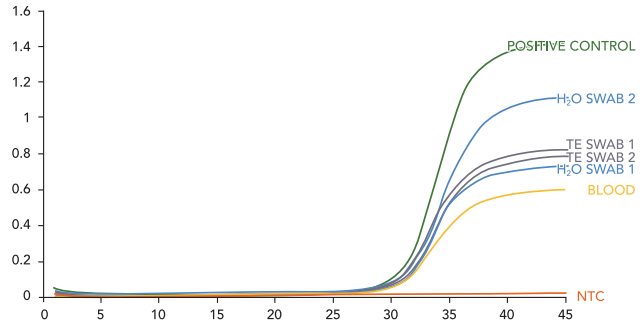
Results (Ct values averaged)

	Air dried
Positive control	30.70
Water swab	30.77
TE swab	30.65
Fresh blood	30.45

Conclusion

Good results were obtained with swabs from air dried blood, with Ct values similar to fresh blood. Results were the same irrespective of which buffer was used to swab the sample.

Air Dried Blood



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Arcis Hair Follicle Nucleic Acid Extraction Protocol

Forensic analysis of hair samples in order to extract DNA is a method commonly used for the purpose of identification in both criminal investigations as well as parental DNA testing. Genetic identification and profiling is commonly through analysis of nuclear DNA. Despite the possibility of remnants of nuclear DNA in the hair shaft (in cut hairs or naturally shed hairs), analysis with this specific part of the hair will likely not yield results. Hair follicles are usually required to yield DNA as they contain keratinocytes which contain nuclear DNA.

Methods to extract DNA from hair follicles have frequently involved complex enzymatic digestions in solutions ranging from proteinase K to laundry detergent! Here we describe a protocol using the Arcis Sample Prep Kit - a ready to use, room temperature kit comprising two reagents enabling pre-analytical processing of samples. In 3 minutes, with no prior sample preparation, the kit allows you to go from follicle to downstream nucleic acid investigations without the need for isolation or purification. The kit is suitable for untreated specimens and specimens that have been frozen. The DNA released is ready for immediate use in PCR or other molecular applications. The Arcis Sample Prep Kit is intended for in vitro identification of nucleic acids or diagnostic use.

The following protocol describes the extraction of DNA from single hair follicle samples and the subsequent analysis of the genetic material extracted by the Arcis Sample Prep Kit.

Storage conditions

Tubes are shipped and stored at room temperature. Samples which have been lysed in buffer 1 are stable at room temperature for 30 days.

Materials provided

Materials provided	Quantity	No. Reactions
Tube 1 Lysis Buffer	1	48
Tube 2 Wash Buffer	1	48

Samples

Samples tested were from multiple samples of single hair follicles.

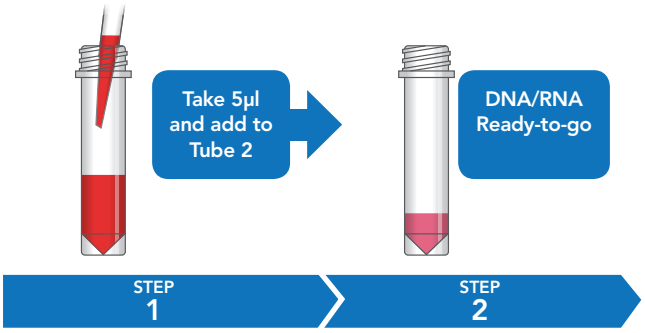
Method

- Single hair follicles were dropped into the buffer 1 Lysis solution (150µl) and agitated briefly to release the nucleic acids.
- The sample was then incubated for 1 minute at room temperature. After this 5µl was transferred to 20µl of Tube 2 reagent to give a 1:4 volume ratio and mixed thoroughly.
- 5µl of the resultant solution was then added to the PCR master mix for a final reaction volume of 25µl

PCR conditions

Initial denaturation 95°C 10 min, denaturation 95°C 15 sec, annealing 60°C 60 sec, 45 cycles on LC480 II Lightcycler. Fluorescence readings acquired in the VIC channel (540-580nm) at the annealing step.

hRNase P PCR Mix	Vol µl
Master Mix 2x	12.5
F Primer (50µM)	0.4
R Primer (50µM)	0.5
Probe (1µM)	0.5
Water	6.2
Template (tube 2)	5

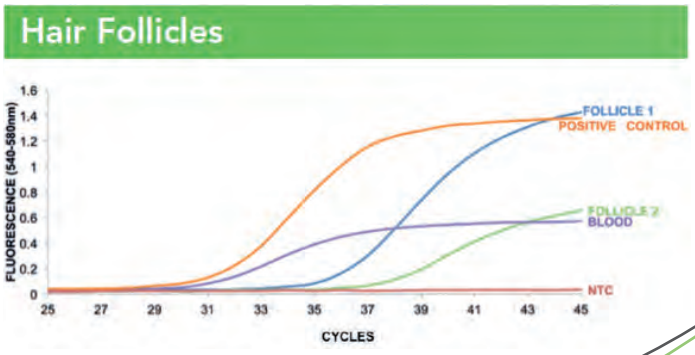


Results (Ct values averaged)

	Sample
Positive control	31.14
Hair follicle 1	35.26
Hair follicle 2	36.98
30µl blood	30.50
NTC	No amp

Conclusion

The Ct values obtained were between 35 and 37. The amplification curves were prominent and easily detected. This test may be near the limit for detection for this assay.



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Arcis Saliva Nucleic Acid Extraction Protocol

Saliva is increasingly used as a bio-fluid for rapid diagnostics. Sample collection is non-invasive and easy to administer and there are several commercial collection kits on the market. Here we look at the release of DNA from saliva samples following Arcis treatment and assess the DNA for quality, quantity and compatibility with qPCR. Furthermore, the saliva samples will be assessed for stability in Arcis reagent 1, with samples stored at room temperature and at 40°C for real-time and accelerated stability testing.

Sample Collection

Donors rinsed their mouths with water 10 minutes prior to providing samples. Donors provided samples of approximately 2ml of saliva into 15ml Falcon tubes. These samples were vortexed mixed prior to testing

Storage conditions

Tubes are shipped and stored at room temperature.
Samples which have been lysed in buffer 1 are stable at room temperature for 30 days.

Materials provided

Materials provided	Quantity	No. Reactions
Tube 1 Lysis Buffer	1	48
Tube 2 Wash Buffer	1	48

Method

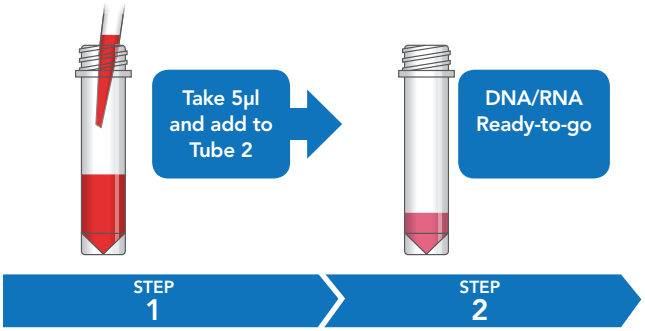
Protocol	Volume of Saliva (µl)	Volume of Reagent 1 (µl)	Ratio of Tube 1: Tube 2
1 (Standard)	30	170	1:4
2	60	140	1:4
3	90	110	1:4
4	30	170	1:2

- Samples of saliva were added to reagent 1 according to the table above
- The sample was then incubated for 1 minute at room temperature. After this 5µl was transferred to Tube 2 (see ratios) and mixed thoroughly.
- 5µl of the resultant solution was then added to the PCR master mix for a final reaction volume of 25µl

PCR conditions

Initial denaturation 95°C 10 min, denaturation 95°C 15 sec, annealing 60°C 60 sec 45 cycles on LC480 II Lightcycler. Fluorescence readings acquired in the VIC (540-580nm) at the annealing step.

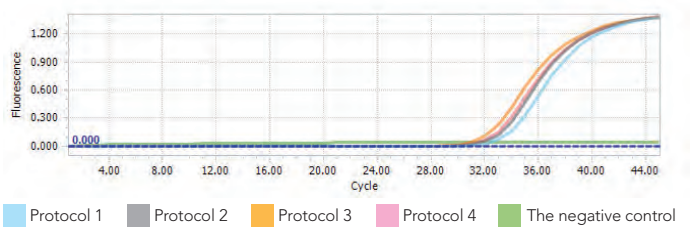
	Vol µl	Vol µl
ABI Master Mix	12.5	750
F Primer (50µM)	0.4	24
R Primer (50µM)	0.4	24
Probe (1µM)	0.5	30
Water	6.2	372
Dispensed per well	20	20
Template (tube 2)	5	5



Results (Ct values averaged)

	1	2	3	4
	32.65	32.02	31.24	31.70
	31.13	32.55	31.50	31.63
	32.45	32.36	32.55	33.08
	31.85	32.12	32.40	33.06
	33.75	32.49	31.78	33.01
	31.56	31.80	31.99	32.91
Average	**32.23**	**32.22**	**31.91**	**32.57**
SD	**0.93**	**0.29**	**0.51**	**0.70**

Amplification Curves



Conclusion

All protocols yielded positive results, indicating that DNA extraction from saliva samples is possible with Arcis reagents. There is little difference in Ct value or amplification between the protocols, indicating robustness of the system with a wide working range of volumes.

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A Novel protocol for processing low volume clinical samples for IL28B polymorphism detection via sequencing and qPCR

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Objectives

Assessment of a novel two-step approach (Arcis Sample Prep Kit) for simple and rapid processing of whole blood clinical samples for the downstream detection of Single Nucleotide Polymorphisms (SNPs) of the IL28B gene. A multiplexed, probe-based qPCR assay was assessed for diagnostic potential in low volume (30µl) clinical samples.

Introduction

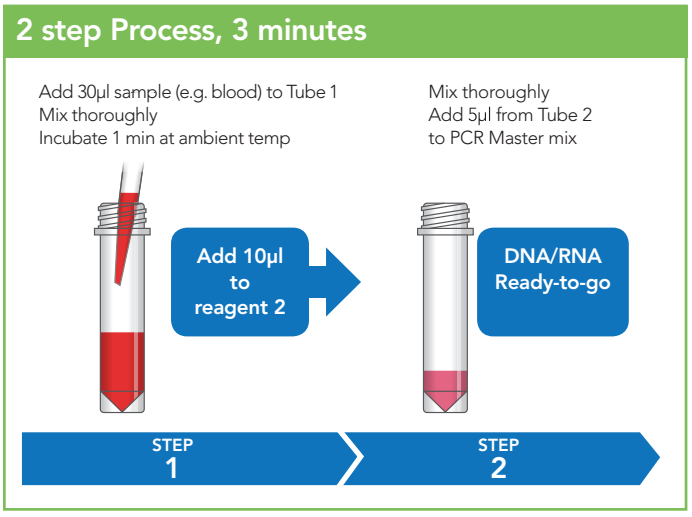
Genome whole association studies (GWAS) have demonstrated that SNP rs12979860, in the IL28B gene is one of the most important prognostic markers for treatment response with pegylated interferon (PegIFN) plus ribavirin (RBV) in chronic hepatitis C patients infected with hepatitis C virus (HCV) genotype 1. The allele composition at rs12979860 position correlates with a significantly higher rate of spontaneous clearance of HCV and with the prospect of an interferon antiviral treatment of the patients (Pegasys, Roche). In particular, patients with the CC genotype showed a two-fold higher sustained virological response rate (SVR) of 55-80% compared with 20-40% for individuals with the CT or TT genotype.

Materials and Methods

This novel two-step approach for DNA extraction was evaluated on low volume whole blood samples (30µl). Extractions were carried out on fresh or frozen blood samples up to 4 months post-collection.

The ultra-fast extraction protocol takes less than 5 minutes: 30µl samples were placed in Arcis Sample Prep Kit reagent 1 (170µl), mixed briefly and then 10µl transferred to 40µl reagent 2. The extract was then ready for PCR.

The PCR was performed on the StepOne™ Real-Time PCR System (Applied Biosystems) with the following thermal profile: activation steps of 2 min at 50°C and 15s at 95°C; followed by 45 cycles of 15 s at 95°C and 1 min at 62°C.



Arcis Sample Prep Kit Protocol

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Results

A: Specific qPCR detection of TT, CT and CC genotypes was shown, see Figure 1.

Specific detection of TT sample →

Specific detection of CT sample →

Specific detection of CC sample →

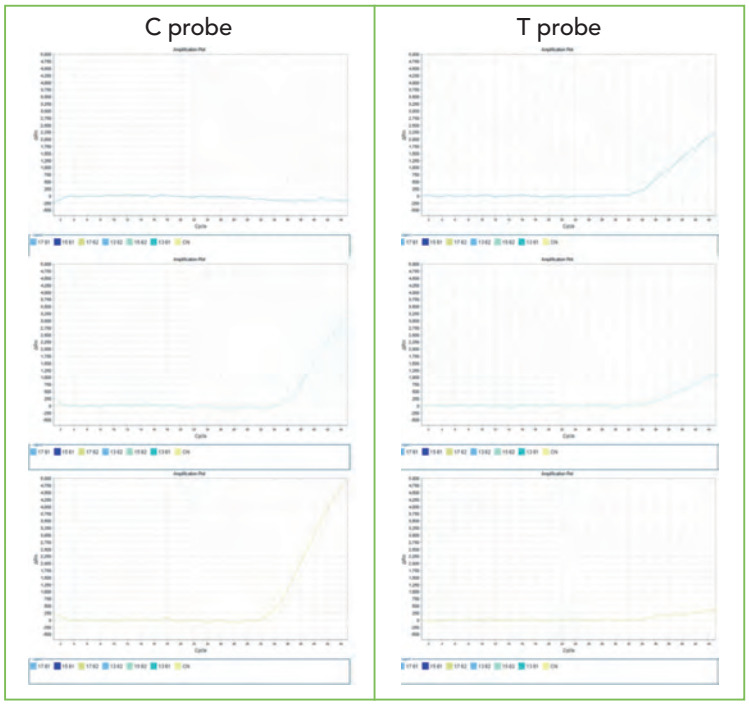

Figure 1: qPCR detection of IL28B genotypes

Results

B: IL28B genotyping by Sanger and Pyrosequencing.

A cohort of 53 patient samples were processed with the Arcis Kit for investigation with Bi-Directional Sanger Dye-Terminator sequencing and Pyrosequencing.

Sanger sequencing was carried out at the sequencing service at University Hospital La Paz, Madrid. The same samples were further sequenced on the PyroMark Q96 ID instrument in SNP Analysis mode (QIAGEN).

Concordant SNP detection was observed in 53/53 samples between the two platforms. See Figure 2 for a typical result.

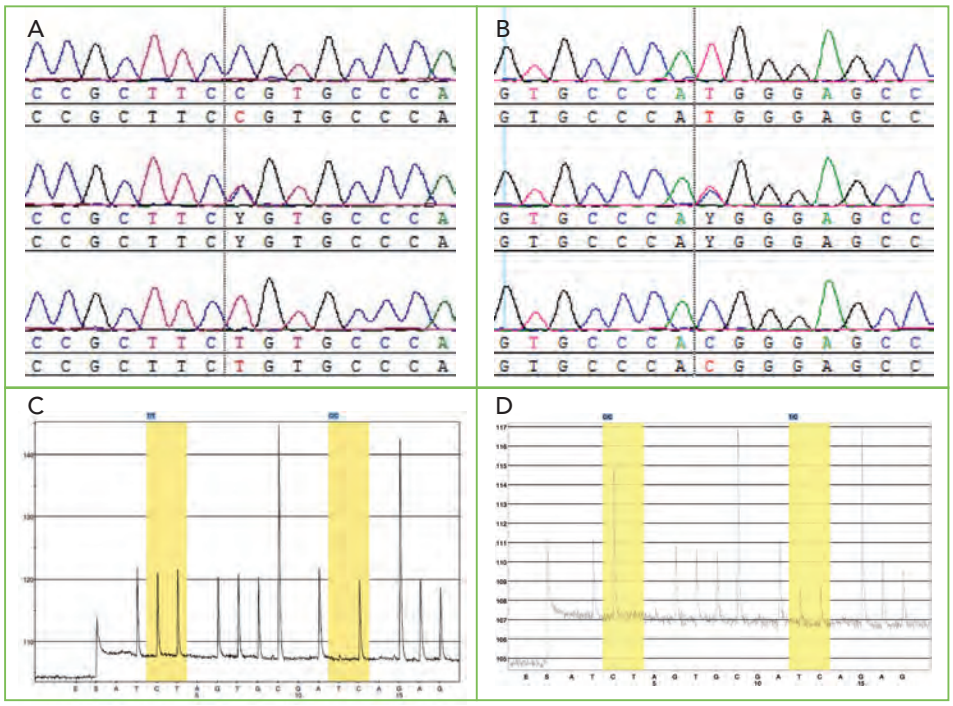

Figure 2: Sanger sequencing (Panel A and B) and Pyrosequencing detection (Panel C and D) of IL28B genotypes. Full concordance was shown in all samples tested

Conclusions

The results obtained using Arcis Prep Sample Kit indicate that the system is suitable for the rapid processing of clinical samples for IL28B SNP detection by sequencing and qPCR. The Arcis Kit reduces pre-analytical processing time to 3 minutes and shows full compatibility with downstream molecular diagnostics.

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Novel protocol for processing clinical samples used for Malaria qRT-PCR diagnostics

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Objectives

Assessment of a novel two-step approach (Arcis Sample Prep 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

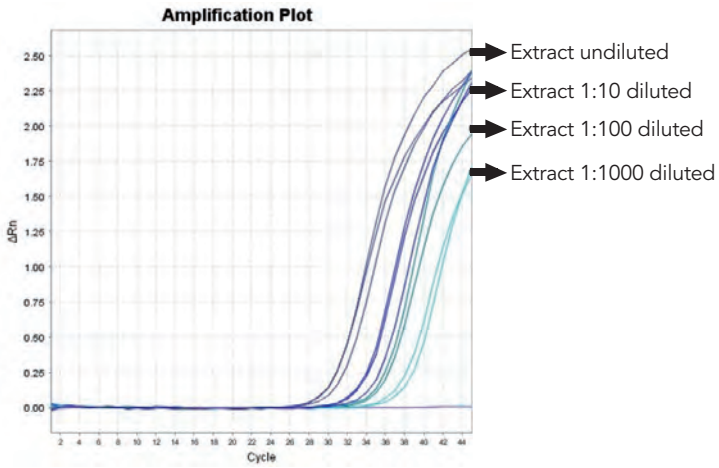
This new two-step approach for extraction and preservation of genetic material was evaluated on malaria infected whole blood samples. The ultra-fast extraction protocol takes less than 5 minutes. Samples (30µl) were placed in Arcis Sample Prep Kit reagent 1 (170µl) and stored at room temp for a period of 26 days. Immediately prior to testing, the 10µl sample was added toReagent 2 (40µl). 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-PCR 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

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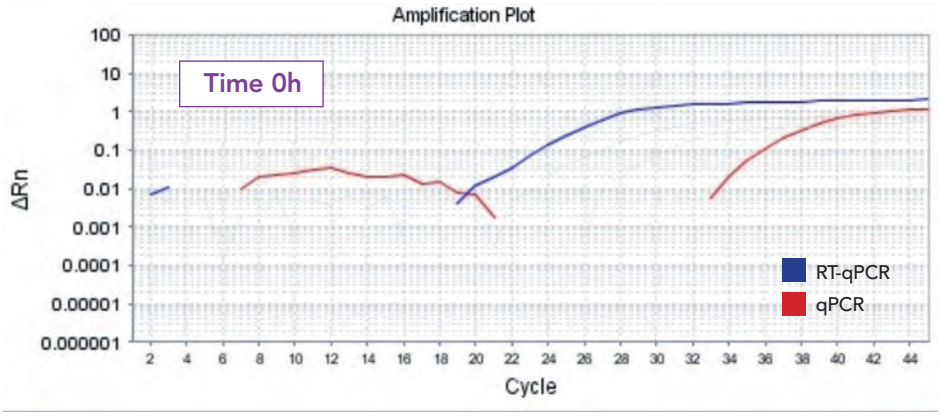
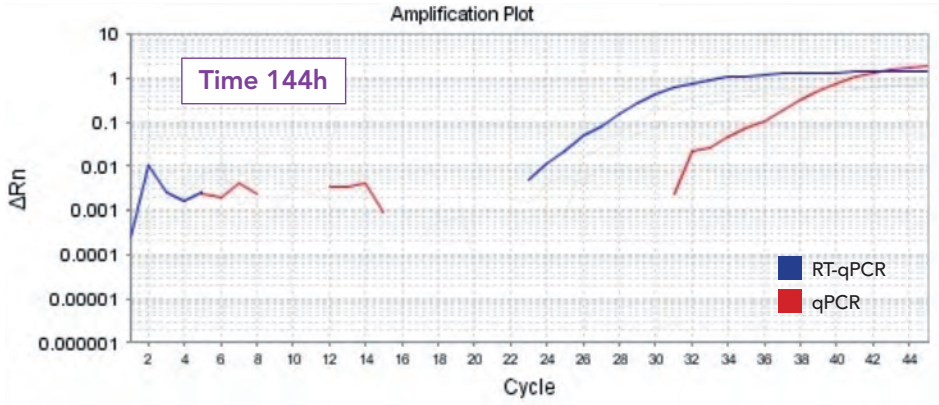
Results continued

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.





Conclusions

The results obtained using Arcis Sample Prep 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 Sample Prep Kit will allow the transer 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.

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