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An efficient and novel technology for the extraction of parasite genomic DNA from whole blood or culture

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Abstract

The aim of this study was to assess pathogen DNA extraction with a new spin column-based method (DNA-XT). DNA from i) whole blood samples spiked with *Plasmodium falciparum* or ii) *Leishmania donovani* amastigote culture was extracted with DNA-XT and compared with that produced by a commercial extraction kit (DNeasy). Eluates from large and small sample volumes were assessed by PCR and spectroscopy. Using a small volume (5 µl) of blood, the DNA-XT and DNeasy methods produced eluates with similar DNA concentrations, 0.63 versus 1.06 ng/µl, respectively. The DNA-XT method produced DNA with lower PCR inhibition than DNeasy. The new technique was also twice as fast and required fewer plastics and manipulations but had reduced total recovered DNA compared with DNeasy.

Methods summary

DNA-XT, which is designed for small sample volumes, uses a 5 min detergent and enzymatic lysis step to release DNA from cells. Contaminating proteins and lipids are then bound to a matrix within a spin column during a 1 min centrifugation step whilst DNA passes directly through.

Keywords Malaria, *Plasmodium*, *Leishmania*, diagnostics, blood, DNA extraction, PCR

Background

DNA extraction is an essential starting point for methodologies such as the polymerase chain reaction (PCR), which is used in the laboratory for molecular biology and clinical diagnostics. It was first achieved in 1869 by Friedrich Meischer [1] but a routine laboratory procedure was not developed until 1958 [2]. Today a variety of methodologies are available (reviewed in [3]). However, commercial spin column purification of DNA from blood and other tissues and fluids is the most common approach used in modern laboratories. The technique, with its origins reported in 1979 [4], uses silica matrices to selectively bind DNA, allowing washing before elution of the purified product.

NanoMal was an EU-funded industrial-academic consortium that was brought together with the aim of developing a simple-to-use, affordable, handheld diagnostic device to detect malaria infection and the parasites' drug resistance status by identifying associated genetic mutations. The cartridge-based assay was designed to use only a finger prick of blood, with results obtained approximately 20 minutes after sampling. The diagnostics platform technology developed by our industrial partner, QuantuMDx Group Ltd., incorporates a novel DNA extraction technique that allows DNA from lysed whole blood (or other sample types) to flow straight through a proprietary material, while cellular debris is retained. The resulting eluate contains DNA that is of a quality that can be used directly in PCR. Therefore, the technique removes the need for additional washing and elution steps, which greatly simplifies the extraction procedure. During the NanoMal project, QuantuMDx created a standalone spin column-based version of this easy-to-use extraction methodology, called DNA-XT. Optimised for human whole blood, small volume samples are lysed and placed over a column of the proprietary material, which is housed within a microcentrifuge

collection tube. Following centrifugation, the resulting eluate contains purified, high quality genomic DNA (gDNA).

Here we examined the performance characteristics of DNA-XT in parallel with a widely used commercial DNA extraction spin column kit for the isolation of parasite DNA from small volumes of i) *Plasmodium falciparum*-infected erythrocytes spiked into human whole blood (as a model for blood infections) and ii) purified *Leishmania donovani* amastigotes (as might be required in a laboratory setting). The two primary objectives were to determine i) the efficiency of gDNA extraction and ii) the quality of the isolated gDNA by undertaking parasite-specific PCR. Secondary objectives were to assess i) the level of operator input, ii) the time required for extractions and iii) the use of reagents/consumables.

Methods

Parasites

P. falciparum 3D7 parasites were cultured in human red blood cell (RBC) suspensions, using RPMI 1640 medium (Sigma-Aldrich; Cat. No. R0883-500ML) supplemented with 2 mM L-glutamine, 35 mM HEPES, 0.5% (*w/v*) Albumax I, 0.2 mM hypoxanthine, and 50 µg/ml gentamycin and maintained at 37°C under 5% CO_2. Parasite growth was followed by microscopic examination of Giemsa stained thin blood smears and maintained at ≤10% parasitaemia, with a ~2% haematocrit. Synchronization of early trophozoite stages was achieved by incubating infected RBCs (iRBCs) in 5% (*w/v*) sorbitol for 10 to 20 min at room temperature [5]. *Leishmania donovani* amastigotes were harvested from the spleens of donor mice. Briefly, female RAG1B6 KO mice, infected with *L. donovani* at least 60 days

beforehand, were humanely killed. At necropsy, spleens were dissected and homogenized and the amastigotes harvested by differential centrifugation [6].

Sample preparation

For *P. falciparum*-infected RBC (iRBC) spiked fresh whole blood, stock iRBCs, with an 8-10% parasitaemia, were diluted in fresh whole blood (Cambridge Biosciences), to attain a parasitaemia of 1%. Two additional 10-fold dilutions were performed to obtain samples with parasitaemias of 0.1% and 0.01%. The parasitaemia for 1% and 0.1% samples were confirmed by microscopy (Additional file 1). Non-spiked fresh whole blood was used as a negative control. As a reference, levels of parasitaemia of 0.1 – 0.2% (5,000 – 10,000 parasites/µl of blood) are generally accepted to be the point at which fever begins and a patient becomes symptomatic for falciparum malaria[7].

For *Leishmania* parasite preparations, parasites were washed twice in either RPMI 1640 (amastigotes) without serum prior to counting and were used at a concentration of 1 x 10^7/ml.

Column purification

For iRBC spiked fresh whole blood, Qiagen's DNeasy® Blood and Tissue and QuantuMDx's DNA-XT™ DNA extraction kits were used to process samples. Samples (iRBC spiked or non-spiked fresh whole blood) were processed following the manufacturer's instructions (blood protocols). For the DNA XT kit, 10 µl of sample was used and for the DNeasy kit, 100 µl was used, termed Q100. Notably, only 40 µl of the 80 µl lysis step is passed though DNA-XT columns after initial processing and thus only 5 µl of the original blood sample is passed through the column (Fig. 1). In light of this, an additional arm of the study used

DNeasy columns with a 5 µl sample volume, termed Q5, to allow a more direct comparison of the two extraction methodologies.

For *Leishmania* parasite preparations, the same two DNA extraction kits were used to process samples. For the DNA-XT kit, 10 µl of sample was used, following manufacturer's instructions (blood protocol) and for the DNeasy kit, 5 µl of sample was used, along with the manufacturer's instructions (nucleated blood protocol).

DNA quantitation

The volume of the eluate (though technically flow-through in the case of the DNA-XT kit) was noted and the DNA concentration determined by fluorescence, using a Qubit spectrophotometer (Thermo Fisher). The two kits have a 5-fold difference in final eluate/flow-through volume, with DNeasy recommending eluting in 200 µl and DNA-XT having a flow-through of ~40 µl.

PCR

PCR targeting *P. falciparum* β tubulin (*Pf*BetaT) was performed, using Phire II polymerase (Thermo Scientific). Two volumes of template (1 µl and 10 µl) were used in parallel reactions with primers (*Pf*BetaT F, 5'-TTGGGGTCCTTCCCCTTTATTGTAT-3' and *Pf*BetaT R, 5'-CAAAGGGGCCAGCACGAACACT-3') at final concentrations of 200 nM. Cycling conditions were 95 °C for 30 s, then 35 cycles of [95 °C for 30 s, 55 °C for 30 s, 68 °C for 30 s], then 68 °C for 10 min.

PCR targeting *L. donovani* kinetoplast DNA (high copy number) and DNA pol I (low copy number) was performed using Phusion polymerase (Thermo Fisher). Volumes of 1 µl and 10

µl *L. donovani* eluate were used, respectively, with primers for the kinetoplast DNA (*Ld*Kinetoplast F2, 5'-CCAATGAAGCCAAGCCAGTG-3' and *Ld*Kinetoplast R2, 5'-GGCTGGTTTTAGATGTGGGC-3') and DNA pol I (*Ld*DNApol I F, 5′-TGTCGCTTGCAGACCAGATG-3′ and *Ld*DNApol I R, 5′-GCATCGCAGGTGTGAGCAC-3′ [8] at final concentrations of 200 nM. Cycling conditions were 98°C for 30 s, then 10 cycles of [10 s 98°C, 10 s 69-59°C Touch down, 2 s 72°C], then 20 cycles of [10 s 98°C, 10 s 59°C, 2 s 72°C], then 72°C for 5 min.

PCR products were electrophoresed on 1% (*w/v*) agarose gels and visualised with UV.

Statistical analysis

For the total eluate DNA concentration, a Student's *t*-test (unpaired, equal variance) was performed using Prism version 6.0h for Macintosh (GraphPad Software).

Results

Extraction

Process timings

The time to complete extractions from eight samples, using each of the kits was recorded. For the DNA-XT kit, the extractions took between 50-60 min, compared with 120-130 min for the DNeasy kit.

Manipulations

The number of manipulations required to perform the extractions was also tallied. For an individual sample, the DNA-XT kit required 14 manipulations, whereas the DNeasy kit

required 25. A manipulation was considered to be any change of tip or tube/wash column or any action (*e.g.* centrifugation or incubation step). In terms of plastics usage for a single sample, the DNA-XT kit used 5 tips and 4 tubes (including the column), while the DNeasy kit required 8 tips and 7 tubes.

Eluate Characteristics

P. falciparum

All three column extraction arms, Q100, Q5 and DNA-XT, had eluates that contained DNA, as measured by fluorescence spectrometry (Table 1). Given that the theoretical amount of human DNA in each sample far exceeds that of any parasite DNA, samples spiked with different levels of iRBCs were analysed both alone and together when comparing yields. Unsurprisingly in the Q100 arm there was a greater yield of DNA than the Q5 arm. This difference was 21-fold (4420 ± 340 vs 205 ± 11 ng; mean ± SEM; n = 12), which is in line with the different volumes of blood processed in the Q100 and Q5 arms, of 100 µl and 5 µl, respectively. Assessing the Q5 and DNA-XT arms where the same amount of sample was passed through the respective column, similar concentrations of DNA were obtained, 0.63 ± 0.04 vs 1.06 ± 0.06 ng/µl, respectively (mean ± SEM; n = 12), with the Q5 arm performing significantly ($p = 0.013$) better, albeit by 1.7-fold (NB, only a single batch of each column type was tested here). Notably, the volume of eluate produced when using each manufacturer's protocol is different; ~200 µl for Q100 and Q5 and ~40 µl for DNA-XT. Thus, the total DNA yield from the Q5 column was higher than that of the DNA-XT column (7.8-fold).

L. donovani

For the *L. donovani* extractions, only the Q5 and DNA-XT protocols were used (*i.e.* those that use 5 µl of sample). Both arms, Q5 and DNA-XT, had eluates that contained detectable, albeit low, levels of DNA, with the exception of one replicate of the Q5 arm (Table 2). Again, similar concentrations of DNA were obtained in the Q5 and DNA-XT arms, 0.06 ± 0.002 vs 0.17 ± 0.01 ng/µl, respectively (mean ± SEM; n = 2/3), with the DNA-XT arm performing slightly better by 2.8-fold. Due to the different eluate volumes, the total DNA yield from the Q5 column was slightly higher than that of the DNA-XT column (1.5-fold).

Parasite DNA quality

P. falciparum

While the DNA extracted in these experiments reflects human DNA predominantly, parasite DNA was collected from spiked samples. Its presence and quality in eluates were determined by PCR detection of *Pf*BetaT. Two different volumes of eluate were used, a low volume (1 µl) and a high volume (10 µl). In most cases, amplicon bands of the appropriate size (388 bp) could be detected in samples in which parasite DNA should be present (Fig. 2). When using the low volume of eluate, the strongest bands were produced from the Q100 eluates. This was to be expected, as the Q100 eluates contain the highest total DNA concentrations (Table 1). Bands were also visible, though fainter, in lanes using Q5 and DNA-XT samples, even at the lowest (0.01%) parasitaemia used. However, when a greater (10-fold) volume of eluate was used only the DNA-XT derived samples resulted in positive amplicon bands, in a parasitaemia-dependent manner. This suggests an apparent inhibition of PCR when using high volumes of the Q5 and Q100 samples. The findings presented in Figure 2 are supported by data from three independent repeats of the experiment (with duplicate technical repeats within each). The number of positive bands in each (see Additional file 2 for PCR gel images not present in main text) are presented in Table 3.

L. donovani

DNA was successfully amplified from low volume (1 µl) eluates of purified *L. donovani* amastigotes derived from Q5 and DNA-XT extraction technologies when targeting the kinetoplast - a high copy number DNA sequence (Fig. 3A). Targeting a single copy number gene, *Leishmania* spp. DNA pol I, a PCR amplicon was obtained with a larger volume (10 µl) of DNA-XT derived eluate, but not with Q5 derived eluate (Fig. 3B).

Discussion

Here, a simple process for the extraction of DNA from small, parasite-containing culture samples was assessed against a standard commercial, spin column-based extraction process that is widely used in research and diagnostic laboratories. A considerable difference between the two techniques is that DNA is retained on the Qiagen DNeasy mini spin column prior to elution whilst the QuantuMDx DNA-XT column retains contaminants and only the DNA is eluted in one centrifugation step. The advantages of this process, as demonstrated in this study, are time savings, reduced plastics use, reduced reagents use (including removing the necessity to use guanidinium chloride), and a reduction in PCR inhibiting contaminants. However, total yields of DNA are much lower generally, when using the DNA-XT protocol.

The DNA-XT methodology is similar to a previously described DNA isolation method that uses a polyaniline-containing silica sorbent [9]. In terms of ease of use, the DNA-XT methodology is also comparable to DNA extraction protocols that use Chelex 100. Both of these alternative DNA extraction techniques have been used for forensic applications and perform well with samples that have low DNA concentrations (*e.g.*[10]; [11]; [12]). In this

study, DNA-XT was used to extract DNA from whole blood spiked with *P. falciparum*-infected RBCs, simulating a clinical diagnostic sample, and purified *L. donovani* amastigotes, as might be used in a research laboratory. In both cases, it was possible to amplify parasite DNA, although the majority of DNA in whole blood extracts would be expected to be human DNA.

Furthermore, with comparable input volumes, the DNA-XT technology compared favourably with the Qiagen extraction kit. Thus, DNA-XT has potential uses within a laboratory, where DNA extraction is required from small sample volumes.

The technology behind DNA-XT was designed for microfluidic-based, point-of-care, molecular diagnostics platforms, where cell lysis and DNA extraction occur within a cassette to provide DNA for microfluidic PCR and subsequent amplicon sensing.

In the case of malaria diagnosis at point-of-care (and other diseases caused by blood-borne pathogens), capillary sampling from finger or heel pricks are the preferred options to generate test samples for parasite detection. Capillary sampling can be safely performed by staff with basic training and is often quicker and less stressful when compared with venepuncture [13]. Capillary sampling has also been used extensively for epidemiological studies of malaria with the use of blood spot collection on filter paper [14–16].

Future perspectives

Since DNA-XT has been demonstrated here to produce high-quality parasite gDNA (seemingly free of the types of PCR inhibitors that frequently contaminate whole blood extracts [17–19]), this technology may also be useful for extracting blood from dried blood

spots, as has been demonstrated for other DNA extraction methodologies [20]. Further applications for high quality DNA extraction include genotyping for therapeutic diagnostics (*e.g.* parasite drug resistance/susceptibility) and epidemiology for monitoring asymptomatic individuals [21,22] and those with sub-microscopic parasitaemia within a population [23]. These could be particularly useful in potential eradication programmes [24–26].

Future work for diagnostic applications for the DNA-XT technology should include patient samples, and a larger sample size, to field-test this new technology. In addition, a more accurate method of DNA quantification should be used in these proof-of-concept studies, namely qPCR.

Executive summary

- A new spin column-based DNA extraction method (DNA-XT) was assessed using i) pathogen-spiked blood samples or ii) pathogen culture.
- The DNA-XT method was quicker, used less plastic and required fewer manipulations, yet the total recovered DNA was less than the comparator.
- Eluates from the DNA-XT method had less PCR inhibition than those of the comparator.

List of Abbreviations

Q5 – 5 µl of blood in Qiagen kit

Q100 – 100 µl of blood in Qiagen kit

PCR – Polymerase Chain Reaction

RDT – Rapid Diagnostic Test

POC – Point-of-Care

*Pf*BetaT – *P. falciparum* β tubulin

iRBC – infected red blood cell

Tables

Table 1: Eluate characteristics for *Plasmodium falciparum* spiked whole blood derived samples

Column		Eluate volume (mean ± SEM, µl)	[DNA] (mean ± SEM, ng/µl)	Total DNA yield (mean ± SEM, ng)	n
Q100	Overall	197 ± 1.4	22.4 ± 1.6	4420 ± 340	12
	1%		22.1 ± 1.5	4320 ± 290	3
	0.1%		21.4 ± 2.2	4260 ± 500	3
	0.01%		24.5 ± 0.5	4880 ± 170	3
	0%		21.5 ± 1.1	4210 ± 210	3
Q5	Overall	194 ± 0.4	1.06 ± 0.06	205 ± 11	12
	1%		1.06 ± 0.02	204 ± 4.5	3
	0.1%		1.09 ± 0.12	211 ± 23	3
	0.01%		1.08 ± 0.03	209 ± 5.6	3
	0%		1.02 ± 0.05	197 ± 10	3
DNA-XT	Overall	42 ± 0.4	0.63 ± 0.04	26.3 ± 1.7	12
	1%		0.65 ± 0.03	28.4 ± 1.1	3
	0.1%		0.59 ± 0.05	24.8 ± 2.3	3
	0.01%		0.68 ± 0.05	28.9 ± 2.6	3
	0%		0.55 ± 0.07	22.8 ± 3.7	3

Table 2: Eluate characteristics for purified *Leishmania donovani* amastigote samples

Column		Eluate volume (µl)	[DNA] (ng/µl)	Total DNA yield (ng)
Q5	Mean ± SEM	193 ± 1.2	0.06 ± 0.002	11± 0.4
	A	191	Too low	nd.
	B	195	0.06	11
	C	193	0.05	10
DNA-XT	Mean ± SEM	42 ± 0.3	0.17 ± 0.01	7.3 ± 0.2
	A	42	0.16	6.6
	B	42	0.11	7.2

C	43	0.19	8.1

nd., Not determined

Table 3: PCR positive outcomes for *Plasmodium falciparum* spiked whole blood derived samples

Human Whole Blood	1% parasitaemia (pos/total)*	0.1% parasitaemia (pos/total)	0.01% parasitaemia (pos/total)	0% unspiked (pos/total)
High volume template Q5	2/6	2/6	2/6	0/6
High volume template Q100	0/6	1/6	0/6	0/6
High volume template DNA-XT	6/6	6/6	6/6	0/6
Low volume template Q100	6/6	6/6	5/6	0/6
Low volume template Q5	5/6	6/6	6/6	0/6
Low volume template DNA-XT	6/6	6/6	5/6	0/6

*pos = gel band positive

Figure legends

Figure 1: DNA-XT protocol. Sample preparation: 10 µl of blood/sample added to 150 µl of lysis buffer; mixture incubated at 55 ˚C for 10 min. Column preparation: 350 µl of buffer added prior to centrifugation; 1,400 *g*, 3 min. 80 µl of incubated lysis buffer/sample added to prepared column and incubated at room temperature for 3 min prior to centrifugation; 1,400 *g*, 3 min.

Figure 2: *Plasmodium falciparum* DNA from spiked whole blood determined by *Pf*Beta tubulin PCR. A. low volume eluate template, 1 µl B. high volume eluate template, 10 µl. M: 100bp marker, NTC: No template control. Percentage denotes the parasitaemia post spiking.

Figure 3: *L. donovani* DNA extracted purified amastigotes and determined by PCR of kinetoplast sequence. A. 1 µl of eluate from each manufacturer's column was used as template. B. 10µl of eluate from each manufacturer's column was used as template. Eluates from 3 separate columns for each manufacturer were used. POS positive control, NTC no template control.

References

1. Dahm R. Friedrich Miescher and the discovery of DNA. *Dev. Biol.* 278(2), 274–288 (2005).
2. Meselson M, Stahl FW. THE REPLICATION OF DNA IN ESCHERICHIA COLI. *Proc. Natl. Acad. Sci. U. S. A.* 44(7), 671–82 (1958).
3. Griffiths L, Chacon-Cortes D. Methods for extracting genomic DNA from whole blood samples: current perspectives. *J. Biorepository Sci. Appl. Med.* 2, 1 (2014).
4. Vogelstein B, Gillespie D. Preparative and analytical purification of DNA from agarose. *Proc. Natl. Acad. Sci. U. S. A.* 76(2), 615–9 (1979).
5. Lambros C, Vanderberg JP. Synchronization of Plasmodium falciparum Erythrocytic Stages in Culture. *J. Parasitol.* 65(3), 418 (1979).
6. Croft SL, Yardley V. Animal Models of Visceral Leishmaniasis. *Handb. Anim. Model. Infect.* , 783–787 (1999).

7. Hanscheid T. Diagnosis of malaria: a review of alternatives to conventional microscopy. *Clin. Lab. Haematol.* [Internet]. 21(4), 235–245 (1999). Available from: http://www.ncbi.nlm.nih.gov/pubmed/10583325.
8. Bretagne S, Durand R, Olivi M, *et al.* Real-time PCR as a new tool for quantifying Leishmania infantum in liver in infected mice. *Clin. Diagn. Lab. Immunol.* 8(4), 828–31 (2001).
9. Kapustin D V., Yagudaeva EY, Zavada LL, *et al.* A Composite Polyaniline-Containing Silica Sorbent for DNA Isolation. *Russ. J. Bioorganic Chem.* 29(3), 281–285 (2003).
10. Kopka J, Leder M, Jaureguiberry SM, Brem G, Boselli GO. New Optimized DNA Extraction Protocol for Fingerprints Deposited on a Special Self-Adhesive Security Seal and Other Latent Samples Used for Human Identification*. *J. Forensic Sci.* 56(5), 1235–1240 (2011).
11. Matai AS, Sijen T. Improving a rapid DNA profiling service regularly applied in forensic casework. *Forensic Sci. Int. Genet. Suppl. Ser.* 4(1), e284–e285 (2013).
12. Walsh PS, Metzger DA, Higuchi R. Chelex 100 as a Medium for Simple Extraction of DNA for PCR-Based Typing from Forensic Material. *Biotechniques*. 54(3) (2013).
13. WHO | WHO guidelines on drawing blood: best practices in phlebotomy. *WHO*. (2017).
14. Bereczky S, Mårtensson A, Gil JP, Färnert A. Short report: Rapid DNA extraction from archive blood spots on filter paper for genotyping of Plasmodium falciparum. *Am. J. Trop. Med. Hyg.* [Internet]. 72(3), 249–51 (2005). Available from: http://www.ncbi.nlm.nih.gov/pubmed/15772315.
15. Corran PH, Cook J, Lynch C, *et al.* Dried blood spots as a source of anti-malarial antibodies for epidemiological studies. *Malar. J.* [Internet]. 7(1), 195 (2008). Available from: http://www.ncbi.nlm.nih.gov/pubmed/18826573.
16. Hsiang MS, Lin M, Dokomajilar C, *et al.* PCR-Based Pooling of Dried Blood Spots for Detection of Malaria Parasites: Optimization and Application to a Cohort of Ugandan Children. *J. Clin. Microbiol.* [Internet]. 48(10), 3539–3543 (2010). Available from: http://www.ncbi.nlm.nih.gov/pubmed/20686079.
17. Akane A, Matsubara K, Nakamura H, Takahashi S, Kimura K. Identification of the heme compound copurified with deoxyribonucleic acid (DNA) from bloodstains, a major inhibitor of polymerase chain reaction (PCR) amplification. *J. Forensic Sci.* [Internet]. 39(2), 362–72 (1994). Available from: http://www.ncbi.nlm.nih.gov/pubmed/8195750.
18. Al-Soud WA, Rådström P. Purification and characterization of PCR-inhibitory components in blood cells. *J. Clin. Microbiol.* [Internet]. 39(2), 485–93 (2001). Available from: http://www.ncbi.nlm.nih.gov/pubmed/11158094.
19. Al-Soud WA, Jönsson LJ, Rådström P. Identification and characterization of immunoglobulin G in blood as a major inhibitor of diagnostic PCR. *J. Clin. Microbiol.* [Internet]. 38(1), 345–50 (2000). Available from: http://www.ncbi.nlm.nih.gov/pubmed/10618113.
20. Strøm GEA, Tellevik MG, Hanevik K, Langeland N, Blomberg B. Comparison of four methods for extracting DNA from dried blood on filter paper for PCR targeting the mitochondrial Plasmodium genome. *Trans. R. Soc. Trop. Med. Hyg.* 108(8), 488–94 (2014).
21. le Fichoux Y, Quaranta JF, Aufeuvre JP, *et al.* Occurrence of Leishmania infantum parasitemia in asymptomatic blood donors living in an area of endemicity in southern France. *J. Clin. Microbiol.* [Internet]. 37(6), 1953–7 (1999). Available from: http://www.ncbi.nlm.nih.gov/pubmed/10325353.

22. Riera C, Fisa R, Udina M, Gállego M, Portus M. Detection of Leishmania infantum cryptic infection in asymptomatic blood donors living in an endemic area (Eivissa, Balearic Islands, Spain) by different diagnostic methods. *Trans. R. Soc. Trop. Med. Hyg.* [Internet]. 98(2), 102–10 (2004). Available from: http://www.ncbi.nlm.nih.gov/pubmed/14964810.

23. Lin JT, Saunders DL, Meshnick SR. The role of submicroscopic parasitemia in malaria transmission: what is the evidence? *Trends Parasitol.* 30(4), 183–190 (2014).

24. Silal SP, Little F, Barnes KI, White LJ. Hitting a Moving Target: A Model for Malaria Elimination in the Presence of Population Movement. *PLoS One* [Internet]. 10(12), e0144990 (2015). Available from: http://www.ncbi.nlm.nih.gov/pubmed/26689547.

25. White LJ, Maude RJ, Pongtavornpinyo W, *et al.* The role of simple mathematical models in malaria elimination strategy design. *Malar. J.* [Internet]. 8(1), 212 (2009). Available from: http://www.ncbi.nlm.nih.gov/pubmed/19747403.

26. Tietje K, Hawkins K, Clerk C, *et al.* The essential role of infection-detection technologies for malaria elimination and eradication. *Trends Parasitol.* [Internet]. 30(5), 259–266 (2014). Available from: http://www.ncbi.nlm.nih.gov/pubmed/24726857.

Reference annotations

*Al-Soud, W. A. and Radstrom, P. (2001) 'Purification and Characterization of PCR-Inhibitory Components in Blood Cells', *Journal of Clinical Microbiology*, 39(2), pp. 485–493. doi: 10.1128/JCM.39.2.485-493.2001.

This paper characterised the components of blood cells which cause PCR inhibition.

*Bereczky, S. *et al.* (2005) 'Short report: Rapid DNA extraction from archive blood spots on filter paper for genotyping of Plasmodium falciparum.', *The American journal of tropical medicine and hygiene*, 72(3), pp. 249–51. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15772315 (Accessed: 8 February 2019)

Described herein is a commonly used method for blood collection and archiving, from which DNA can be extracted, using volumes particularly relevant to the DNA-XT extraction technology.

*Griffiths, L. and Chacon-Cortes, D. (2014) 'Methods for extracting genomic DNA from whole blood samples: current perspectives', *Journal of Biorepository Science for Applied Medicine*. Dove Press, 2, p. 1. doi: 10.2147/BSAM.S46573.

This article describes the current landscape of DNA extraction methods from whole blood samples, including their strengths and drawbacks which is a useful backdrop for assessing new DNA extraction techniques.

*Kapustin, D. V. *et al.* (2003) 'A Composite Polyaniline-Containing Silica Sorbent for DNA Isolation', *Russian Journal of Bioorganic Chemistry*. Kluwer Academic Publishers-Plenum Publishers, 29(3), pp. 281–285. doi: 10.1023/A:1023992701568.

The majority of commercial DNA extraction kits rely on the silica sorbent method described by Kapustin, D.V. *et al.*

**Riera, C. *et al.* (2004) 'Detection of Leishmania infantum cryptic infection in asymptomatic blood donors living in an endemic area (Eivissa, Balearic Islands, Spain) by different diagnostic methods.', *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 98(2), pp. 102–10. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14964810 (Accessed: 8 February 2019).

**Tietje, K. *et al.* (2014) 'The essential role of infection-detection technologies for malaria elimination and eradication', *Trends in Parasitology*, 30(5), pp. 259–266. doi: 10.1016/j.pt.2014.03.003.

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Riera, C. *et al.*, and Tietje, K. *et al.* demonstrate the critical importance of sensitive diagnostics which can be readily employed in the field, so that leishmania and malaria can be detected in asymptomatic patients. Eradication becomes that much more difficult when asymptomatic patients act as reservoirs for these parasites, continuing the spread of disease.

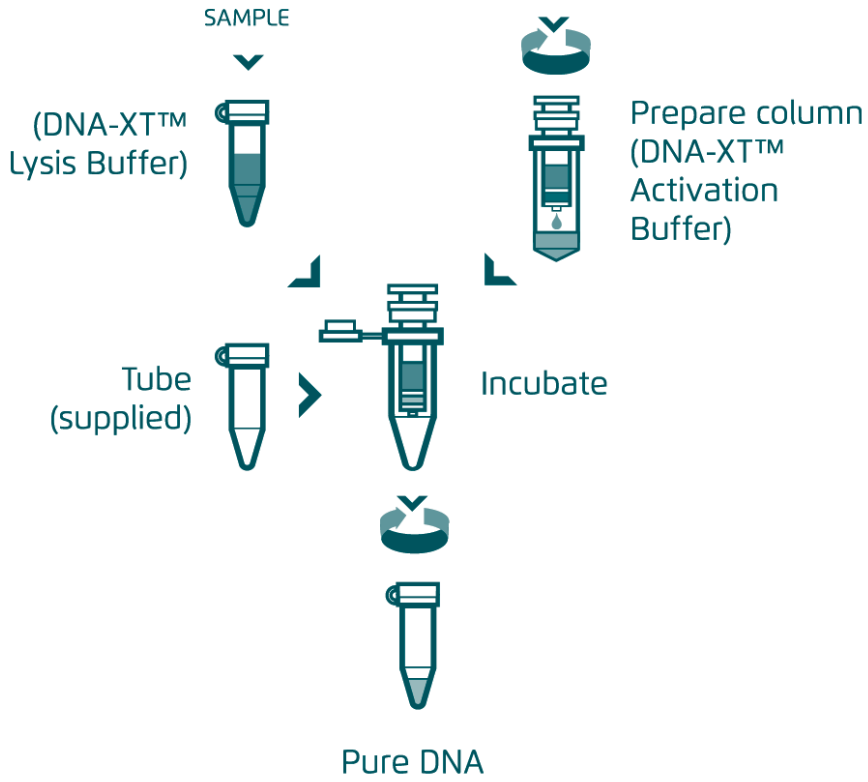


Figure 1: DNA-XT protocol. Sample preparation: 10 µl of blood/sample added to 150 µl of lysis buffer; mixture incubated at 55 ˚C for 10 min. Column preparation: 350 µl of buffer added prior to centrifugation; 1,400 *g*, 3 min. 80 µl of incubated lysis buffer/sample added to prepared column and incubated at room temperature for 3 min prior to centrifugation; 1,400 *g*, 3 min.

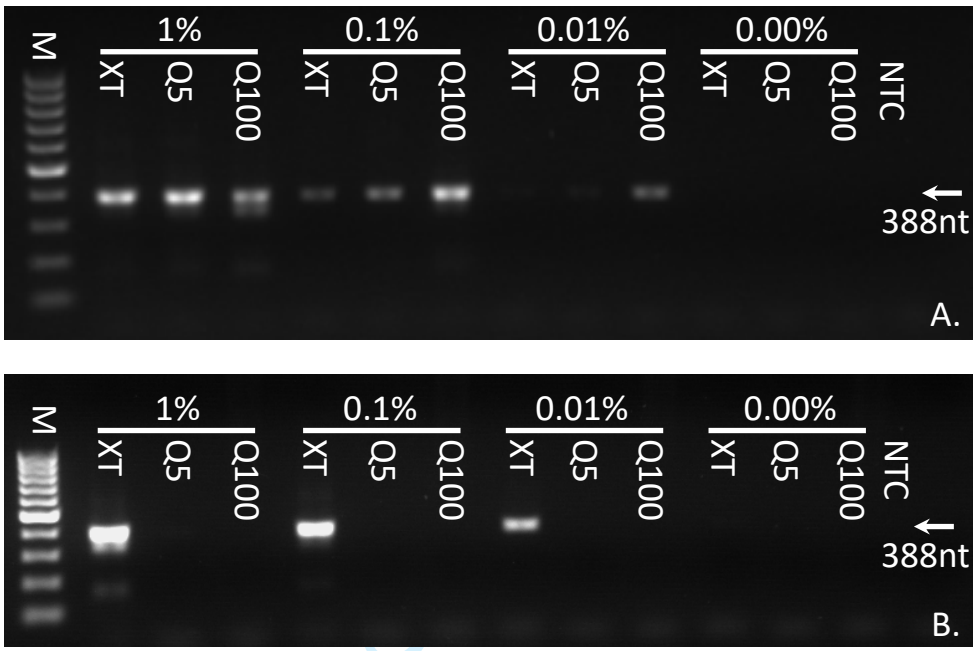


Figure 2: *Plasmodium falciparum* DNA from spiked whole blood determined by *Pf*Beta tubulin PCR. A. low volume eluate template, 1 µl B. high volume eluate template, 10 µl. M: 100bp marker, NTC: No template control. Percentage denotes the parasitaemia post spiking.

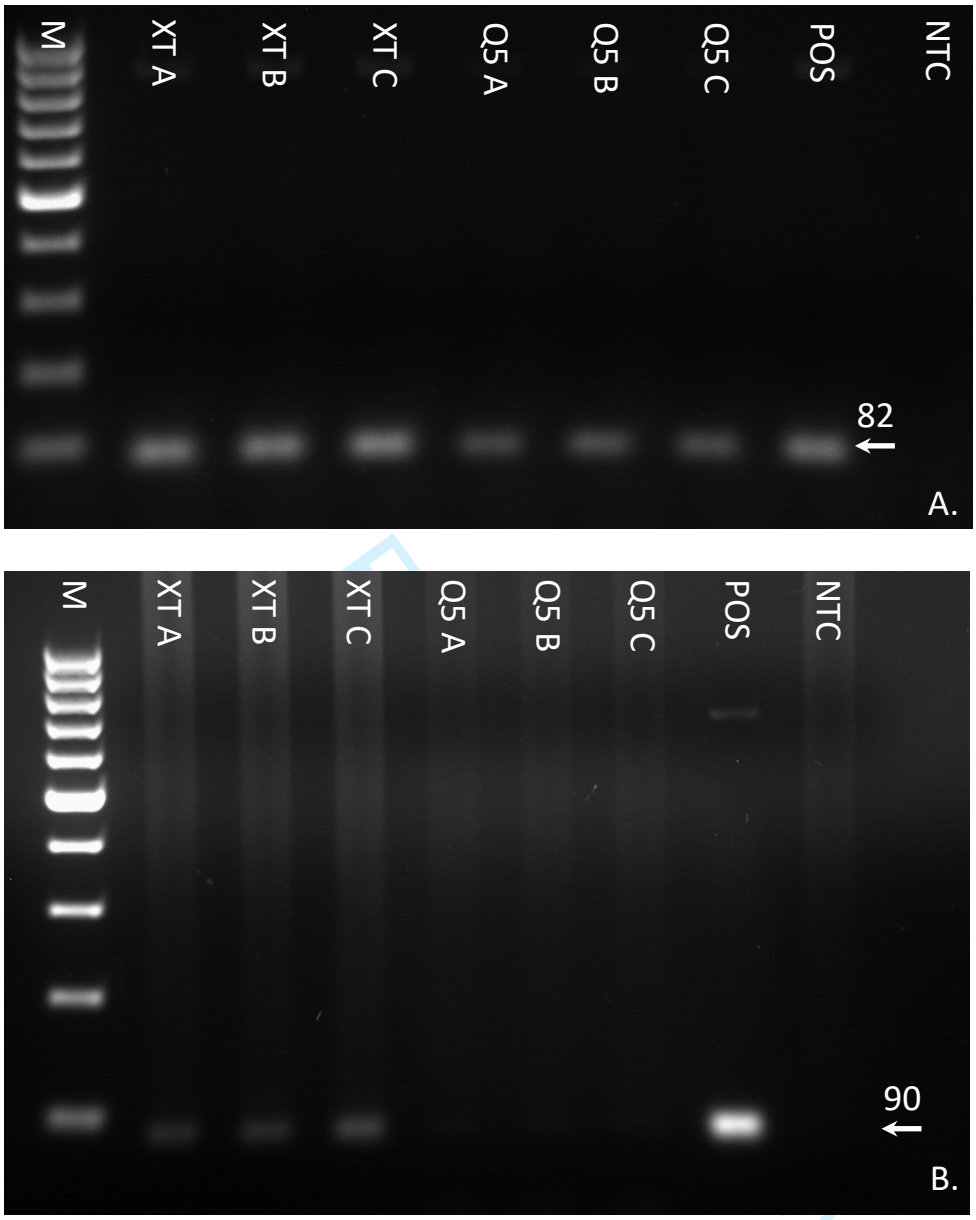


Figure 3: *L. donovani* DNA extracted purified amastigotes and determined by PCR of kinetoplast sequence. A. 1 µl of eluate from each manufacturer's column was used as template. B. 10µl of eluate from each manufacturer's column was used as template. Eluates from 3 separate columns for each manufacturer were used. POS positive control, NTC no template control.

Experiment 1

Parasitaemia, %			
Predicted	Count 1	Count 2	Actual
1	1.16	1.44	1.30
0.1	0.10	0.09496	0.10
0.01	NT	NT	NT
0	NA	NA	NA

Experiment 2

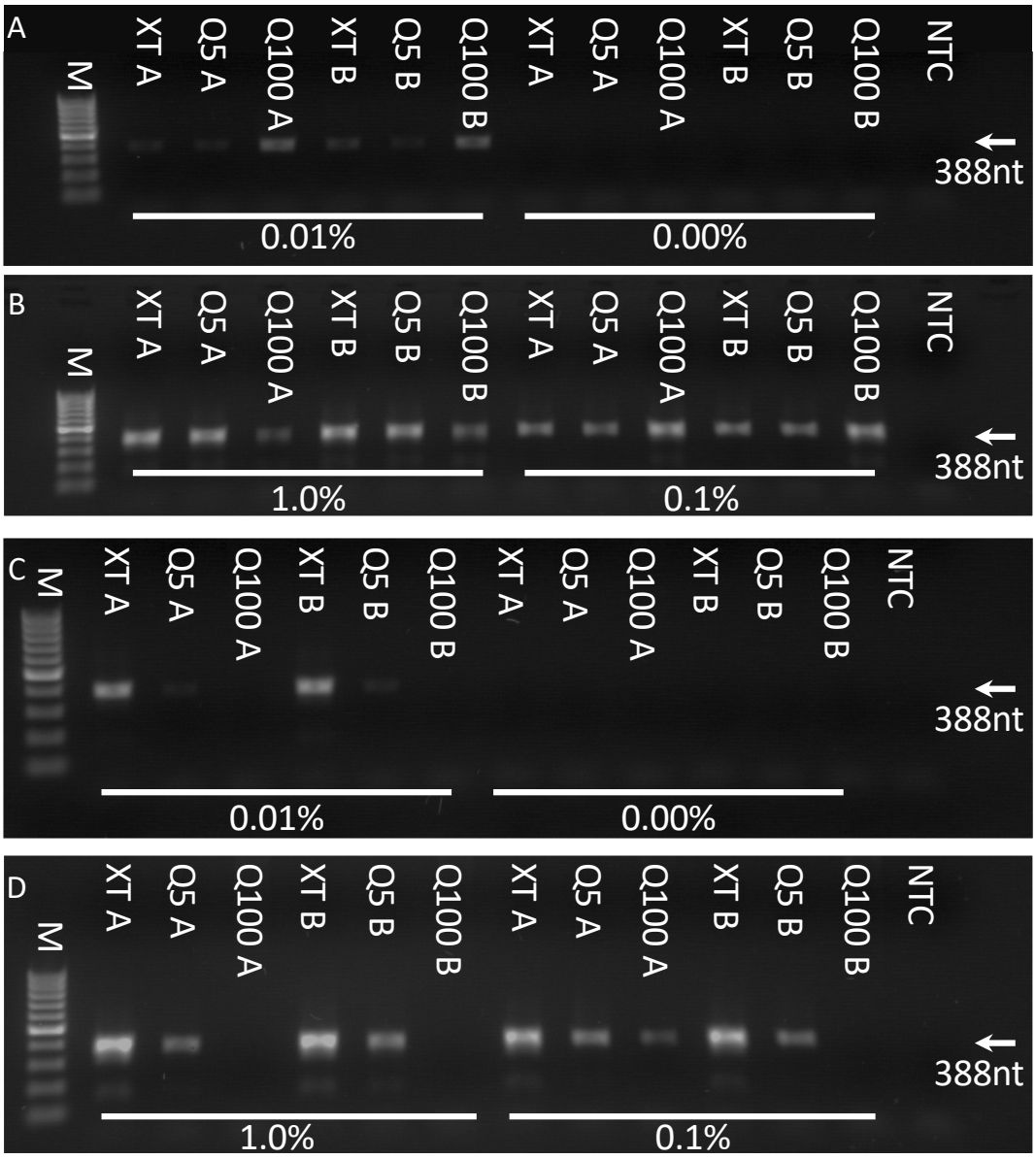
Parasitaemia, %			
Predicted	Count 1	Count 2	Actual
1	0.88	0.79	0.84
0.1	0.09	0.09	0.09
0.01	NT	NT	0.01
0	NA	NA	NA

Experiment 3

Parasitaemia, %			
Predicted	Count 1	Count 2	Actual
1	0.91	0.95	0.93
0.1	0.10	0.10	0.10
0.01	NT	NT	NT
0	NA	NA	NA

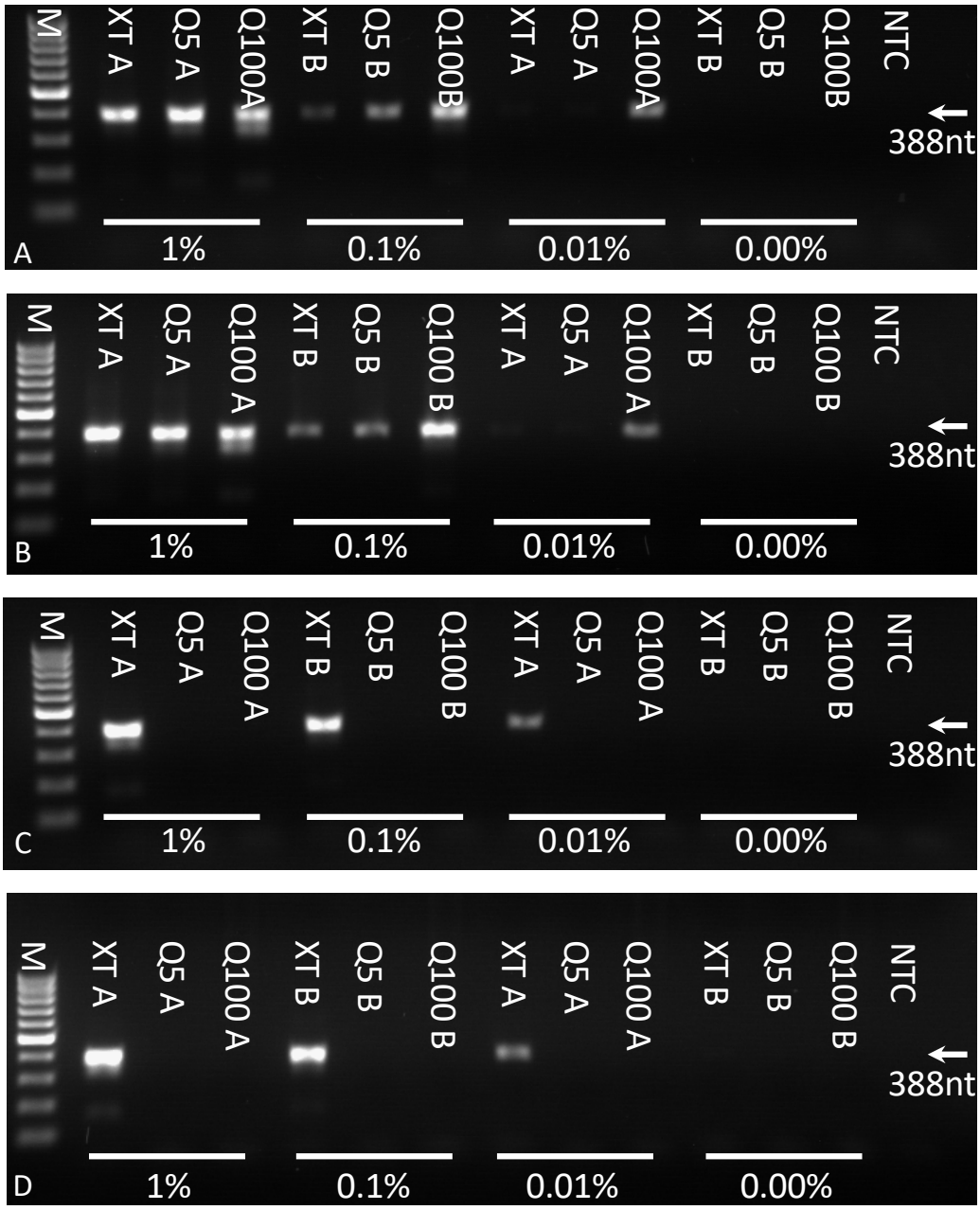
NT: Not tested
NA: Not applicable

Additional file 2



Supplementary Figure 1: *Plasmodium falciparum* DNA from spiked whole blood determined by *Pf*Beta tubulin PCR. A and B. low volume eluate template, 1 µl C and D. high volume eluate template, 10 µl. Two extractions run per parasitaemia condition. M: 100bp marker, NTC: No template control. Percentage denotes the parasitaemia post spiking.

Additional file 2



Supplementary Figure 2: *Plasmodium falciparum* DNA from spiked whole blood determined by *Pf*Beta tubulin PCR. A and B. low volume eluate template, 1 μl C and D. high volume eluate template, 10 μl. Two extractions run per parasitaemia condition. M: 100bp marker, NTC: No template control. Percentage denotes the parasitaemia post spiking.