

## Review Article

# Comparative assessment of genomic DNA extraction processes for *Plasmodium*: Identifying the appropriate method

Riti Mann, Supriya Sharma, Neelima Mishra, Neena Valecha & Anupkumar R. Anvikar

National Institute of Malaria Research (ICMR), New Delhi, India

## ABSTRACT

*Plasmodium* DNA, in addition to being used for molecular diagnosis of malaria, find utility in monitoring patient responses to antimalarial drugs, drug resistance studies, genotyping and sequencing purposes. Over the years, numerous protocols have been proposed for extracting *Plasmodium* DNA from a variety of sources. Given that DNA isolation is fundamental to successful molecular studies, here we review the most commonly used methods for *Plasmodium* genomic DNA isolation, emphasizing their *pros* and *cons*. A comparison of these existing methods has been made, to evaluate their appropriateness for use in different applications and identify the method suitable for a particular laboratory based study. Selection of a suitable and accessible DNA extraction method for *Plasmodium* requires consideration of many factors, the most important being sensitivity, cost-effectiveness and, purity and stability of isolated DNA. Need of the hour is to accentuate on the development of a method that upholds well on all these parameters.

**Key words** DNA isolation; malaria; molecular tools; *Plasmodium*; protocols

## INTRODUCTION

Malaria, a parasitic disease caused by the protozoans belonging to family Plasmodiidae and genus *Plasmodium*, is a major global health problem. Since 2005, the number of malaria infections has decreased following new initiatives in malaria control, increased urbanization, and overall economic development; however, the data on malaria are still alarming<sup>1</sup>. Malaria globally affects about 198 million people annually and accounts for nearly 5,84,000 deaths<sup>1</sup>. Laboratory diagnosis is important to the prevention, control and adequate treatment of malaria. Malaria diagnosis can not rely solely on the clinical manifestations of the disease because the signs and symptoms imitate other infections<sup>2</sup>. Microscopic examination of Giemsa-stained blood smears remains the gold standard for malaria diagnosis; however rapid diagnostic tests (RDTs) have also been successfully implemented<sup>3-4</sup>. Accurate diagnosis can not be achieved by microscopy or RDTs alone at present, as they both have certain drawbacks. Microscopy, for instance requires a skilled technician to read the slides and show poor sensitivity in detection of low-level parasitaemia with a detection limit of approximately 50 parasites/ $\mu$ l<sup>4-5</sup>. RDTs, however, are user-friendly can sometimes be inaccurate at diagnosis, are

relatively expensive and fail to detect low-density infections<sup>4</sup>. They are not much useful in monitoring the response to antimalarial drugs due to their inability to quantify the degree of parasitaemia<sup>6-7</sup>. Molecular methods introduced for malaria diagnosis can overcome these restraints, with their ability to detect submicroscopic and mixed infections<sup>3, 8</sup>. Compared with microscopy and RDTs, molecular tests are much more sensitive at detecting malaria parasites with detection limits as low as 5 parasites/ $\mu$ l<sup>3, 9</sup>. With this level of sensitivity, molecular methods are capable of malaria detection in asymptomatic carriers and are useful in the context of malaria eradication programmes<sup>8</sup>. The most routinely used molecular method for detecting malaria parasites is polymerase chain reaction (PCR) assay, whose potency lies in accurate identification at the species and subspecies level, and detection of low level parasitaemia and mixed infections<sup>8, 10</sup>. PCR assay has captured special attention in malaria diagnosis as it is highly sensitive and specific<sup>4</sup>.

Isolation of high-quality genomic DNA is an important step in determining the suitability of a particular molecular method in malaria research<sup>11</sup>. Choice of a molecular method for malaria diagnosis depends to a large extent on the purity, stability and integrity of the genomic DNA used for analysis. High purity DNA is ideal as con-

	Whole Blood	Filter paper spots	Blood clots	Blood smears	RDTs
PROS	Collected blood can directly be used for isolation  Yields high quality DNA  Easy to handle	Ease of collection, storage and transportation	Isolation of DNA from a disregarded source material	Source of historical DNA for studying drug resistance in malaria parasites	Isolated DNA can be used for quality control testing of RDTs  Can be stored in plastic bags, thereby reducing the risk of contamination
CONS	Difficult to store and transport	Possibility of contamination via poor handling by field workers  Proper storage conditions are required	Complicated handling procedures	Risk of contamination during staining and microscopy  Proper storage conditions are required	No possibility of re-extraction  A limited amount of blood is stored on them
METHODS	Rapid boiling Phenol-chloroform GTC-phenol-chloroform Boil and spin Chelex-100 Microwave irradiation Isotachophoresis Commercial kits	Chelex-100 InstaGene TE-based Methanol based Commercial kits	Clot dispersion by centrifugation, high speed shaking, etc.	Slides treatment, followed by DNA extraction using known isolation methods	Simple elution Chelex-100 Phenol-chloroform Commercial kits

Fig. 1: Sources for isolation of *Plasmodium* DNA, their pros and cons, and methods commonly used for extraction from respective source.

taminated DNA often fails to give accurate, consistent and reproducible results. Over the years, numerous approaches have been developed to isolate *Plasmodium* DNA from an array of sources (Fig. 1). Selection of an isolation method decides the quality of DNA and the sensitivity of downstream applications<sup>12</sup>. Contemplating that DNA isolation is the first crucial step necessary for molecular studies on malaria, this review ponders the methods available for genomic DNA isolation from *Plasmodium*, and attempts to compare them and evaluate their appropriateness for use in different applications. Additionally, some of the advantages and disadvantages of these methods are discussed focusing on their sensitivity, cost-effectiveness and labor intensiveness.

Ideally blood samples stored on filter papers is considered the most preferred source for *Plasmodium* DNA. This device facilitates easy sample collection, long-term storage at room temperature, easy transportation; while the DNA captured remains protected from degradation<sup>13</sup>. Detection sensitivity achieved with blood spot filter papers varies with the method of DNA extraction employed<sup>13</sup>. The use of commercially available blood filter paper collection cards like the FTA card is required for regulatory clinical trials on antimalarial drug efficacy against malaria parasites<sup>14</sup>. Improved malaria surveillance and effective implementation of malaria eradication programs requires exploration of alternative sources of parasite DNA; such as frozen blood clots, smears and RDTs.

Frozen blood clots are not the preferred source for isolating DNA because of their troublesome handling procedures and insignificant yield<sup>12</sup>. There are plentiful reports on isolation of *Plasmodium* genomic DNA from stained blood smears<sup>11, 15-19</sup>. DNA isolation from smears is advantageous in resource-limited setups with a scarcity of facilities for blood collection and storage<sup>17</sup>. The old smears are a valuable source of historical material, proving useful for investigating the spread of drug resistance among malaria parasites<sup>17</sup> and facilitating the study of genetic variations acquired by parasite populations over time<sup>15</sup>. The use of RDTs as a source of *Plasmodium* DNA was first specified by Veron and Carme<sup>20</sup>, thereafter many protocols were introduced.

#### DNA isolation methods

A genomic DNA isolation procedure from eukaryotic cells generally includes three important steps: degradation of cell membranes, partitioning of the DNA from all other cell components (proteins, metabolic substances and cell wall debris) and protection of DNA integrity during the isolation process (Fig. 2). Molecular diagnosis of malaria via blood sample analysis broadly entails blood sample collection, genomic DNA isolation and downstream analysis. Hemoglobin in red blood cells interferes with the PCR amplification process; therefore, DNA should be extracted from blood samples before further analysis<sup>4, 21</sup>. Based on whether the sample is avail-

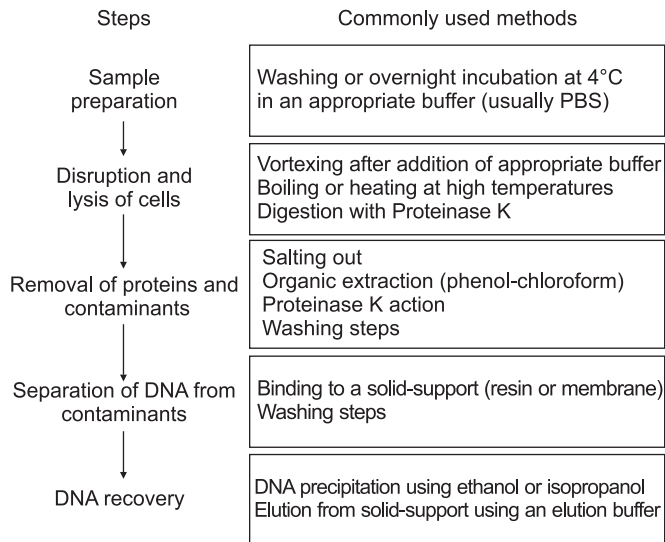


Fig. 2: Basic steps in DNA isolation protocols.

able as whole blood, filter paper spots, RDT samples or blood smear slides there are numerous methods for *Plasmodium* DNA isolation.

**Chemical and matrix based methods:** One of the few methods initially described for *Plasmodium* DNA isolation is based on rapid boiling methodology<sup>22</sup>. It is a simple and rapid method requiring a small blood volume and a short processing time of about 50 min. This method includes washing with sodium phosphate solution, which removes hemoglobin, an inhibitor of Taq polymerase in PCRs. This method has a detection limit of ~200 parasites/μl, is inexpensive, provides an overall sensitivity of about 62% and offers increased sensitivity to the detection of multiple infections<sup>22-24</sup>. Deterioration in the DNA quality after long-term storage is a critical issue associated with this method<sup>23</sup>.

Another conventional manual method of genomic DNA isolation from *Plasmodium* is the Chelex-100 protocol<sup>25</sup>. This method is based on the ability of Chelex resin to bind all cellular components except DNA, thereby allowing the DNA to be extracted from the remaining solution. This method has a sensitivity of about 30 parasites/μl (and 2 parasites/μl when applied to DNA isolation from RDTs), is relatively inexpensive and suitable for detection of low-density parasitaemia<sup>26-27</sup>. This method is moderately labor-intensive and the isolated DNA purity is lower as compared to commercially available kits<sup>28</sup>. Additionally, the DNA is highly susceptible to multiple cycles of freezing and thawing after storage<sup>29</sup>. The Chelex based technique has been successfully employed for DNA isolation from blood spots and for parasite DNA isolation from *Anopheles* spp mosquito specimens<sup>30</sup>. Parasite DNA isolation from mosquito specimens

is important for surveillance of drug-resistant *Plasmodium* sp. alleles in mosquitoes for malaria control programmes<sup>31</sup>.

Cox-Singh *et al*<sup>32</sup> suggested the use of InstaGene matrix for malaria parasite DNA extraction and reported it to be more efficient than Chelex at removing PCR inhibitors, but Strøm *et al*<sup>33</sup> established that both matrices had similar effects. Comparatively, the InstaGene matrix has the advantage of using template volumes of up to 20 μl, whereas with Chelex method, volumes above 10 μl inhibit PCR amplification<sup>32</sup>. In terms of their labour-intensiveness, Chelex-100 method involves only one heating step, whereas with InstaGene matrix two heating steps are required<sup>33</sup>. Another chemical method for isolation of *Plasmodium* DNA in routine use involves the use of hazardous organic solvents, phenol and chloroform<sup>31</sup>. Despite being labour-intensive, phenol-chloroform extraction method is cost-effective as no expensive chemicals or instruments are required and has also been applied for DNA isolation from RDTs. In fact this method gave best extraction yields among the methods compared by Veron and Carme<sup>20</sup>. In guanidine isothiocyanate (GTC) phenol chloroform method of *Plasmodium* DNA isolation, the blood samples are initially prepared using GTC followed by subsequent phenol-chloroform extraction. This is a lengthy and time-consuming method, with a high risk of contamination from the large number of pipetting and tube changing steps<sup>24</sup>. The boil and spin method is used for extracting *Plasmodium* DNA from whole blood samples<sup>34</sup>. A Tris-EDTA (TE) based method and methanol-based method can be used for isolating *Plasmodium* DNA from blood spots<sup>13</sup>. TE-based method yields sensitivity in the range of 93–100% as per Bereczky *et al*<sup>13</sup>, but performance was poor when assessed by Miguel *et al*<sup>30</sup> and Strøm *et al*<sup>33</sup>. Methanol-based approach performs almost similar to TE-based method<sup>30</sup>.

**Isotachophoresis (ITP)-based methodology:** Isotachophoresis has been used for DNA extraction from malaria parasite-infected RBCs in a reasonable time of approximately 30 min<sup>21</sup>. Briefly, the infected RBCs are lysed to release parasite nucleic acids by heating at high temperature (95°C) after addition of proteinase K. The resultant lysate, along with TE buffer, is loaded onto the capillary, and an electric field is applied to focus the DNA at the interface between the leading and trailing electrolytes according to its electrophoretic mobility (the flow direction is from the leading to the trailing electrolyte). Other contaminants like proteins and PCR inhibitors remain unfocused in the trailing electrolyte well. The focused DNA eventually moves to the leading electrolyte well, from where it can be extracted for further use after

monitoring by epifluorescence microscopy. As malaria parasites are harder to lyse than host cells, a higher lysis temperature and increased extraction time via pressure-driven counter flow are required for isolating *Plasmodium* DNA. The study by Marshall *et al*<sup>21</sup> clearly shows the applicability of ITP as a microfluidic malarial parasite DNA preparation method. This method with the detection limit of 500 parasites/ $\mu$ l could possibly be incorporated into malaria diagnostic systems in clinical settings.

**Irradiation based methodology:** Aside from the various mechanical and chemical methods available, there are few simpler methods suggested for malarial parasite DNA isolation like the microwave irradiation method. This method has consistently produced reliable results for DNA extraction from whole blood samples in less than 3 min and is reported to be easy to perform, fast and cost-effective<sup>4</sup>. With this method, 10  $\mu$ l of blood is collected in a 0.5 ml tube and irradiated at 800 W for 2 min or until condensed droplets are visible on the inner walls of the tube. The clear watery solution on the walls can be used directly for downstream purposes or 30  $\mu$ l of phosphate-buffered saline (PBS) can be added to the sample for long-term storage. Smaller tubes with a capacity less than 0.5 ml cannot be used as they can break when irradiated or may get destroyed via air expansion.

**Isolation using commercial kits:** Among the many available kits for DNA isolation from blood samples, here we have compared those cited in literature specifically for *Plasmodium*. The most widely used is the QIAamp Blood Extraction kit for DNA isolation from whole blood samples<sup>35</sup>. The DNeasy Blood and Tissue Kit (Qiagen) is another commercially available kit<sup>36</sup>. It is a multipurpose kit used for DNA extraction from tissue samples, nucleated and non-nucleated blood, and from cultured cells. DNA extraction from blood spots can be accomplished by using the commercially available QIAamp DNA mini kit<sup>33, 37</sup>. The PURE (Procedure for Ultra Rapid Extraction) DNA extraction kit is used for *Plasmodium* DNA isolation with subsequent detection using LAMP<sup>34</sup>. Another commercially available kit is the Gentra Puregene Blood Kit<sup>38</sup>. The DNA obtained using this kit is very pure with an average  $A_{260}/A_{280}$  ratio of 1.8. A modified protocol based on this kit has been employed for clot dispersion by Lundblom *et al*<sup>12</sup>. DNA extracted using Promega Wizard Genomic DNA Purification Kit was used as the gold standard by Miguel *et al*<sup>30</sup>, for comparative study of different isolation protocols for *Plasmodium*.

**Isolation from blood clots, smears and RDTs:** Many mechanical and chemical techniques are suggested for clot dispersion; however obstacles exist in obtaining a good quality and quantity of DNA using these processes<sup>12, 39-41</sup>.

With malaria parasites in particular, optimal clot disruption is essential for obtaining parasite DNA with high PCR sensitivity<sup>12</sup>. Simple high-speed shaking that result in clot dispersion has produced good results<sup>12</sup>. This method is based on linear top-to-bottom shaking of tubes at 3450 oscillations/min in a cell disruptor after addition of RBC lysis buffer to the clot. After clot dispersion, DNA extraction can be performed using standard protocols, the most common being the use of commercially available kits<sup>12</sup>.

Generally, DNA isolation from slides involves scraping the smear surface after adding a suitable buffer to it, followed by DNA extraction using any standard *Plasmodium* DNA isolation method. Chelex-based approach is proved not to be sensitive enough for parasite detection using real time PCR in DNA isolated from smears<sup>19</sup>. The approach described by Kimura *et al*<sup>15</sup> is capable of successfully isolating DNA from smears with sample parasitaemias above 0.03%. Edoh *et al*<sup>17</sup> suggested a simple washing and boiling method for DNA preparation from stained blood smears, which gave reliable results for high parasitaemia samples but not for those with low-level parasitaemias.

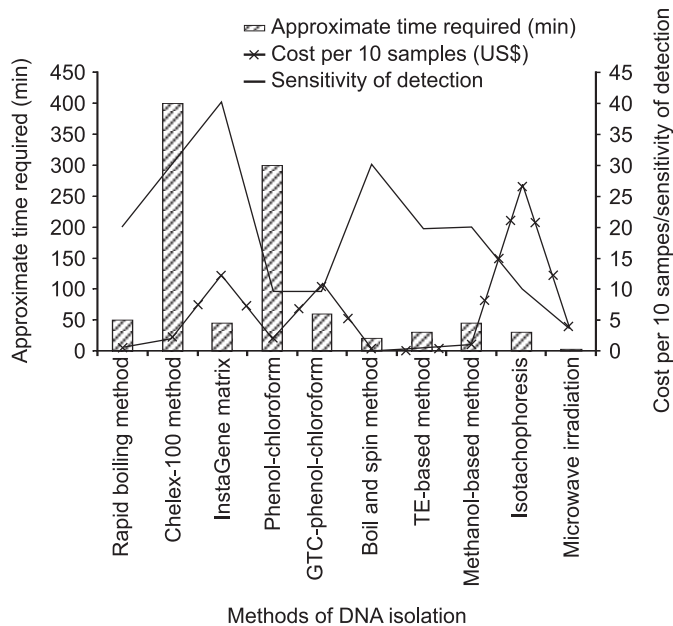
Apart from the phenol-chloroform method and commercially available kits already described, Cnops *et al*<sup>42</sup> suggested an adapted simple elution method and Morris *et al*<sup>27</sup> suggested a modified version for 'isolation of DNA from fresh or frozen whole blood' for DNA isolation from RDTs.

## DISCUSSION

The perfect DNA isolation method should be sensitive, reproducible, quick, trouble-free to use and should minimize the use of specialized instrument or biochemical knowledge. It should shun probable cross contamination of samples and also cause least risk to user. Any protocol, whether it entails a chemical method or a physical approach proves itself worthy only when it yields uncontaminated DNA of sufficient yield for downstream molecular study, preferably in a short time. Cost-effectiveness is a critical factor if the isolation is to be performed in an under developed or developing country. Reviewing the available literature for malaria parasite DNA extraction, we found that researchers on the whole use standard protocols<sup>27, 30</sup> and incorporate alterations wherever necessary according to their own requirements<sup>31, 33</sup>.

The benefits of chemical and matrix based methods lie in the fact that these do not require specialized instruments and are cost-effective as compared to the commercial kit based methods. Only drawback associated with





**Fig. 3:** Assessment of DNA extraction methods for *Plasmodium*: Graph depicting the sensitivity of detection, cost-effectiveness and time required for each method on a relative scale. Approximate costs are calculated from the purchase price of reagents and information specified in published data. These figures do not include equipment cost, reaction failure rates and loss of samples. Detection sensitivity is estimated based on relative analysis of published data and the values obtained are extrapolated on a scale of 0–100.

these methods is the lack of high sensitivity (Fig. 3). A sensitivity and specificity value of  $\geq 95\%$  is considered acceptable for laboratory diagnosis<sup>30</sup>. Amid the methods discussed here, a good level of sensitivity is observed for InstaGene matrix and microwave irradiation based methods with a detection limit of 0.6 and 5 parasites/ $\mu\text{l}$ , respectively. Another noteworthy factor while selecting an appropriate isolation method is time requirement. For rapid diagnosis of malaria, least time should be required from sample collection to molecular analysis. Therefore, the DNA isolation method needs to be as quick as possible. Irradiation-based approach demands minimum time amongst all the methods considered here. The use of isotachopheresis for whole blood samples was found to be applicable in DNA isolation method with a possibility of being used as malaria diagnosis tool in clinical settings<sup>21</sup>. Further, research needs to be done for separating human DNA present as a contaminant while performing isolation using this method. A well-aimed strategy would be to remove the leucocytes before loading sample onto the capillary. Table 1 highlights the advantages, disadvantages and parasite detection limits of different DNA isolation methods for *Plasmodium*.

A summary of the commercial kits commonly used

for *Plasmodium* DNA isolation is presented in Table 2, with emphasis on their DNA yield, limit of detection, cost-effectiveness and time requisite. These methods however, provide sensitivity towards detection of malaria parasite, but are expensive and sometimes very labour-intensive. Despite of their high cost, commercial kits are the most commonly used means for isolating *Plasmodium* DNA because of their less failure rates and sample loss.

Among the different methods studied for clot dispersion, high-speed shaking resulted in a better degree of clot dispersion, higher DNA yields and a better level of PCR detection for parasite DNA<sup>12</sup>. The method is straightforward, rapid, requires little manual handling and minimizes DNA loss as beads are used for tissue homogenization<sup>12</sup>. The mechanical noise accompanying the use of this method is a major drawback, a factor which requires careful consideration concerning the operating space<sup>12</sup>.

For isolating *Plasmodium* DNA from blood smears, use of a Qiagen DNA extraction kit gave better results than the Chelex and boiling methods<sup>19</sup>. Specifically, for thin smears, affinity purification using DNA preparation kits upheld other methods<sup>17</sup>. Thick blood films (TBFs) with higher numbers of parasites gave improved results over thin smears with lower parasitaemia<sup>16</sup>. As compared to DNA isolated from whole blood (sensitivity and specificity of 96 and 76%, respectively), DNA extracted from smears provides a reduced sensitivity value of 78% and specificity of 86%<sup>11</sup>.

The most appropriate method for extracting DNA from RDTs has not been clearly defined, as different evaluation studies have reported various methods to be the most appropriate<sup>20, 27, 42–43</sup>. Long-term or short-term RDT storage at ambient conditions has no effect on the yield of DNA extracted from the device<sup>20, 42</sup>. The quality of DNA isolated is not predicated by the RDT brand, but the RDT design does affect the DNA extraction efficiency<sup>27, 42</sup>. In addition to comparisons of the different RDT brands, the RDT compartment best suitable for DNA isolation has been optimized<sup>27, 42–43</sup>. A combination of the proximal part of the nitrocellulose strip and the conjugate pad gives better results than using each RDT fragment separately<sup>42</sup>. Morris *et al*<sup>27</sup> showed the quality of DNA obtained from RDTs and filter paper samples to be the same.

Broad-scale collection of smears and used RDTs may facilitate enhanced malaria case detection and drug resistance surveillance<sup>27</sup>. Overall, the quality of DNA obtained from smears and RDTs is similar, as confirmed by sensitive detection techniques like real-time PCR on DNA isolated from both sources<sup>19, 42</sup>. Nonetheless, with these

Table 1. DNA extraction techniques for *Plasmodium*, their advantages, disadvantages, parasite detection limits and mean multiplicity of infection (LAMP: Loop mediated isothermal amplification)

Extraction method	Advantages	Disadvantages	Parasite detection/ $\mu$ l of blood (detection technique)	Mean multiplicity of infection
Rapid boiling method	<ul style="list-style-type: none"> <li>• Inexpensive</li> <li>• Nominal blood volume required</li> </ul>	<ul style="list-style-type: none"> <li>• Contamination risk</li> <li>• Long-term storage affects DNA quality</li> </ul>	200 (Nested PCR)	2.17 $\pm$ 0.57
Chelex-100 method	<ul style="list-style-type: none"> <li>• Low cost</li> <li>• Not labour-intensive</li> <li>• Highly sensitive and specific</li> </ul>	<ul style="list-style-type: none"> <li>• Template volumes &gt; 10 <math>\mu</math>l inhibit amplification</li> </ul>	6 (Nested PCR) 0.16 (Mitochondrial PCR)	2 $\pm$ 0.29
InstaGene matrix	<ul style="list-style-type: none"> <li>• Highly efficient at removing PCR inhibitors from blood</li> </ul>	<ul style="list-style-type: none"> <li>• Relatively expensive</li> <li>• Labour-intensive</li> </ul>	0.6 (Nested PCR) 0.16 (Mitochondrial PCR)	NA
Phenol-chloroform	<ul style="list-style-type: none"> <li>• Reasonably priced</li> </ul>	<ul style="list-style-type: none"> <li>• Time-consuming</li> </ul>	NA	NA
GTC-phenol-chloroform	<ul style="list-style-type: none"> <li>• Cost-effective</li> </ul>	<ul style="list-style-type: none"> <li>• Time-consuming</li> <li>• Prone to contamination</li> </ul>	NA	1.75 $\pm$ 0.53
Boil and spin method	<ul style="list-style-type: none"> <li>• Rapid</li> <li>• Economical</li> <li>• Requires only boiling and centrifugation</li> </ul>	<ul style="list-style-type: none"> <li>• Low detection specificity</li> </ul>	$\geq 1$ (LAMP) <sup>45</sup>	NA
TE-based method	<ul style="list-style-type: none"> <li>• Rapid</li> <li>• Simple</li> <li>• Inexpensive</li> <li>• Low risk of cross-contamination</li> </ul>	<ul style="list-style-type: none"> <li>• Less sensitive</li> </ul>	200 (Mt PCR)	2 $\pm$ 0.26
Methanol-based method	<ul style="list-style-type: none"> <li>• Low-priced</li> </ul>	<ul style="list-style-type: none"> <li>• Less sensitive</li> </ul>	NA	1.4 $\pm$ 0.27
Isotachophoresis	<ul style="list-style-type: none"> <li>• Suitable for automated <i>Plasmodium</i> DNA isolation</li> </ul>	<ul style="list-style-type: none"> <li>• Epifluorescence microscopy required for monitoring</li> </ul>	500 (Quantitative PCR)	NA
Microwave irradiation	<ul style="list-style-type: none"> <li>• Easy to do</li> <li>• Rapid</li> <li>• Cost-efficient</li> <li>• Suited to field settings</li> </ul>	<ul style="list-style-type: none"> <li>• Contamination risk if tubes explode</li> <li>• Difficult to retrieve condensed droplets from tube walls</li> </ul>	5 (LAMP) <sup>45</sup>	NA

Table 2. Comparison of the commercial kits used widely for *Plasmodium* DNA isolation (LAMP: Loop mediated isothermal amplification) (Costs per sample are calculated from the purchase price of kits and information specified in the published data. These figures do not take into consideration factors like equipment cost, reaction failure rates, necessary repeat reactions and loss of samples)

Commercial kit	Approximate time required (min)	Cost per sample (US\$)	DNA yield ( $\mu$ g/100 $\mu$ l)	Parasite detection/ $\mu$ l blood (detection technique)
Qiagen QIAmp Blood Extraction Kit	30	3.47	6	Mean multiplicity of infection: 1.58 $\pm$ 0.52
Qiagen DNeasy Blood & Tissue Kit	35	3.08	5	NA
Qiagen QIAamp DNA Mini Kit	30	4.59	6	2 (Mitochondrial PCR), 1 (LAMP)
Eiken PURE DNA Extraction Kit	20	NA	NA	$\geq 1$ (LAMP) <sup>45</sup>
Qiagen Gentra Puregene DNA Isolation Kit	40	8.21	3.5	NA
Promega Wizard Genomic DNA Purification Kit	60	1.82	5	NA

sources, the necessity of collecting blood samples separately for DNA isolation is bypassed. In contrast to RDTs, with blood smears there are contamination risks associated with staining, microscopic examination and storage<sup>44</sup>.

Among several approaches for malaria detection, molecular amplification methods such as PCR provide unlimited sensitivity and quantitative data reflecting the

level of infection. These methods however require careful sample preparation to remove amplification inhibitors and to render the purified DNA suitable for thermal cycling conditions. Advances are progressively made towards development of more convenient to use and unique lab-on-chip instruments named Micro Total Analysis Systems. These allow complete analysis starting from

sample collection to its preparation followed by molecular detection, and demonstrate the possibility of being integrated into automated instruments<sup>46</sup>.

## CONCLUSION

This review underlines the most widely used chemical and kit based methods of *Plasmodium* genomic DNA extraction focusing on their sensitivity and highlights their perspective with respect to the source of blood used. It also summarizes and compares the studies for *Plasmodium* nucleic acid extraction methods published to date. Up until now there is no solidarity on a gold standard method for *Plasmodium* DNA extraction, and they all fluctuate in many different features. Studies judging the DNA extraction techniques and bringing to light their potency and limitations are inadequate, and to our knowledge till date there is no publication that appraises all approaches in terms of all promising attributes. Hence, it is tricky to opt for the best method available. Presently, no all-purpose kit is available that is cost-effective, avoids the use of hazardous reagents, requires little time, and most importantly, could be used on a wide range of blood sources like RDTs cassettes, RDTs strips and glass slides. Based on our review there is much need of a user friendly and broad-spectrum extraction technique. As only small blood volumes are usually available, the method should be sensitive, quick, reliable, reproducible, and easy to perform.

## ACKNOWLEDGEMENTS

The authors are thankful to National Institute of Malaria Research (NIMR) for providing facilities. SS thanks Indian Council of Medical Research for the award of Senior Research Fellowship and Goa University for providing her PhD registration.

## REFERENCES

- World Malaria Report 2014. Geneva: World Health Organization Press 2014; doi:10.1007/s00108-013-3390-9 (Accessed on June 19, 2015).
- Tangpukdee N, Duangdee C, Wilairatana P, Krudsood S. Malaria diagnosis: A brief review. *Korean J Parasitol* 2009; 47(2): 93–102.
- Evidence Review Group on Malaria Diagnosis in Low Transmission Settings. *Malaria Policy Advisory Committee Meeting*, 12–14 March 2014. Geneva, Switzerland: WHO Headquarters 2014.
- Port JR, Nguetse C, Adukpo S, Velavan TP. A reliable and rapid method for molecular detection of malarial parasites using microwave irradiation and loop mediated isothermal amplification. *Malar J* 2014; 13: 454.
- Chiodini PL. Malaria diagnostics: Now and the future. *Parasitology* 2014; 141: 1873–79.
- Parija SC. PCR for diagnosis of malaria. *Indian J Med Res* 2010; 132: 9–10.
- Rubio JM, Buhigas I, Subirats M, Baquero M, Puente S, Benito A. Limited level of accuracy provided by available rapid diagnosis tests for malaria enhances the need for PCR-based reference laboratories. *J Clin Microbiol* 2001; 39(7): 2736–7.
- Fontecha GA, Mendoza M, Banegas E, Poorak M, De Oliveira AM, Mancero T, *et al.* Comparison of molecular tests for the diagnosis of malaria in Honduras. *Malar J* 2012; 11: 119.
- Cordray MS, Richards-Kortum RR. Review: Emerging nucleic acid-based tests for point-of-care detection of malaria. *Am J Trop Med Hyg* 2012; 87(2): 223–30.
- Miller RH, Obuya CO, Wanja EW, Ogutu B, Waitumbi J, Luckhart S, *et al.* Characterization of *Plasmodium ovale curtisi* and *P. ovale wallikeri* in western Kenya utilizing a novel species-specific real-time PCR assay. *PLoS Negl Trop Dis* 2015; 9(1): e0003469.
- Ebrahimzadeh A, Mohammadi S, Polshakan Mir A. Nested polymerase chain reaction (PCR) on fixed stained slides in comparison to whole blood as a source of DNA in southeast of Iran. *J Trop Dis* 2014; 2(3): 136.
- Lundblom K, Macharia A, Lebbad M, Mohammed A, Färnert A. High-speed shaking of frozen blood clots for extraction of human and malaria parasite DNA. *Malar J* 2011; 10: 229.
- Bereczky S, Mårtensson A, Gil JP, Färnert A. Rapid DNA extraction from archive blood spots on filter paper for genotyping of *Plasmodium falciparum*. *Am J Trop Med Hyg* 2005; 72(3): 249–51.
- Methods and techniques for clinical trials on antimalarial drug efficacy: Genotyping to identify parasite populations. *Informal consultation organized by the Medicines for Malaria Venture and cosponsored by the World Health Organization*, 29–31 May 2007. Amsterdam, The Netherlands: World Health Organization 2007.
- Kimura M, Kaneko O, Inoue A, Ishii A, Tanabe K. Amplification by polymerase chain reaction of *Plasmodium falciparum* DNA from Giemsa-stained thin blood smears. *Mol Biochem Parasit* 1995; 70(1995): 193–7.
- Alger J, Acosta MC, Lozano C, Velasquez C, Labrada LA. Stained smears as a source of DNA. *Mem Inst Oswaldo Cruz* 1996; 91(5): 589–91.
- Edoh D, Steiger S, Genton B, Beck HP. PCR amplification of DNA from malaria parasites on fixed and stained thick and thin blood films. *Trans R Soc Trop Med Hyg* 1997; 91: 361–3.
- Scopel KKG, Fontes CJF, Nunes AC, Horta MF, Braga EM. Low sensitivity of nested PCR using *Plasmodium* DNA extracted from stained thick blood smears: An epidemiological retrospective study among subjects with low parasitaemia in an endemic area of the Brazilian Amazon region. *Malar J* 2004; 3: 8.
- Cnops L, Esbroeck M Van, Bottieau E, Jacobs J. Giemsa-stained thick blood films as a source of DNA for *Plasmodium* species-specific real-time PCR. *Malar J* 2010; 9: 370.
- Veron V, Carme B. Short report: Recovery and use of *Plasmodium* DNA from malaria rapid diagnostic tests. *Am J Trop Med Hyg* 2006; 74(6): 941–3.
- Marshall LA, Han CM, Santiago JG. Extraction of DNA from malaria-infected erythrocytes using isotachopheresis. *Anal Chem* 2011; 83: 9715–8.
- Foley M, Ranford-Cartwright LC, Babiker HA. Rapid and simple method for isolating malaria DNA from fingerprick samples of

- blood. *Mol Biochem Parasit* 1992; 53(1992): 241–4.
23. Sultan DM, Khalil MM, Abdouh AS, Doleh WF, Al Muthanna AAM. Imported malaria in United Arab Emirates: Evaluation of a new DNA extraction technique using nested PCR. *Korean J Parasitol* 2009; 47(3): 227–33.
  24. Henning L, Felger I, Beck HP. Rapid DNA extraction for molecular epidemiological studies of malaria. *Acta Trop* 1999; 72(1999): 149–55.
  25. Wooden J, Kyes S, Sibley CH. PCR and strain identification in *Plasmodium falciparum*. *Parasitol Today* 1993; 9(8): 303–5.
  26. Singh B, Cox-Singh J, Miller AO, Abdullah MS, Snounou G, Rahman HA. Detection of malaria in Malaysia by nested polymerase chain reaction amplification of dried blood spots on filter papers. *Trans R Soc Trop Med Hyg* 1996; 90: 519–21.
  27. Morris U, Aydin-Schmidt B, Shakely D, Mårtensson A, Jörnham L, Ali AS, *et al.* Rapid diagnostic tests for molecular surveillance of *Plasmodium falciparum* malaria—assessment of DNA extraction methods and field applicability. *Malar J* 2013; 12: 106.
  28. Hwang J, Jaroensuk J, Leimanis ML, Russell B, McGready R, Day N, *et al.* Long-term storage limits PCR-based analyses of malaria parasites in archival dried blood spots. *Malar J* 2012; 11: 339.
  29. Greenspoon SA, Scarpetta MA, Drayton ML, Turek SA. QIAamp spin columns as a method of DNA isolation for forensic case-work. *J Forensic Sci* 1998; 43(5): 1024–30.
  30. Miguel RB, Coura JR, Samudio F, Suárez-Mutis MC. Evaluation of three different DNA extraction methods from blood samples collected in dried filter paper in *Plasmodium* subpatent infections from the Amazon region in Brazil. *Rev Inst Med Trop Sao Paulo* 2013; 55(3): 205–8.
  31. Musapa M, Kumwenda T, Mkulama M, Chishimba S, Norris DE, Thuma PE, *et al.* A simple Chelex protocol for DNA extraction from *Anopheles* spp. *J Vis Exp* 2013; 71: 1–7.
  32. Cox-Singh J, Mahayet S, Abdullah MS, Singh B. Increased sensitivity of malaria detection by nested polymerase chain reaction using simple sampling and DNA extraction. *Int J Parasitol* 1997; 27(12): 1575–7.
  33. 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* 2014; 108: 488–94.
  34. Foundation for Innovative New Diagnostics (FIND), Geneva. Switzerland; Eiken Chemicals Co. Ltd., Tokyo, Japan; and Hospital for Tropical Disease (HTD), London, United Kingdom. *Manual of standard operating procedures for malaria LAMP DNA extraction methods* 2012. Available from: [http://www.finddiagnostics.org/export/sites/default/programs/malaria-afs/docs/SOPs\\_LAMP\\_Malaria\\_AUG12.pdf](http://www.finddiagnostics.org/export/sites/default/programs/malaria-afs/docs/SOPs_LAMP_Malaria_AUG12.pdf) (Accessed on June 20, 2015).
  35. Qiagen, Germany: QIAamp® DNA Mini Kit and QIAamp DNA Blood Mini Kit Handbook 2003. Available from: [http://www.tufts.edu/~mcourt01/Documents/QIAGEN\\_protocol.pdf](http://www.tufts.edu/~mcourt01/Documents/QIAGEN_protocol.pdf) (Accessed on June 20, 2015).
  36. Qiagen, Germany: DNeasy® Blood and Tissue Handbook for purification of total DNA from animal blood/animal tissue 2006. Available from: [http://mvz.berkeley.edu/egl/inserts/DNeasy\\_Blood\\_&\\_Tissue\\_Handbook.pdf](http://mvz.berkeley.edu/egl/inserts/DNeasy_Blood_&_Tissue_Handbook.pdf) (Accessed on June 20, 2015).
  37. Qiagen, Germany: Blood Mini Handbook Sample & Assay Technologies—Sample and Assay Technologies 2012. Available from: <https://www.qiagen.com/us/resources/resourcedetail?id=67893a91-946f-49b5-8033-394fa5d752ea&lang=en> (Accessed on June 20, 2015).
  38. Qiagen, Germany: Gentra Puregene Handbook 2011. Available from: <http://web.emmes.com/study/hbb/public/EN-Gentra-Puregene-Handbook-2011.pdf> (Accessed on June 20, 2015).
  39. Wong SSF, Kuei JJ, Prasad N, Etsemaye A, Mendoza GA, Pemberton TJ, *et al.* A simple method for DNA isolation from clotted blood extricated rapidly from serum separator tubes. *Clin Chem* 2007; 53(2): 522–4.
  40. Clements DN, Wood S, Carter SD, Ollier WER. Assessment of the quality and quantity of genomic DNA recovered from canine blood samples by three different extraction methods. *Res Vet Sci* 2008; 85(2008): 74–9.
  41. McCulloch E, Ramage G, Jones B, Warn P, Kirkpatrick WR, Patterson TF, *et al.* Don't throw your blood clots away: Use of blood clot may improve sensitivity of PCR diagnosis in invasive aspergillosis. *J Clin Pathol* 2009; 62: 539–41.
  42. Cnops L, Boderie M, Gillet P, Van Esbroeck M, Jacobs J. Rapid diagnostic tests as a source of DNA for *Plasmodium* species-specific real-time PCR. *Malar J* 2011; 10: 67.
  43. Ishengoma DS, Lwitiho S, Madebe RA, Nyagonde N, Persson O, Vestergaard LS, *et al.* Using rapid diagnostic tests as source of malaria parasite DNA for molecular analyses in the era of declining malaria prevalence. *Malar J* 2011; 10: 6.
  44. Aubouy A, Carne B. *Plasmodium* DNA contamination between blood smears during Giemsa staining and microscopic examination. *J Infect Dis* 2004; 190: 1335–7.
  45. Hopkins H, González IJ, Polley SD, Angutoko P, Ategeka J, Asiimwe C, *et al.* Highly sensitive detection of malaria parasitemia in a malaria-endemic setting: Performance of a new loop-mediated isothermal amplification kit in a remote clinic in Uganda. *J Infect Dis* 2013; 208: 645–52.
  46. Gascoyne P, Jutamaad S, Mathuros R. Microfluidic approaches to malaria detection. *Acta Trop* 2004; 89(3): 357–69.

Correspondence to: Dr Anupkumar R. Anvikar, Scientist 'E', National Institute of Malaria Research (ICMR), Sector – 8, Dwarka, New Delhi–110 077, India.  
E-mail: [anvikar@gmail.com](mailto:anvikar@gmail.com)

Received: 4 July 2015

Accepted in revised form: 25 August 2015