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REVIEW ARTICLE

Comparison between different methods of DNA isolation from dried blood spots for determination of malaria to determine specificity and cost effectiveness

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Abstract DNA extraction from filter paper by using different methods was compiled through a thorough review of many research articles published in various journals. When performing malaria epidemiological surveys in remote area, it is difficult to collect blood samples and transport it. In field particularly in remote area where facilities for storing and processing of samples does not exist, there surveillance and diagnosis of malaria is very difficult. In this review we are focused upon four simple methods of DNA isolation from the field collected blood and mosquito abdomen blood meal spotted on Whatman No. 1 or No. 3 filter paper. The main DNA isolation methods are Chelex-100, Tris-EDTA (TE) buffer; Methanol based DNA extraction and Phosphate buffer saline (PBS) using Lysis buffer and Phenol–Chloroform method. Efforts have been taken to identify the methods which are cost-effective and take less time to extract DNA from dried blood spots (DBS) and whole mosquitoes. The purpose of this paper is to update the knowledge and find a method to extract DNA from DBS which will be specific, rapid, cost-effective, less time consuming and feasible for epidemiological survey in remote area.

Keywords Extraction · Chelex-100 · TE-Buffer · Methanol · PBS

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Introduction

Surveillance and diagnosis of malaria is difficult, in remote inaccessible area where facilities for storing and processing of samples do not exist. When performing epidemiological or diagnostic surveys in remote area, it is difficult for collection, storage and transportation of samples (Wheat 2001). The new method by using dried blood spots (DBS) can solve all the problems (Mastronardi et al. 2015). Samples, such as finger-prick blood and mosquito blood meal, are easily, quickly and safely collected onto filter paper, stored at room temperature and easily transported to the testing laboratory. As very small amount of blood sample's collected on filter paper, it requires intense diagnosis to achieve accurate result with specificity and sensitivity (Pieter et al. 2014).

Although a thick smear is the gold standard for malaria diagnosis, this method is not efficient for the patients who have asymptomatic *Plasmodium* infections and for this identification it required trained technician for proper staining (Coleman et al. 2002). Accurate quantification of low-density parasitemia from DBS samples is also important (Tran et al. 2014). Molecular methods such as the Polymerase Chain Reaction (PCR) are efficient for the sensitivity and specificity of *Plasmodium* diagnosis (Snounou et al. 1993). Pure and uncontaminated DNA is required for getting a good PCR results. For epidemiological studies DNA extraction procedure is very important. For blood DNA extraction procedure, the blood samples should be kept immediately in freezing condition after collection. This requires a laboratory with electrical connection which is not available in remote areas (Miguel et al. 2013). DBS method is useful for molecular epidemiologic studies, drug monitoring, diagnostic screening, and genetic analysis studies from remote area (Eun et al.

2014). In scientific research the use of stored DBS samples combined with clinical information from medical registries is an ideal resource in a large population studies (Eun et al. 2014). DBSs have offered many advantages in areas where infrastructure is not appropriate for sample storing, processing and for cold chain transportation (Hollegaard et al. 2011).

In this review we have focused upon four simple methods of DNA isolation, those are Chelex-100, TE buffer, Methanol based DNA extraction and PBS (Phosphate buffer saline) using Lysis buffer and Phenol–Chloroform methods. Many researchers use different costly chemicals and tools to extract better quantity of DNA. In remote endemic areas all those facilities are not available to extract good quantity and quality of DNA, because it needs high degree of specificity. Above mentioned methods may serve as effective techniques for collection and transportation of blood samples from such areas. The aim of this paper is to update the knowledge and find a method for extraction of blood and mosquitoes DNA from DBS which is specific, rapid, cost-effective and feasible for studies in resource poor settings.

Sample collection and storage

In DBSs, collection of finger or heel-prick blood is easy and convenient method which requires minimal training. After drying for 10 min, it stored in a zipped bag with desiccant to reduce humidity damage and kept at room temperature. In case of mosquitoes, abdomen part of the full-fed mosquitoes are spotted on Whatman Filter paper 3MM and dried at room temperature (Panda et al. 2018).

If DBSs are stored in freezers, it will be dried thoroughly after being brought to room temperature to avoid condensation inside the bag (Tuaillon et al. 2010; Solmone et al. 2002). The filter strips are covered with a transparent film so that any cross contamination can be avoided between samples. Samples on DBS can be stored up to as long as 5 years if stored at optimal and aseptic conditions (Hwang et al. 2012).

Biosafety issues

While collecting, preserving and transporting DBS samples safety regulation should be followed as DBS contain dried blood. However, DBS is safer and easy to carry, and it can be transferred as non-regulated, unrestricted materials (CDC 2012). More evaluation is needed to validate potential Infectiousness of different pathogens on DBS (Prado et al. 2005; Reitmeyer et al. 1993; Evengard et al. 1989).

DNA extraction from DBS

Ivar Christian Bang (1869–1918) the father of modern clinical microanalysis, who gave the idea of using blood collected on a paper card made of cellulose (Bang 1986).

By using DBS, many problems like the introduction of the polymerase chain reaction, the production of monoclonal antibodies and expression of synthetic proteins have been solved (Parker and Cubitt 1999). DBS now a new method adopted in malaria studies (as a rapid diagnostic tests), as a source of DNA for PCR-based detection and genotyping of parasites.

DBS is a method where blood samples from infected humans or persons with symptoms of the disease is collect on filter papers and can be used to extract DNA using different techniques. Similarly abdomen of full fed mosquito can be spotted on a filter paper and can be stored or analysed by isolating DNA from it (Panda et al. 2018). It can be assayed for a wide range of data that include sporozoite detection, vector species detection and feeding behaviour of the vector etc.

Depending upon the pore size and thickness, different types of filter paper brands are available consisting of 100% cellulose. Generally two filter papers Whatman no 1 and Whatman 3 MM (Whatman, Maidstone, UK) are used widely (Mei et al. 2010).

Different techniques to isolate DNA from DBS

Although many techniques and procedures are available for extracting DNA from DBS samples we are going to cover only four of them here which are simple to conduct, cost effective and yet have a high degree of specificity and yield.

These four methods are Chelex-100 method, TE buffer method, Methanol extraction method and Phenol–Chloroform method (Bereczky et al. 2005; Baidjoe et al. 2013; Golassa et al. 2013).

CHELEX-100 method to extract DNA from DBS

Chelex 100 is a chelating resin with high affinity for polyvalent metal ions like Mg^{2+} and it was developed for extraction of DNA from forensic samples (Walsh et al. 1991). The procedures are rapid, simple; do not involve any organic solvents and multiple tube transfers (Mahittikorn et al. 2005; Bereczky et al. 2005). Chelex 100 is better than using proteinase K and phenol–chloroform extraction to extract DNA from semen and very small bloodstains. When prepared by DBS method DNA extracted from bloodstains seems less prone to contain PCR inhibitors.

Sample processing and storage

Extraction of DNA from a DBS sample is very fast and cost-effective when Chelex-100 molecular grade resins was used. They are very effective at binding DNA which can be separated simply by centrifugation.

The procedure and protocol differs from lab to lab and person to person and is optimized as per the sample and requirements. The general procedure followed to isolate DNA from DBS is as follows.

The general procedure is to put DBS punches of diameter 2–3 mm cut from Whatman 3MM filter paper on which blood samples were collected. It is then placed in 5% Chelex 100 which is already preheated to 100 °C. 5% Chelex means 5 g of Chelex 100 beads dissolved in autoclaved water or 0.05 g in 1 ml of autoclaved water. Each sample of DBS punches should be poured with at least 50–60 Chelex beads per sample. The samples along with the preheated Chelex beads are incubated in 99 °C for a few minutes. While incubated at 99 °C the DNAs from the sample binds strongly with the Chelex beads. Chelex also protects the DNA by binding into cations like Mg^{+2} which are essential cofactors of DNases. After incubating at 99 °C the samples are centrifuged at 12,000 rpm for the Chelex beads to settle down leaving the aqueous part above with the DNAs. After boiling, the Chelex-DNA preparation is stable and can be stored at 4 °C for 3–4 months (Walsh et al. 1991; Kain and Lanar 1991). The principle is after breaking open cells; polar resin beads bind polar cellular components while DNA and RNA remain in water solution above Chelex. However, the heating steps do denature the double helix, and the resulting single-stranded DNA is less stable in storage. These freshly collected supernatants are again centrifuged and the supernatant aqueous layer with the DNAs is collected and stored (Walsh et al. 1991).

This technique approximately takes 30 min to complete. The DNA extraction procedure from the Chelex beads are comparatively cheaper than other DNA isolation kits or Chemicals and the whole procedure needs only heating and incubation at 99 °C followed by two successive centrifugations at room temperature. It is widely used in extraction of DNA from forensic samples and also from rice grains (Butler 2005; Chunwongse and Martin 1993). Chelex protects the sample from DNases. DNA extracted using Chelex 100 Resin is suitable for PCR.

Chelex without proteinase-K method have been used to extract DNA from protozoan parasites such as *T. Gondii* (Mahittikorn et al. 2005) and *P. falciparum* (Bereczky et al. 2005). It has been proposed that this method is suitable for low parasitemia in low endemic settings (Morris et al. 2013).

TE buffer method to extract DNA from DBS

In molecular biology (procedures involving DNA, cDNA or RNA), TE buffer is commonly used. TE-Buffer composed of Tris, a common pH buffer, and EDTA, a molecule that chelates cations like Mg^{2+}. The purpose of TE buffer is to solubilize DNA or RNA, while protecting it from degradation. EDTA inactivates DNase, by binding to metal cations required by this enzyme (Yagi et al. 1996).

Sample processing and storage

For extracting DNA samples from DBS, samples soaked in TE buffer and incubated at 55 °C for a minutes (1 ml of 1 M Tris base (pH 8.0) and 0.2 ml EDTA (0.5 M) for 100 ml solution). Then punches of DBS cut from the collected sample are incubated with the preheated TE buffer at 97 °C to elute the DNA. Then the sample is centrifuged at 12,000 rpm in room temperature for 2 min and the supernatant is collected. It is then stored at 4 °C for later use or stored at − 20 °C when required to store for longer period (Bereczky et al. 2005).

DNA extraction using this method requires less man effort and takes approximately 45 min to complete the whole procedure and Tris buffer is a good source to store DNAs for longer period in a pH stable state. It requires incubation at 55 °C and 97 °C followed by one successive centrifugation hence a very cheap and effective way to isolate DNA in resource poor areas.

Based on nuclease studies from the 1980s, the pH is usually adjusted to 7.5 for RNA and 8.0 for DNA. The respective DNA and RNA nucleases are supposed to be less active at these pH values, but pH 8.0 can safely be used for storage of both DNA and RNA. Genomic and plasmid DNA can be stored in TE Buffer at 4 °C (39.2 °F) for short-term use, or − 20 °C (− 4 °F) to − 80 °C (− 112 °F) for long-term storage (Ross 1990; Alex 2018).

Methanol for DNA extraction from DBS

Methanol is a methyl alcohol which is relatively in expensive, lots of compounds dissolve in it, relatively free of regulation compared to ethanol, easily evaporated so it is commonly used (Fiedler et al. 2005).

Sample processing and storage

In this extraction method, DBS punches cut out from the collected filter paper and it is soaked in absolute methanol. After incubating the sample at room temperature for a minute the methanol is removed and the sample is dried. Alike ethanol, methanol can easily dissolve polar solvents and elutes DNA. The samples are dried and autoclaved

water is added. The samples are mashed and incubated at 97 °C for 10 min for DNA elution. The samples are then transferred and stored at 4 °C (Gil et al. 1995; Opes et al. 2003).

Methanol is very cheap in comparison to other chemicals used in DNA isolation and available abundantly. The whole procedure takes approximately 30 min and incubated at 97 °C without the use of centrifugation.

Phenol–chloroform method of DNA extraction from DBS

Phenol–chloroform extraction is a liquid–liquid extraction technique in molecular biology used to purify nucleic acids and eliminate proteins and lipids.

Phenol and Chloroform are highly non-polar solvents. Whereas water is a very polar solvent. These properties of the above liquids are basic principle of extracting DNA through this method.

Sample processing and storage

DBS are cut into small punches and are placed with Lysis buffer (containing KCl, $MgCl_2$, Tris Cl, and Tween-20) and Proteinase K overnight. The components of Lysis buffer degrade the cellular integrity and the cell is lysed. Proteinase K being derived from Keratin protein effectively degrades the proteins present in the mixture after cell lysis.

Next day, the cellular debris is removed by centrifugation and the supernatant is collected and equal volume of phenol is mixed. The protein and organic portion mix well with the non-polar phenol part while DNAs remain in the aqueous phase. The phenol portion is discarded after centrifugation and phenol: Chloroform is added into the mixture in 1:1 ratio. This increases the density of the non-polar portion of the mixture which helps in better separation of the aqueous and phenol–chloroform part. After the separation of these two phases Chloroform: Isoamyl alcohol in 24:1 ratio is mixed to remove any foam produced by the proteins and the aqueous phase is separated through successive centrifugations at 10,000 rpm. This whole procedure is carried out at 4 °C to prevent the naked DNA present in aqueous medium from degrading. At the third day naked DNA is eluted by applying cold ethanol and isolated from the aqueous phase by centrifugation at 12,000 rpm. Then the ethanol is removed and the samples are again centrifuged with 70% ethanol for best results. The samples are dried and stored by adding autoclaved water at 4 °C. This whole procedure is accomplished at 0 °C to ensure the integrity of naked DNA and to preserve and prevent it from degrading (Fraga et al. 2004).

This procedure is carried out in 3 days in 3 different temperatures. First the sample is crushed and washed with Phosphate Buffer Saline (PBS). PBS is an isotonic

Table 1 A brief summary of all methods discuss in the article

Sl no.	Techniques	Reagents required	Procedure	Total time required	DNA yield	Specificity	Cost per sample (US $)
1	Chelex-100	5% Chelex-100	One heating and incubation at 99 °C followed by two successive centrifugation	30 min	67–306 ng/µl (Sjoholm et al. 2007)	93% (Bereczky et al. 2005; Miguel et al. 2013)	$0.16 (Strøm et al. 2014)
2	TE-Buffer	10 mM Tris pH 8.0 with HCl, 1 mM EDTA	Incubation at 55 °C and 99 °C followed by one successive centrifugation	45 min	87 ng/µl (Bereczky et al. 2005)	100% (Bereczky et al. 2005)	$1 per 96 extractions (Krystal et al. 2013)
3	Methanol	Methanol	Incubation at 97 °C without use of centrifugation	30 min	19.60 ng/µl (Suzanne 2016)	73% (Bereczky et al. 2005)	–
4	Phenol–Chloroform	KCL, $MgCl_2$, Tris–cl, Tween-20, Proteinase-K, Phenol–Chloroform, Isoamyl alcohol, 70% ethanol	Lysis for 12 h, and use of Centrifuge machine for several time	1 day or 3 days	87 ng/µl (Nguyen et al. 2012)	–	$5 per 96 extractions (Krystal et al. 2013)

detergent with pH slightly alkaline ranging in between 7.4 and 8.0. Being an isotonic solvent it does not disturb the integrity of the cell and the sample is easily washed because of its detergent ability.

This method of DNA extraction, either from DBS or from whole mosquito results in good yield of DNA, while being advantageous at storing the DNA sample for as long as 6 months after extraction.

Conclusions

For the surveillance and diagnosis of infectious diseases, filter paper plays an important role for the last 50 years. DBS also plays a key role in the diagnosis of parasitic infections. Extraction of DNA from dried blood spot is very easy and time consuming. Although microscopy is a gold standard to detect malaria but PCR techniques have greater sensitivity and specificity than microscopic examination. DBS performs as one of the most successful way to collect and store samples from infected persons who live in inaccessible areas. DBS serves as a very cheap and good source of collection of samples and it can be easily transported to laboratory. The above mentioned methods are most commonly used methods to isolate DNA from collected samples. All the above mentioned procedures have a very high degree of specificity and cost-effective when compared to DNA isolation Kits and blood slide examination procedures.

Among the above four methods, all are nearly equal in their specificity while they may vary from minimal to large extent in case of yield and longevity of the DNA samples. Among the four methods described, Phenol–Chloroform method is more time taking and costlier. Chelex-100 method found to be more rapid, cost-effective and less time consuming method compare to other blood stains collected in a filter paper (Table 1).

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest regarding the publication of this study.

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