Methods for extracting genomic DNA from whole blood samples: current perspectives

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Abstract: Deoxyribonucleic acid (DNA) extraction has considerably evolved since it was initially performed back in 1869. It is the first step required for many of the available downstream applications used in the field of molecular biology. Whole blood samples are one of the main sources used to obtain DNA, and there are many different protocols available to perform nucleic acid extraction on such samples. These methods vary from very basic manual protocols to more sophisticated methods included in automated DNA extraction protocols. Based on the wide range of available options, it would be ideal to determine the ones that perform best in terms of cost-effectiveness and time efficiency. We have reviewed DNA extraction history and the most commonly used methods for DNA extraction from whole blood samples, highlighting their individual advantages and disadvantages. We also searched current scientific literature to find studies comparing different nucleic acid extraction methods, to determine the best available choice. Based on our research, we have determined that there is not enough scientific evidence to support one particular DNA extraction method from whole blood samples. Choosing a suitable method is still a process that requires consideration of many different factors, and more research is needed to validate choices made at facilities around the world.

Keywords: genomic DNA extraction, whole blood samples, solution-based DNA extraction, solid-phase DNA extraction, cost-effectiveness, time efficiency

Introduction

Human health studies in the field of molecular biology require the use of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein samples. Successful use of available downstream applications will benefit from the use of high-quantity and high-quality DNA. Therefore, nucleic acid extraction is a key step in laboratory procedures required to perform further molecular research applications. It is essential to choose a suitable extraction method, and there are a few considerations to be made when evaluating the available options. These may include technical requirements, time efficiency, cost-effectiveness, as well as biological specimens to be used and their collection and storage requirements.¹

Whole blood is one of many different available sources to obtain genomic DNA (gDNA), and it has been widely used in facilities around the world. Therefore, we will focus on DNA extraction protocols using whole blood samples. Issues regarding collection, storage, and manual handling of human whole blood specimens escape the scope of this publication and will not be covered. However, they are important and they should be considered, as they could potentially impact on the performance and success of any DNA extraction technique chosen.
Initial development of DNA extraction techniques

Friedrich Miescher was the first scientist to isolate DNA while studying the chemical composition of cells. In 1869, he used leukocytes that he collected from the samples on fresh surgical bandages and conducted experiments to purify and classify proteins contained in these cells. During his experiments he identified a novel substance in the nuclei, which he called “nuclein”. He then developed two protocols to separate cells’ nuclei from cytoplasm and to isolate this novel compound, nowadays known as DNA, which differed from proteins and other cellular substances. This scientific finding, along with the isolation protocols used, was published in 1871 in collaboration with his mentor, Felix Hoppe-Seyler. However, it was only in 1958 that Meselson and Stahl developed a routine laboratory procedure for DNA extraction. They performed DNA extraction from bacterial samples of Escherichia coli using a salt density gradient centrifugation protocol. Since then, DNA extraction techniques have been adapted to perform extractions on many different types of biological sources.

DNA extraction methods follow some common procedures aimed to achieve effective disruption of cells, denaturation of nucleoprotein complexes, inactivation of nucleases and other enzymes, removal of biological and chemical contaminants, and finally DNA precipitation. Most of them follow similar basic steps and include the use of organic and nonorganic reagents and centrifugation methods. Finally, they have developed into a variety of automated procedures and commercially available kits.

Initially, we will discuss protocols and steps aimed to achieve cell lysis, inactivation of cellular enzymes, denaturation of cellular complexes, and DNA precipitation, which require similar procedures and/or reagents during DNA extraction from whole blood samples. Key differences in steps aiming to remove biological and chemical contaminants will be highlighted when we discuss each protocol in detail.

As previously mentioned, lysis of cells is a common step in most DNA extraction protocols, and it is commonly achieved through the use of detergents and enzymes. Sodium dodecyl sulfate (SDS) and Triton X-100 (Sigma-Aldrich, St Louis, MO, USA) are examples of popular detergents used to solubilize cell membranes. Enzymes are also combined with detergents to target cell surface or cytosolic components. Proteinase K is a commonly used enzyme used in various protocols to cleave glycoproteins and inactivate RNases and DNases. Other denaturants such as urea, guanidinium salts, and chemical chaotropes have also been used to disrupt cells and inactivate cellular enzymes, but these can impact on quality and nucleic acid yield.

DNA precipitation is achieved by adding high concentrations of salt to DNA-containing solutions, as cations from salts such as ammonium acetate counteract repulsion caused by the negative charge of the phosphate backbone. A mixture of DNA and salts in the presence of solvents like ethanol (final concentrations of 70%–80%) or isopropanol (final concentrations of 40%–50%) causes nucleic acids to precipitate. Some protocols include washing steps with 70% ethanol to remove excess salt from DNA. Finally, nucleic acids are resuspended in water or TE buffer (10 mM Tris, 1 mM ethylenediaminetetraacetic acid [EDTA]). TE buffer is commonly used for long-term DNA storage because it prevents it from being damaged by nucleases, inadequate pH, heavy metals, and oxidation by free radicals. Tris provides a safe pH of 7–8, and EDTA chelates divalent ions used in nuclease activity and counteracts oxidative damage from heavy metals.

Main types of DNA extraction methods from human whole blood samples

Table 1 shows the main categories and subcategories of DNA extraction methods from whole blood samples that are generally used in research facilities worldwide. Laboratory reagents commonly used for each stage of the nucleic acid extraction protocol are included in this table in order to highlight similarities and differences between them.

DNA extraction techniques included in Table 1 will be discussed in more detail in the following sections, along with a brief summary of the technique history and background. In recent years, some of these protocols have been adapted to microdevices that develop miniaturized total chemical analysis systems or microfluidic genetic analysis microchips. However, we will limit the scope of our review to those techniques that are available for macroscale nucleic acid extraction.

Solution-based DNA extraction methods

As previously mentioned, solution-based protocols have two main approaches: 1) solution-based methods using organic solvents and 2) those based on a salting out technique. Further description of both methods follows.

Solution-based DNA extraction methods using organic solvents

DNA extraction protocols using organic solvents derived originally from a series of related RNA extraction methods.
Some of the main steps used in these methods are: 1) cell lysis undertaken by adding a detergent/chaotropic-containing solution, including SDS or N-Lauroyl sarcosine; 2) inactivation of DNases and RNases, usually through the use of organic solvents; 3) purification of DNA and removal of RNA, lipids, and proteins; and 4) resuspension of extracted nucleic acids.\textsuperscript{1,5,11}

This method was initially developed in 1977 when an RNA extraction technique using guanidium isothiocyanate was used by Ullrich et al\textsuperscript{12} to isolate plasmid DNA. This technique was later modified by Chirgwin et al\textsuperscript{13} in 1979. It required the use of guanidium thiocyanate and long hours of ultracentrifugation through a cesium chloride cushion. In an effort to improve this method, Chomczynski and Sacchi\textsuperscript{14,15} developed in 1987 a protocol for RNA extraction using guanidium thiocyanate–phenol–chloroform and much shorter centrifugation. This last RNA extraction protocol was able to isolate RNA, DNA, and proteins, but in order to be used as a DNA extraction technique, guanidium thiocyanate–phenol–chloroform was later replaced by a mixture of phenol, chloroform, and isoamyl alcohol, as the former solvent did not completely inhibit RNase activity.\textsuperscript{11} Phenol is a carbolic acid that denatures proteins quickly, but it is highly corrosive, toxic, and flammable. This organic solvent is usually added to the sample and then, using centrifugal force, a biphasic emulsion is obtained. The top hydrophilic layer contains diluted DNA, and the bottom hydrophobic layer is composed of organic solvents, cellular debris, proteins, and other hydrophobic compounds. DNA is then precipitated after centrifugation by adding high concentrations of salt, such as sodium acetate, and ethanol or isopropanol in 2:1 or 1:1 ratios. Excess salt can be removed by adding 70% ethanol, and the sample is then centrifuged to collect the DNA pellet, which can be resuspended in sterile distilled water or TE buffer (10 mM Tris; 1 mM EDTA pH 8.0).\textsuperscript{1,5}

Because these techniques involve the use of toxic and corrosive organic solvents, safety is a main concern. Personal protective equipment, safety measures involving the use of a biohazard hood, and training are required. Phenol–chloroform needs to be equilibrated to an adequate pH, and protocol conditions should be optimized.\textsuperscript{7} In an effort to improve the safety and ease of use of these protocols, certain modifications have been introduced in order to avoid physical contact with solvents. These include incorporating a silica gel polymer\textsuperscript{16} or replacing solvents with other substances like benzyl alcohol.\textsuperscript{17}

### Solution-based DNA extraction methods using salting out

Some nucleic acid extraction techniques that avoid the use of organic solvents have also been developed over the years.\textsuperscript{1,6,11} In 1988, Miller et al\textsuperscript{18} published a protocol that achieved DNA purification through protein precipitation at high salt concentration. The traditional protocol involves initial cell disruption and digestion with SDS–proteinase K, followed by the addition of high concentrations of salts, usually 6 M sodium chloride. The mixture is then centrifuged to allow proteins to precipitate to the bottom, with the supernatant containing DNA then transferred to a new vial. DNA is then precipitated using ethanol or isopropanol in the same manner as described for organic solvent methods.\textsuperscript{1,18–20}
However, the use of proteinase K can be time consuming and expensive when compared with other reagents used in different solution-based approaches, so there have been a few attempts to find alternative reagents for deproteinization of DNA. In 1991, Lahiri and Nurnberger developed a DNA extraction protocol from blood samples that eliminated the use of organic solvents and prolonged incubation with proteinase K. Their protocol used Nonidet™ P-40 (NP-40; Sigma-Aldrich, St Louis, MO, USA) to lyse blood cells and high salt buffers and 10% SDS to inactivate and remove contaminants. Another protocol is the modified salting out method published in 2005 by Nasiri et al., which replaced proteinase K digestion with the use of laundry powder. This modified technique has been successfully used as a DNA extraction protocol in many facilities around the world.

Solid-phase DNA extraction methods

Purification of DNA using the liquid/solid-phase approach can be traced back to 1979, when Vogelstein and Gillespie used silica in a glass powder form in their protocol to purify DNA fragments previously separated by agarose gel electrophoresis. Solid-phase extraction methods for DNA extraction from blood samples were initially described in 1989 by McCormick, who published a technique using siliceous-based insoluble particles, chemically similar to phenol, which interact with proteins to allow DNA purification. A number of different procedures using the liquid/solid DNA extraction approach have been developed since then and are used in the majority of commercially available extraction kits.

These techniques will absorb DNA under particular pH and salt content conditions through any of the following principles: 1) hydrogen binding in the presence of a chaotropic agent to a hydrophilic matrix, 2) ionic exchange using an anion exchanger under aqueous conditions, and 3) affinity and size exclusion mechanisms. Most of these methods follow a series of similar steps to achieve cell disruption, DNA adsorption, nucleic acid washing, and final elution. Most solid-phase techniques use a spin column to bind nucleic acid under centrifugal force. Spin columns are made of silica matrices, glass particles or powder, diatomaceous earth, or anion exchange carriers, and these compounds generally need to be conditioned using buffer solutions at a specific pH to turn them into the required chemical form. Blood cells previously degraded using particular lysis buffers are applied to the columns and centrifuged, and the DNA binds to the column aided by pH and salt concentration conditions provided by binding solutions. Some proteins and other biochemical compounds may also bind to the column, and they are later removed using washing buffers containing competitive agents during a series of washing steps. DNA is finally eluted in sterile distilled water or TE buffer.

DNA extraction methods using silica and silica matrices

Silica matrices have unique properties for DNA binding. They are positively charged and have high affinity toward the negative charge of the DNA backbone. High salt conditions and pH are achieved using sodium cations, which bind tightly to the negatively charged oxygen in the phosphate backbone of DNA. Contaminants are removed with a series of washing steps, followed by DNA elution under low ionic strength (pH ≥7) using TE buffer or sterile distilled water. Commercially available kits using a silica-based approach are manufactured by Clontech Laboratories, Inc., Mountain View, CA, USA (NucleoSpin™); MO BIO Laboratories, Inc., Carlsbad, CA, USA (UltraClean™); and Sigma-Aldrich, St Louis, MO, USA (GenElute™), among others. In these protocols, blood samples are incubated for a few minutes with a lysis buffer. Most protocols take about 40 minutes to 1 hour to complete, producing high yields of DNA with minimum contamination.

A substance that contains high amounts of silica (up to 94%) known as kieselguhr, diatomite, or diatomaceous earth has also been used for DNA purification. It was initially described by Boom et al. in 1990. It binds DNA in the presence of chaotropic agents, followed by washing with a buffer containing alcohol, and finally DNA is eluted in a low salt buffer or sterile distilled water. Quantum Prep® (Bio-Rad Laboratories, Hercules, CA, USA) is an example of a DNA extraction product developed using diatomaceous earth.

DNA extraction kits have also evolved, and they are incorporated into semi- and fully automated equipment able to perform protocols from sample lysis to some downstream applications like polymerase chain reaction (PCR), such as BioRobot EZ1® Advanced (QIAGEN) and Biomek® 4000 Laboratory Automation Workstation (Beckman Coulter, Inc., Brea, CA, USA), among others. Less risk of pipetting error, reduced number of sample transfers, and less protocol time are among the advantages of these devices. However, they should be carefully considered, given the high cost of some of the available choices of equipment. They have also been incorporated into miniaturized total chemical analysis systems, which are silicon microchips, where DNA purification separation and detection are achieved.
DNA extraction using anion exchange resins

Positively charged chemical substances able to bind to negatively charged nucleic acids or contaminants or enzymes, such as nucleases, are called anion exchange resins, and they have also been used as part of DNA extraction protocols from blood samples.  

Chelex® 100 resin (Bio-Rad Laboratories, Hercules, CA, USA) is made of styrene divinylbenzene copolymers that contain paired iminodiacetate ions. It is used in DNA extraction protocols as a chelating ion exchange resin that binds polyvalent metal ions such as nucleases commonly used in DNA extraction from forensic samples. The initial laboratory protocol, using blood as a biological source, was described by Walsh et al in 1991. Based on this initial approach, other protocols have been developed to perform nucleic acid extraction from whole blood samples. They require small sample volumes (under 1 mL of blood) and are usually performed in a single tube reaction with different steps and reagents involved. Blood samples could be lysed using proteinase K and/or incubation at high temperature, and removal of contaminants is achieved by adding Chelex® 100 resin, which precipitates them. Single-stranded DNA is obtained and remains suspended in the supernatant, which can be immediately used in downstream application or can be transferred to a new vial for long-term storage.

Seligson et al used anion exchange materials as part of their invention to isolate nucleic acid samples from a variety of sources, including whole blood samples. Seligson et al’s protocol uses a column containing a resin with positively charged diethylaminoethyl cellulose groups on its surface to bind negatively charged phosphates of the backbone of DNA. The strength of DNA binding to the column, as well as RNA and other impurities, can be altered through salt concentrations and pH conditions of buffers used in this nucleic acid isolation protocol. Contaminants such as protein and RNA can be washed from the DNA-containing column using medium salt buffers.

DNA extraction methods using magnetic beads

Nucleic acid extraction techniques using magnetic separation have been emerging since the early 1990s. They were originally used to extract plasmid DNA from bacterial cell lysates by Hawkins et al in 1994 and in 2006 by Saiyed et al, who developed and validated a protocol using naked magnetic nanoparticles for genomic DNA extraction from whole blood samples.

Magnetic particles are made of one or several magnetic cores, such as magnetite (Fe₃O₄) or maghemite (gamma Fe₂O₃), coated with a matrix of polymers, silica, or hydroxyapatite with terminal functionalized groups. In the protocol developed by Saiyed et al, 30 µL of whole blood is mixed with an equal volume of 1% (weight/volume [w/v]) SDS solution. The tube is mixed by inversion two or three times and incubated at room temperature for 1 minute. Ten microliters of magnetic nanoparticles is added to this mixture, followed by the addition of 75 µL of binding buffer (1.25 M sodium chloride and 10% polyethylene glycol 6000). The solution is mixed by inversion and allowed to rest for 3 minutes at room temperature, and the magnetic pellet is immobilized using an external magnet to discard the supernatant. The magnetic pellet is washed with 70% ethanol and dried. The magnetic pellet is resuspended in 50 µL of TE buffer, and magnetic particles bound to DNA are eluted by incubation at 65°C with continuous agitation.  

Choosing the appropriate protocol

Choosing the appropriate protocol

The ideal extraction method should fit the following criteria: it should be sensitive, consistent, quick, and easy to use, and depending on the country in which it is used it may be important to minimize specialized equipment or biochemical knowledge. It should also pose minimum risk to users, as well as avoid possible cross-contamination of samples. Finally, and most importantly, the DNA extraction technique chosen should be able to deliver pure DNA samples ready to be used in downstream molecular applications.

The quality and quantity of genomic DNA extracted from blood samples is a key feature most facilities consider when choosing a protocol. Measuring ultraviolet light absorbance using spectrophotometry at different wavelengths (230 nm, 240 nm, 260 nm, and 280 nm) is an initial quick and efficient way of determining purity and concentration of nucleic acid samples. Concentration is usually calculated from DNA absorbance reading at 260 nm using Beer–Lambert law. Purity of nucleic acid samples is assessed in a 260/280 absorbance ratio, and values in the range of 1.8–2.0 are generally considered acceptable. The 260/230 absorbance ratios between 2.0 and 2.2 are also considered to be adequate as a secondary measure of purity for DNA.

Lahiri et al published a study in 1991 where they compared ten solution-based extraction methods for DNA extraction using whole blood as a source. They compared a protocol previously developed by their group (method 10a and 10b), which required no use of organic solvents or enzyme digestion, against nine other methods previously used.
published and used for DNA extraction from blood. In their study, Lahiri et al\(^{14}\) extracted whole blood samples from five individuals in triplicate using the aforementioned methods. They determined DNA concentration from samples using spectrophotometry absorbance reading at 260 nm and assessed quality through 260/280 absorbance ratio and electrophoresis on agarose gel, as well as restriction of enzyme digestion and southern blot. A summary of some of the protocol features, as well as findings, is presented in Table 2.

All protocols tested were able to isolate DNA with relatively good purity (260/280 ratios from 1.7 to 1.94), but DNA obtained with methods 2, 5, and 6 showed different amounts of degradation evidenced in gel electrophoresis. Seven of the protocols tested, methods 3–9, required use of organic solvents and/or hazardous substances such as phenol–chloroform or chloroform. Methods 1 and 2 did not use organic compounds, but method 1 was the most time-consuming. It required overnight incubation with proteinase K, a problem solved in protocol 2 by incubating samples for 30 minutes with both proteinase K and RNase A, reducing DNA extraction time to 5 hours. Method 10 (version a and b) was the quickest of all DNA isolation methods (1 hour) and removed enzyme digestion and the use of organic solvents/hazardous substances. Both versions of this protocol were able to recover similar or higher DNA yields than the other tested protocols, with about comparable 260/280 ratios. Based on their study findings, Lahiri et al\(^{14}\) were able to conclude that the DNA extraction method they developed was the quickest and safest of the solution-based methods tested, recovering DNA of comparable quality and quantity.

Abd El-Aal et al\(^{48}\) compared a combination of manual and automated extraction methods for DNA extraction from whole blood samples. Their study included six techniques: phenol–chloroform purification, DNA extraction using microwave thermal shock, DNA extraction with Wizard Genomic DNA Purification Kit (Promega Corporation), magnetic separation (LC MagNA Pure Compact Instrument; Roche Diagnostics GmbH, Manheim, Germany) both manually and partially automated using the Precision\(^{TM}\) XS Microplate Sample processor from Biotek Instruments (Vermont, USA), and finally they modified the Wizard SV 96 Genomic DNA Purification Kit (Promega Corporation), combining it with magnetic separation using the MagNA Pure purification method. They extracted 96 blood samples and used 100 µL as the initial volume. However, they failed to mention how many samples were extracted using each method and the number of experimental replicates performed. Their results showed DNA extracted for each protocol with final concentrations ranging from 0.50 µg/µL to 0.98 µg/µL. Although phenol–chloroform, manual magnetic separation, and the combined Promega–MagNA Pure method showed relatively similar DNA concentrations (0.72–0.79 µg/µL), the magnetic separation and microwaving technique achieved the highest and lowest DNA concentrations, 0.98 µg/µL and 0.50 µg/µL, respectively. In their comparisons, they established five categories for simplicity of extraction: extremely simple, simple, less simple, more simple, and difficult, but their system can be confusing because they failed to present criteria used for each category. However, they categorized phenol–chloroform as difficult and automated MagNA Pure as extremely simple, using their previously mentioned category system. They also have five categories for cost of each protocol, with the automated MagNA Pure technique as the most expensive and microwaving as the cheapest method. Their costing categories were also not defined and there is no actual mention of specific costs for each method in their study. Based on previously mentioned findings, they concluded that magnetic separation using an automated protocol

<table>
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<tr>
<th>Method</th>
<th>Enzyme digestion</th>
<th>Hazardous reagents</th>
<th>RNase treatment</th>
<th>Time (hours)</th>
<th>260/280 ratio</th>
<th>DNA yield (µg)</th>
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</thead>
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<td>No</td>
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<tr>
<td>10b</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<td>Yes</td>
<td>Yes</td>
<td>3</td>
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<td>No</td>
<td>Yes</td>
<td>5</td>
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<td>No</td>
<td>Overnight</td>
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<td>No</td>
<td>Overnight</td>
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<td>Yes</td>
<td>No</td>
<td>Overnight</td>
<td>1.72</td>
<td>55</td>
</tr>
</tbody>
</table>

**Note:** Reprinted from J Biochem Biophys Methods 1992;25(4). Lahiri DK, Bye S, Nurnberger Jr Jr, Hodes ME, Crisp M. A non-organic and non-enzymatic extraction method gives higher yields of genomic DNA from whole-blood samples than do nine other methods tested. 193–205. Copyright © 1992, with permission from Elsevier.\(^{14}\)

**Abbreviations:** DNA, deoxyribonucleic acid; RNase, ribonucleic acid.
performed best in terms of simplicity of extraction, purity of extracted DNA, and speed, even though it is the one with the highest cost. They also concluded that it was essential to optimize any method chosen and they recommended the use of magnetic separation, because it required minimal starting material and it was both cost-effective and user-friendly. However, as previously stated, there is no mention of how cost-effectiveness was determined.\(^4\)

Lee et al\(^4\) extracted DNA from 22 whole blood samples using three automated extraction systems. All three protocols compared were based on solid-phase extraction techniques: QIAamp® Blood Mini Kit (QIAGEN, Hilden, Germany) with QIAcube®, which uses a silica membrane and resins within a spin column to bind DNA, and two other protocols that are based on magnetic-based DNA isolation techniques MagNA Pure LC Nucleic Acid Isolation Kit I with MagNA Pure LC (Roche Diagnostics GMBH, Mannheim, Germany) and Magtration-Magnazorb DNA Common Kit-200N with Magtration System 12GC (Precision System Science Co, Ltd, Tokyo, Japan). DNA concentration was measured by spectrophotometry and purity was assessed by 260/280 ratio, DNA electrophoresis on agarose gel, and PCR. Statistical analysis was performed to validate study results, and they are summarized in Table 3.

Results showed no statistical difference between DNA concentrations obtained among the three commercial methods, but DNA purity was slightly lower for the Magtration-Magnazorb DNA Common Kit-200N when compared with the other two methods. DNA extracted was of similar quality based on results from PCR and electrophoresis on agarose gel. Therefore, they concluded that effectiveness for all systems was equivalent and that they all produced acceptable nucleic acid isolation.\(^4\)

Table 4 was adapted from a review published by Carpi et al\(^5\) in 2011, where they reviewed DNA extraction methods used in a wide range of biological sources, including six methods used on whole blood samples. A summary of the three evaluated features for nucleic acid isolation methods, such as use of toxic compounds, cost per sample, and time required, is shown in Table 4.

Based on the methods included in Table 4, it can be noted that the magnetic bead-based method is the quickest DNA extraction protocol, requiring over 30 minutes to be performed, whereas all the other protocols require more than 3 hours. Also, the magnetic bead-based method is the most expensive one, costing more than US$5 per sample extracted. However, there is no mention in this review of the differences when comparing quality and quantity of nucleic acid isolated for each method.\(^1\)

Chacon-Cortes et al\(^6\) evaluated cost-effectiveness and time efficiency of three available DNA extraction techniques from whole blood samples: a traditional salting out method, a modified salting out method, and a commercially available kit based on a solid-phase DNA extraction method QIAamp® DNA blood maxi kits (QIAGEN® Pty Ltd, Clifton Hill, VIC, Australia). The modified salting out protocol\(^7\) replaced the sample overnight incubation step from the traditional salting out method,\(^8\) required for contaminant removal using protease K, with the use of laundry detergent to reduce time of extraction to about 1 hour. Five microliters of whole blood from six breast cancer patients was

<table>
<thead>
<tr>
<th>DNA extraction method</th>
<th>Toxic compounds</th>
<th>Cost estimate per sample (US$)</th>
<th>Time required (hours)</th>
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</thead>
<tbody>
<tr>
<td>Phenol–chloroform method</td>
<td>Phenol, chloroform</td>
<td>&lt;5</td>
<td>&gt;3</td>
</tr>
<tr>
<td>Silica gel method</td>
<td>Phenol, chloroform</td>
<td>&lt;5</td>
<td>&gt;3</td>
</tr>
<tr>
<td>Benzyl alcohol method</td>
<td>Benzyl alcohol</td>
<td>&lt;5</td>
<td>&gt;3</td>
</tr>
<tr>
<td>Salting out method</td>
<td>None</td>
<td>&lt;5</td>
<td>&gt;3</td>
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<tr>
<td>Magnetic bead-based method</td>
<td>None</td>
<td>&gt;5</td>
<td>&gt;0.5</td>
</tr>
</tbody>
</table>

Table 5 Summary of results from comparative study of DNA extraction methods by Chacon-Cortes et al

<table>
<thead>
<tr>
<th>DNA extraction method</th>
<th>Average final gDNA yield (µg)</th>
<th>Average 260/280 ratio</th>
<th>Cost estimate per sample (AUD)</th>
<th>Time required (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Traditional salting out method</td>
<td>32.65</td>
<td>1.75</td>
<td>3.65</td>
<td>Overnight</td>
</tr>
<tr>
<td>Modified salting out method</td>
<td>40.47</td>
<td>1.75</td>
<td>1.9</td>
<td>1</td>
</tr>
<tr>
<td>QIAGEN® DNA blood maxi kits (QIAGEN® Pty Ltd, Clifton Hill, VIC, Australia)</td>
<td>61.86</td>
<td>2.02</td>
<td>12.3</td>
<td>1</td>
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</table>


Abbreviations: AUD, Australian dollars; gDNA, genomic deoxyribonucleic acid.

manually extracted using each protocol, and techniques were compared in terms of quality and quantity of DNA extracted, as well as cost and time required. DNA quantity was measured using spectrophotometry, and DNA quality was assessed by both 260/280 ratio and agarose gel electrophoresis of PCR product.

A summary of findings is presented in Table 5. As shown in Table 5, QIAGEN QIAamp DNA Blood Maxi Kits produced the highest yield and 260/280 ratio from all methods evaluated, with an average measure of 61.86 µg and 2.02, respectively, but traditional and modified salting out protocols both produced similar results in terms of DNA yield and 260/280 ratios. However, statistical analysis using analysis of variance showed that DNA yield and 260/280 ratio results were not significantly different for all three methods (P-value of 0.110 and 0.05, respectively). On the other hand, the traditional salting out method required an overnight incubation step, and the QIAGEN QIAamp DNA Blood Maxi Kit was the most expensive of all the methods included, costing about 12.30 Australian dollars, almost seven times the modified salting out protocol. Therefore, based on these findings, the modified salting out protocol developed by Nasiri et al proved to be the most cost-effective and time-efficient technique to isolate gDNA from whole blood samples. Unfortunately, no other solid-phase extraction methods for DNA extraction were included in this study.

Conclusion

DNA extraction has evolved for the past 145 years and has developed into a diversity of laboratory techniques. This review highlights the currently available methods for DNA extraction from whole blood samples, and it summarizes comparison studies using different nucleic acid extraction approaches published to date. DNA extraction has evolved from solution and solid-phase manual techniques initially performed manually into incorporating these into automated methods. There is no consensus on a gold standard method for DNA extraction from whole blood samples, and they all differ in many different aspects. Studies comparing extraction techniques and highlighting their strengths and weaknesses are limited, and to our knowledge there is no publication that evaluates all approaches in terms of all possible features. Therefore, it is very difficult to determine the best choice available. Facilities around the world usually choose a method based on the availability of equipment, samples, and reagents, as well as considering speed, extraction efficiency and quality, technical requirements, and cost, but based on our review findings there is not enough scientific evidence to support these choices.

Disclosure

The authors of this publication certify that there are no conflicts of interest with any financial or scientific organization regarding the material discussed in the manuscript.

References


