A simplified method for DNA extraction from human blood clot: A familiarized diagnostic tool in forensic analysis

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Abstract:

We have simplified the conventional techniques used to extract DNA from clotted blood by salting out technique, in such away some simple chemical treatments (SDS and NaCl) of cellular lysate of clot substituted the hazardous organic solvents such as phenol. Distilled water played an important role in eliminating almost all RBCs contaminating the specimens. Differences were not identified between clotted blood procedure and non-clotted blood procedure. Therefore, this method might be used in routine clinical laboratories for forensic analysis such as finger printing and other tests.

طريقة مبسطة لعزل الدنا من خثرة دم البشر: أداة تشخيصية مألوفة في التحليل الجنائي

الخلاصة

قمنا بتبسيط التقنيات التقليدية المستخدمة لعزل الدنا من الدم المتخثر بواسطة تقنية التمليح الخارجي، بحيث تستبدل بعض المعاملات الكيميائية البسيطة (أس دي أس و كلوريد الصوديوم) للمستخلص الخلوي للخثرة المذيبات العضوية الخطيرة كما في الفينول. لعب الماء المقطر دوراً مهماً في إزالة معظم كريات الدم الحمر الملوثة للعينات. لم يوجد هنالك فروقات ملحوظة بين حزم الدنا في خطوات عمل الدم المتخثر مقارنة بخطوات عمل الدم الغير متخثر. ولهذا السبب، ربما من الممكن أن تستخدم هذه الطريقة في المختبرات السريرية الروتينية للتحليل الجنائي كما في بصمة الدنا واختبارات أخرى.

Introduction:

In international courts, blood has been exploited as a source for crime evidence more then one decade ago (1), and it is most commonly used as evidence in crimes of violence, and has become widespread in paternity cases (2). Since blood in crime seen is found as a clot (dry stain), many researchers were isolated DNA from blood clot (3) but they used either sophisticated separatory tubes (4) or they used special centrifuges which had big rotors (special to 50 ml tubes) not exists in local laboratories (5). Walsh et al, 1989 mentioned that DNA extracted from blood could only be

detected through specific, sophisticated laboratory methods (6). In addition, some of these DNA extraction methods provide DNA that is only suitable for PCR (7). Other methods, although they provide high-quality genomic DNA, are cumbersome and tedious, they involve homogenization of clot (8,9). Homogenization probes aid tubes need very careful cleaning and chemical treatment between the samples to avoid cross-contamination.

As long as blood that left at the crime seen was presented in a clotted state, easy methods should be developed to enhance DNA detection with simple commercially available tools avoiding the sophisticated counterparts, to make the extraction procedure more familiar in use. Therefore this paper describes how much beneficial this method is in forensic analysis.

Materials and methods:

All steps except the final one (visualizing step) were undertaken in biology department / college of science for women / Babylon university.

Reagents used: Sucrose, and bromophenol blue were from BDH, England. Boric acid LUDECO, Belgium. Disodium ethylene diamine tetra-acetic acid (sodium EDTA) Scharlau, European Union. Sodium dodecyl sulfate (SDS), and sodium chloride (NaCl) were from Carlo Erba reagents, India. Tris-base Fluka, Switzerland. Proteinase K was purchased from Promega, USA. Agarose was from high media, India. Ethidium bromide was purchased from Sigma, USA. Isopropanol was from Carlo Erba reagents - India. Absolute alcohol and Triton X 100 were from Riedel – USA.

The method mentioned here was derived from our simple and non-hazardous modified method we have used in previous paper (10) but with some modifications suitable to clotted blood, in addition to the repetition of washing of the whole blood with distilled water (11).

Samples of 5 ml of blood were collected 6 apparently healthy donators and placed immediately in 10 ml sterile tubes, and allowed to clot at room temperature. After the blood was clotted, the specimens were placed into sterile plastic Petri-dishes and sliced with scalpel into small pieces with a diameter less than 1mm, then suspended in 10 ml of cold solution contain 38 mmol NaCl, 10 mmol EDTA, and 5 mmol Tris.cl (pH=8) and placed in a sterile 10 ml centrifugation tubes and centrifuged at 4000 rpm for 10 min at room temperature. Supernatants were removed and the pellets were re-suspended in 10 ml of autoclaved distilled water to destroy the RBCs. Tubes were centrifuged at 4000 rpm for 10 min at room temperature. The later two steps were repeated several times until the red color of specimens was disappeared. Then pellets suspended with 10 ml of a second buffer contained 1% Triton X-100, 0.15 mol of NaCl , 10 mmole of EDTA, and 5 mmol Tris.cl (pH=8), centrifuged at 4000 rpm for 10 min at room temperature later the pellets treated with a lytic buffer (0.05 M Tris-Cl, 0.5% SDS, 0.1 M NaCl, 0.001 M Na2EDTA pH 7.2) contain 100 µg / ml proteinase K. The mixture was incubated overnight at 37˚C.

After digestion was complete, 200 µl of 25% SDS and 4 ml of 5 M NaCl were added to each tube and shaken vigorously for 15 seconds, followed by centrifugation at 6000 rpm for 10 minutes. The precipitated

protein pellet was left at the bottom of the tube and the supernatant containing the DNA was transferred to another 15 ml polypropylene tube. Exactly 1 volume of freeze cold (-20˚C) isoprobanol was added and the tubes were inverted several times until the DNA was precipitated. The precipitated DNA strands were removed with a Pasteur-pipette and washed with 70% ethanol, then transferred to a 1.5 ml micro-centrifuge tube containing 400 µl of TE buffer (10 mM Tris-HCl, 0.2 mM Na2EDTA, pH 7.5). The DNA was allowed to dissolve for 15 minutes at room temperature and stored in -20˚C until electrophoresis.

Agarose gel electrophoresis conditions: Electrophoresis was taken place in 27x17.5x10 cm tank dimensions, 15x15 cm tray dimensions, 15x1.0 mm comb dimensions (agarose electrophoresis unit was purchased from Labnet international corporation, Korea). Agarose concentration was 1%. 25 µl DNA suspension was applied to the gel after mixing with 5 µl (1X) loading buffer (40% w/v sucrose and 0.25% w/v bromophenol blue). Electrophoresis was taken place under 70 V, 20 mA at room temperature for 3 hours. Agarose gel was stained with 10 mg/ml ethidium bromide for 30 minutes, then placed in UV light translluminator (Herolab – Germany), submitted to 256 nm wavelength and photographed by 7.2 Mega pixel digital camera (Sony-Japan).

Results and discussion:

In the routine clinical laboratory, large amounts of uncoagulated blood are collected and the blood clot usually is discarded. In molecular biology, particularly in forensic analysis, researchers was make use of clotted blood as only remaining source for DNA isolation (12). But DNA in procedures described in many papers was obtained after extraction with hazardous organic solvents (phenol-chloroform). However, the techniques may be difficult or impractical. While other techniques were time consuming, using many chaotropic reagents, RNA removal steps, or large volume of samples and reagents not suitable in the clinical analysis (13). When we compared the DNA extracted from clotted blood (b – g in figure 1) with the DNA extracted from EDTA-anticoagulated blood (a in figure 1), there wasn t any significant different between these two types of extraction except some variations occurred among individuals. This finding indicates that this simple previously mentioned method can be used for extraction of DNA from both clotted as long as non-clotted blood.

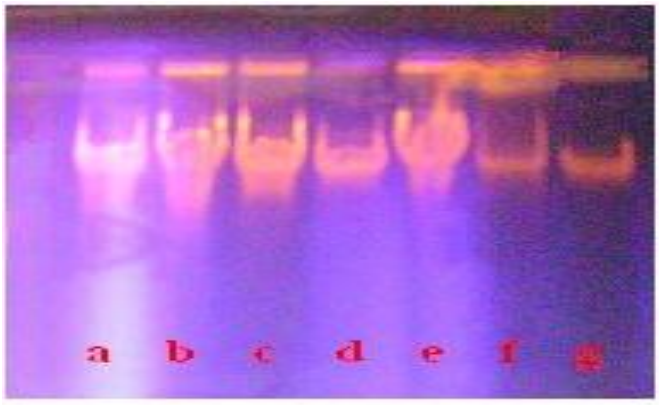

Figure (1): Electrophoresis of 1% Agarose gel electrophoresis displaying genomic DNA prepared from clotted blood using the low cost non-hazardous DNA extraction method. All lanes (from a to g) were run at 70 V/ 20 mA for 3 hrs. DNA specimens were made visible after staining with 10 mg/ml ethidium bromide and visualization under 256 nm. The direction of electrophoresis was from top to bottom.

Despite of Garg *et al.*, 1996 notion in which they were described slicing clot in a small pieces with a scalpel as very cumbersome and exposes laboratory personnel directly to blood-contaminated sharp instruments (14), the slicing of the blood clot masses with a sterile scalpels was practical with increase handling of specimens of clotted blood. However precautions should be taken when dealing with specimens of that kind, but not to the extent of considering slicing process as non-practical preparation as it was mentioned by Garg *et al.* 1996 particularly when we keep in mind how much the slicing procedure was simple and economic compared with the cost-effective and sophisticated immunological micro-pores nylon meshes method of Garg et al., 1996(14).

However, the utilization of simple devices in this procedure, was an extension of our previous paper in which we were demonstrated the possibility of DNA extraction from blood (non-clotted) in a non-hazardous, relatively low cost method, made it possible to extract DNA from clotted blood with the same efficiency as well. The absence of the hazardous carcinogenic solvents (such as phenol) was played an important role in popularizing this simple method in forensic analysis. But still, so far, the procedure was not tested yet to see weather it was suitable for restriction fragment length polymorphism (RFLP) analysis.

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