Short report

Rapid and simple method for preparation of genomic DNA from easily obtainable clotted blood

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Abstract

A method was developed for the preparation of genomic DNA from clotted blood that is usually discarded after extraction, for other laboratory tests. The method, which involves proteinase K digestion, salt/chloroform extraction and 90% ethanol precipitation of DNA from clotted blood, is rapid, simple, and easy because it does not impose an extra burden on the patient.

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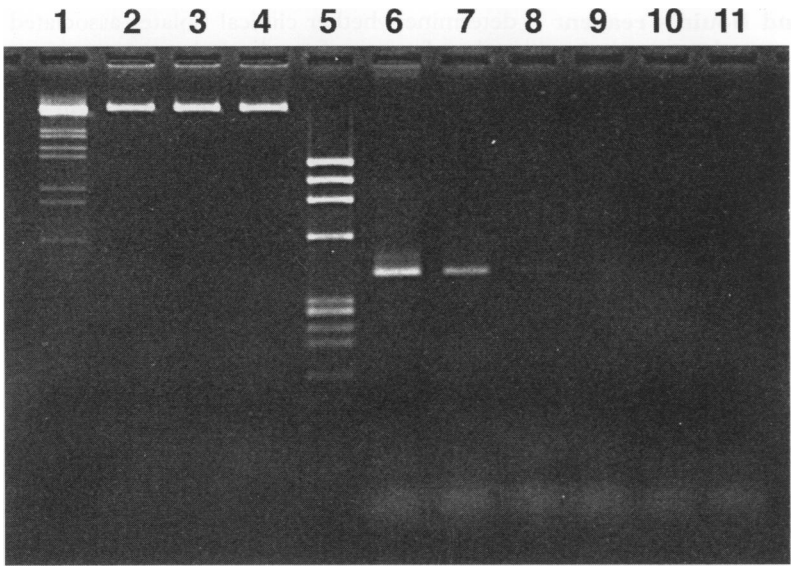
Genetic tests or screening for diseases or disease susceptibility are based on blood as a source of genomic DNA. Genomic DNA is usually prepared from white cells or whole blood.[1] However, isolation of white cells requires density gradient centrifugation (using a medium such as Ficoll-Hypaque), and this is very time consuming when many samples have to be prepared. Even when whole blood is used, the amount collected usually exceeds that required, and an anticoagulant (such as EDTA) must be added. Direct polymerase chain reaction (PCR) amplification from blood has also been devised,[2 3] but the sample cannot be stored as DNA for further investigation. On the other hand, clotted blood left after collection of serum for other laboratory tests is usually discarded. We therefore developed a method of using this clotted blood for genetic analysis. This method frees the patient from any inconvenience involved in the taking of surplus blood, and is especially useful for children.



DNA incubated at 37°C and amplified PCR products electrophoresed on 2% agarose. Lane 1: λDNA/Hind III and EcoRI digest; lanes 2–4: no incubation, and after one week and one month of incubation of the DNA at 37°C; lane 5: φX174 DNA/HaeIII digest; lanes 6–10: PCR amplification of 1, 0·1, 0·01, 0·001, and 0·0001 μl of the extracted DNA dissolved in 100 μl TE buffer; lane 11: negative DNA control (no template DNA). The 410 base pair DNA fragments were amplified by PCR using the following temperature cycle profile in a thermal reactor (Minicycler, MJ Research, Massachusetts, USA): 30 cycles of 30 seconds at 94°C, 30 seconds at 60°C, 60 seconds at 70°C.

Method

Throughout the process, 1·5 ml tubes are used so that many samples can be prepared easily and at the same time. Lysis solution (360 μg/ml proteinase K, 150 mM sodium chloride, 50 mM EDTA, 2% sodium dodecyl sulphate, divided into 250 μl aliquots in 1·5 ml tubes and stored at −20°C) can be prepared beforehand. Two hundred microlitres of fresh blood clot or a freeze-thawed clot is aspirated with a 2 ml disposable serum pipette using rather strong negative pressure, mixed with lysis solution, and incubated at 55–65°C for three hours with periodic mixing, avoiding aggregation of the clot at the bottom of the tube. After incubation, 150 μl of saturated NaCl (nearly 6 M) and 600 μl of chloroform are added and mixed vigorously for 10 minutes by inverting the tube. Phase separation is achieved by centrifugation at 5000 rpm for five minutes. The upper 400 μl of the aqueous phase is transferred to a fresh 1·5 ml tube containing 800 μl of 90% ethanol. The pellet is recovered by centrifugation of 5000 rpm for five minutes. After washing with 70% ethanol, the pellet is resuspended in 100 μl of TE (10 mM TRIS-HCl, 1 mM EDTA) buffer.

Results

We modified the salt/chloroform extraction method for nucleated cells[3] and found it suitable for extracting DNA from clotted blood. The extracted DNA is colourless, not sheared, and free of protein, pigment, salt and RNA. The amount of DNA comprises

about 10 μg, enough for more than 100 PCRs. No degradation was found after incubation at 37°C for one month (figure), indicating the long term stability of the sample at 4°C. The clotted blood before DNA extraction can be stored at −20°C in the tube used for serum separation for up to a month, and can be transferred on dry ice to other laboratories.

Discussion

The salt/chloroform method is better than the use of phenol, because it avoids having to use a dangerous solvent. However, unlike the procedure described before,[4] neither absolute ethanol nor propanol are recommended for DNA precipitation because it also precipitates salts and blood pigments, which inhibit polymerases and restriction enzymes. This method is simple, rapid, economical, and within the scope of most clinical laboratories. It facilitates the collection of many samples for studies of genetic disorders because it uses normally discarded blood clots, and is widely applicable to medical, genetic, epidemiological, and forensic studies.

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Comparison of formalin and Bouin's reagent for fixation of coagulase negative staphylococcal biofilm

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Abstract

Methodological modifications, particularly the use of different fixatives, may account for discrepancies between studies of the relation between virulence and biofilm production *in vitro* by isolates of coagulase negative staphylococci. The efficacy of formalin and Bouin's reagent for fixing coagulase negative staphylococcal biofilms in a microtitre tray assay was compared. The optical density of stained adherent growth by three strains was reduced by an average of 20% following fixation with 10% formaldehyde compared with Bouin's reagent. This difference seemed to be mainly because of increased background staining and blackening of the biofilm when Bouin's reagent was used. Formalin fixation was also effective at identifying early and late biofilm production in adherence growth kinetic experiments with 10 coagulase negative staphylococcal clinical isolates.

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Coagulase negative staphylococci adhere to and accumulate on polymer surfaces by producing extracellular slime, forming biofilms. Although authors have frequently referred to the *in vitro* measurement of coagulase negative staphylococcal slime production, the most commonly used methods[1] primarily examine surface accumulation of bacterial cells, rather than slime per se.[2] The term "biofilm" has been used here as a compromise, accepting that cell accumulation is the main parameter under investigation. Many groups have examined biofilm formation by coagulase negative staphylococci in vitro to determine whether clinical isolates associated with infection of medical devices are more adherent than control strains, with conflicting results.[1 3–8] Methodological differences in the commonly used microtitre tray assay of adherent growth are possible causes of some of these discrepant results.[1 3–5 7 8] In particular, methods used to fix the coagulase negative staphylococcal biofilm to the bottom of the wells in the microtitre tray after washing may not be equally effective.[9] The original report of the microtitre tray assay described the use of Bouin's reagent as a fixative.[1] This reagent, however, is potentially explosive, relatively expensive, and less readily available than alternatives such as formalin and glutaraldehyde, which have been used as fixatives in some cases.[8 10] This study compares the efficacy of formalin and Bouin's reagent for the fixation of coagulase negative staphylococcal biofilms in vitro.

Method

Twelve coagulase negative staphylococcal clinical isolates were obtained from patients with peritonitis undergoing continuous