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.

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 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...
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 primarily examine surface accumulation of bacterial cells, rather than slime per se. 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. Methodological differences in the commonly used microtitre tray assay of adherent growth are possible causes of some of these discrepant results. 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. The original report of the microtitre tray assay described the use of Bouin’s reagent as a fixative. 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. 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