Improved method for direct PCR amplification from whole blood

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Many PCR diagnostic tests are based on blood as the biological material. DNA is usually extracted fron blood according to the phenol/chloroform method (1). This however is time consuming and there is also a risk of introducing contamination during the preparation. An alternative is to PCR amplify directly from the blood sample. Previous investigators have reported that the maximum volume of blood that can be amplified directly is 2 μl (2) and 8 μl (3) as volumes of blood any higher than this will inhibit the PCR reaction. It is desirable to directly amplify from larger volumes of blood to increase the sensitivity of the reaction particularly when the target DNA of interest in the blood sample is present in low quantities. We describe here a simple and efficient method to amplify DNA directly from larger volumes of whole blood samples up to 45 μl.

PCR amplification was performed directly from volumes of whole blood up to 45 μl in a 100 μl reaction. This is more than a twenty-fold increase than in the volume previously reported (2). Blood volumes of 1 μl, 2 μl, 5 μl, 10 μl, 20 μl, 30 μl, 40 μl, 45 μl, 50 μl were made up to 50 μl with sterile water in a 0.5 ml PCR tube and the tube was overlaid with ~100 μl of oil (Sigma). The blood was then heated to 95°C for 15 minutes to lyse the cells. After the incubation step 50 μl of a 2×PCR cocktail was added (20 mM Tris−HCl (pH 8.0), 100 mM KCl, 8 mM $MgCl_2$, 0.02% Gelatin, 0.2% Triton X-100, 0.25 mM dNTP mixture, 150 ng of each primer and 1.5 units of Taq, polymerase (Promega)). The primers used in the PCR reaction amplify a 224 base pair fragment from the Insulin Growth Fartor-1-gene (IGF-1). The following are the primer sequences:

GP 1: 5′-CTCACTGTCACTGCTAAA-3′
GP 2: 5′-AAGAAATCACAAAAGCAGC-3′

Amplification conditions were 94°C for 30 seconds, 50°C for 30 seconds and 72°C for 1 minute over 15 cycles, 20 μl of each amplified product was run on a 2% agarose gel stained with ethidium bromide (Figure 1).

The results show that pre-treatment of the blood at 95°C for 15 minutes prior to the addition of the PCR components allows amplification of up to 45 μl of blood. This volume is a more than twenty-fold increase than reported by Mercier *et al.* (2) and nearly a six-fold increase than reported by Panaccio *et al.* (3). These results suggest that some of the PCR inhibitory factors in blood may be inactivated by the pretreatment step. Thus it appears as if the addition of the PCR cocktail after the preheating step is crucial to the success of this method. This method has been successful with all blood samples tested and different targets for the PCR (data submitted but not shown).

ACKNOWLEDGEMENTS

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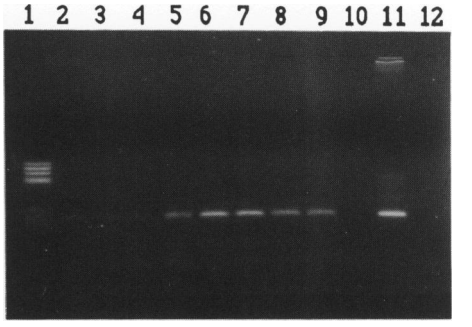

Figure 1: Direct PCR amplified fragments electrophoresed on a 2% agarose gel. Lane 1: pBR322 HaeIII digest. Lanes 2−10 direct amplification of whole blood: 1 μl, 2 μl, 5 μl, 10 μl, 20 μl, 30 μl, 40 μl, 45 μl, 50 μl. Lane 11: PCR amplification of 1 μg of purified human DNA. Lane 12: negative PCR control (absence of target DNA in the reaction).