PCR based diagnosis in the presence of 8% (v/v) blood

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The Polymerase Chain Reaction (PCR) has proven to be an extremely powerful research tool (1). However its full potential as a diagnostic agent still remains to be proven. A major limitation of PCR based diagnostic tests is the inhibition of Taq polymerase by many substances found in clinical material. To date, blood, mucus, urine, sperm and faecal material are some of the body fluids/products that have been shown to inhibit Taq polymerase. This makes PCR-based diagnosis directly from clinical material difficult and therefore most PCR-based tests designed, rely on a DNA or target (bacteria or virus) purification step (2). This increases the labour input and cost of the test.

As little as 1% (v/v) of blood is enough to totally inhibit Taq polymerase (Fig. 1). This inhibition is not dependent on the concentration of magnesium ions (results not shown). An interesting finding was that different thermo-polymerases differ dramatically in their ability to function in the presence of clinical material. Whilst Taq polymerase was totally inhibited by the presence of as little as 1 µl of blood, Tth polymerase, from *Thermus thermophilus* HB8 was able to successfully amplify a specific 1.3 kb target sequence in PCR reaction mix containing up to 4% (v/v) blood. Clearly there are no specific inhibitors of DNA polymerase activity in whole blood. A further increase in the amount of blood inhibited the ability of Tth polymerase to amplify DNA. It is believed that this loss of DNA polymerase activity is due to the entrapment of target DNA by the large amount of coagulated organic material present in the blood containing PCR samples. Thus the target DNA is inaccessible to the DNA polymerase. The addition of one unit of the single stranded DNA binding protein, T4 gene 32 protein, further increases the accessibility of Tth polymerase to the target DNA. In the presence of one unit of T4 gene 32 protein, Tth DNA polymerase, can specifically amplify DNA from whole bacteria in the presence of 8% (v/v) blood. Using Tth polymerase and T4 gene 32 protein it is possible to amplify target sequences directly from clinical material without the need to purify the target DNA, thus avoiding a time consuming and costly step.

REFERENCES

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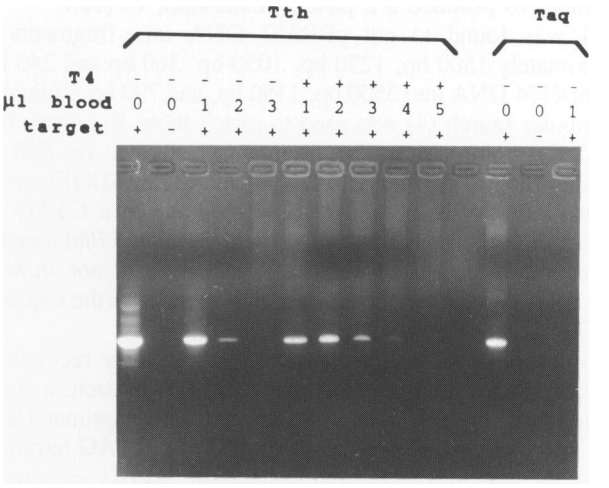

Figure 1. Photograph of an 1.5% agarose gel stained with ethidium bromide. Each track contains the product of a 50 µl PCR reaction containing 200 µM of dATP, dCTP, dTTP and dGTP (Pharmacia, Sweden), 0.2 µM of forward and reverse *C. fetus* specific oligonucleotide primers (P1f and P1r), 10 mM Tris-pH 9.0 (at 25°C), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% Gelatin, 0.1% Triton X-100 and one unit of Taq polymerase (Promega, USA) or Tth polymerase (Toyobo, Japan) as indicated. The amount of whole blood added to each sample is indicated. The presence of T4 (one unit of T4 gene 32 protein (Boehringer Mannheim, West Germany)) and Target (approximately 1×10^3 *Campylobacter fetus fetus* bacteria) are also indicated. PCR conditions were as follows: 30 cycles: 95°C for 30 seconds; 50°C for 30 seconds; 70°C for 60 seconds.