Storing and preserving blood and other tissue samples suitable for shipping or later DNA extraction, to be used in PCR assays and other procedures, are usually cumbersome. This often results in a poor quality of DNA and a low yield, due to the cellular lysis and further degradation of genetic material. Several methods, such as cooling, freezing (1), fixing (5), drying (6), freeze-drying (9), and the use of microbiome and enzyme inhibitors (1, 2, 7), have been used to preserve the DNA from field samples. The results are mixed; the majority of these methods do not prevent cellular and DNA damage, and most of the methods are useful only in preserving samples for short periods. This report introduces an alternative method for tissue preservation with an antifreeze solution that can be kept in freezing temperatures for a prolonged period of time. Preserving the integrity of blood and tissue samples dramatically improves the quality and yield of the extracted DNA.

MATERIALS AND METHODS

Several ratios (from 0 to 30%) of autoclaved propylene glycol and ethylene glycol (Sigma Chemical Co., Gaithersburg, Md.), each alone or combined in equal parts (kept at room temperature), were mixed by gentle inversion with Na2EDTA-containing peripheral goat and human blood and directly placed in three freezers at 4, 20°C and 70°C for up to 6 months. After the best concentration of antifreeze was determined, it was tested with other samples, such as Brucella. Clinical samples taken from Brucella-infected goats and human samples, the detection of b-globin was performed as an internal control for the extracted DNA, using previously described procedures (3).

RESULTS AND DISCUSSION

The solution that better preserved cells was 20% ethylene glycol-propylene glycol (E/P20). Samples kept at either 4 or 20°C showed excellent cellular preservation and therefore high and good yields of genomic DNA. Samples kept at 70°C and those mixed with propylene glycol alone or with concentrations of ethylene-glycol below 15% became frozen. Cells and DNA of samples stored in those solutions were greatly damaged, as judged by their microscopic and macroscopic appearance, electrophoresis results, and PCR performance. EG15 to EG30 showed complete erythrocyte lysis and 1 to 2 lymphocytes per field, and treatments E/P15, E/P20, and E/P25 showed 300 or more erythrocytes and about 2 leukocytes per field; E/P30 showed an average of fewer than 50 erythrocytes and 1 leukocyte per field (Table 1). When DNA yield and quality were evaluated, E/P20 was shown to be the best cell-preserving solution as judged by readings of optical density at 260 and 280 nm, gel appearance, and PCR performance. As shown in Fig. 1, DNA extracted from blood stored in E/P20 at

**Antifreeze Solution Improves DNA Recovery by Preserving the Integrity of Pathogen-Infected Blood and Other Tissues**

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Preserving blood samples for shipping and later DNA extraction has been performed by cooling, freezing, drying, freeze-drying, and protease treatment, among other methods. Most methods to preserve field samples for further DNA extraction do not prevent cellular and DNA damage or are useful only in preserving them for short periods. This report introduces a novel method for blood and tissue that allows preservation in freezing temperatures for a prolonged period of time. The solution reported here (20% ethylene glycol-propylene glycol) preserves cells and tissues integrity, as judged by microscopic analysis, and improves DNA yield and quality.
−20°C for 6 months yielded 34 µg of DNA per 400 µl of blood (lane C), helping to preserve 77% of the cellular DNA contained in the preserved cells compared with the control (feshly collected blood), which yielded an average of 43.7 µg of DNA per 400 µl (lane A). The lowest DNA yield (lane B) was obtained from blood stored for six months at −20°C with no antifreeze (less than 0.06 µg of DNA per 400 µl of blood). Moreover, tissues taken from Brucella-infected goats and stored in the described antifreeze solution also conserved the histopathologic characteristics associated with this pathogen.

The direct correlation between cell intactness and DNA yield and quality seems logical; performance of most methods of DNA extraction from blood relies on the primary lysis of red cells to flush their debris and concentrate by centrifuging the DNA-containing white cells before their rupture. Freezing causes all cells contained within blood samples to burst, diminishing the DNA yield. Other effects of noncontrolled cell lysis are the degrading mechanisms acting on organic molecules, such as DNA, and the difficulty in separating contaminants that remain, binding DNA and causing loss of samples and inhibition of various reactions. We have found this procedure quite convenient in several ways: (i) other people can withdraw field samples and store them to be processed at a later convenient date, (ii) researchers can collect samples, store them in any house freezer, and send them in large batches to be analyzed in specialized remote laboratories, (iii) samples can be repeatedly withdrawn from a freezer, to be tested many times, without suffering any alteration, and (iv) it allows use of the same DNA extraction procedure that is applied for fresh samples. The method described here may be tested in further studies regarding quantification of cell populations, protein and RNA preservation, and other biochemical determinations.

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