

**THE COMPARISON AND VALIDATION OF FTA®, CHELEX, AND QIAGEN EXTRACTION METHODS
ON BLOOD SPOTTED FTA® CARDS FOR THE ANALYSIS OF THE 13 CORE STR LOCI**

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Blood spotted FTA® cards are frequently the method of choice for preservation and storage of reference samples from suspects, victims, and convicted offenders. The advantages of the cards are well known, and include the inactivation of blood borne pathogens, the binding of DNA to the matrix of the card, and their recommended storage at room temperature. This paper describes the comparison of four DNA extraction methods on FTA® cards.

Traditionally, a small punch from the FTA® card is washed according to the manufacturer's protocol and amplified directly without quantitation of the DNA. Our laboratory validated this procedure, which turned out to be problematic in casework. The quantity of DNA on the 1.2mm punch is quite variable. The absence of a quantitation mechanism often results in electropherograms with off-scale data, even though the number of cycles was reduced to 25. Furthermore, presumably due to inhibitory factors present in the card, preferential amplification of the smaller alleles sometimes results in partial and/or unbalanced profiles seen both among and within loci. This is evident by a downward slope of peaks from the smaller to the larger loci, and unbalanced heterozygote peak ratios. Although DNA is supposedly permanently bound to the FTA® Card, a standard 200 µl Chelex Extraction results in high quantities of DNA in the extract. This allows the DNA to be quantitated and a controlled amount to be amplified. Like others, however, we found that in a significant number of cases, inhibitors prevented amplification of the standard 1 ng of DNA. To overcome this, we validated a 400 µl Chelex Extraction, which sometimes alleviates the problem. However, there were still a number of samples for which we got no profile or an incomplete profile. The Qiagen extraction of FTA® cards has proved to be the best method. Full profiles were obtained from specimens which gave no results with the previously described methods. Additionally, the balance among the 13 loci is greatly improved, as is the heterozygote peak height ratio.