DNA EXTRACTION FOR PCR: PHENOL/CHLOROFORM VS. CHELEX - A COMPARATIVE STUDY

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INTRODUCTION

Chelex 100 is a chelating resin with high affinity for polyvalent metal ions which can cause degradation of DNA at high temperatures (1). Boiling of DNA in the presence of Chelex prevents the degradation of DNA.

The Chelex method has been described as a simple, rapid, non hazardous and efficient method to extract DNA (1, 2, 3). To test whether it would be feasible to replace the phenol/chloroform extraction procedure routinely used in our lab by the Chelex method, we extracted DNA with both methods from various forensically relevant stains. The DNA was then amplified (Apo B 3'locus) and the results compared regarding to reliability of amplification, speed, sensitivity to inhibitors and range of DNA concentration which will amplify.

MATERIAL AND METHODS

With small modifications the Chelex extraction method was performed as described by Walsh et al. (2). The stains (stamps, cigarette butts, 6 cm² of saliva samples on filter paper, 5 mm² of sperm stains, one third of swabs with vaginal epithelian cells and different amounts of blood) were cut into small pieces and incubated for one hour in 1 ml of PBS. The fabric or paper was then removed by centrifuging the PBS containing the cells through a small hole into a second Eppendorf tube. The cells were pelleted at 9000 g for 5 min and the supernatant discarded. 100 μl of 5 % Chelex and 5 μl of 10 mg/ml Proteinase K was added to the cell pellet which was then vortexed at high speed and incubated for at least one hour at 56°C. After vortexing again the cells were incubated in a boiling water bath for 8 min, vortexed again and then three different DNA concentrations (30, 5 and 1 μl or - for post-coital samples - 5, 1 and 0.2 μl) were amplified at the Apo B-3'locus as described (4). If necessary the DNA was purified by centrifugation through Centricon 100 tubes or ethanol precipitation was performed.
In the case of post-coital samples sequential DNA extraction was performed as described (2).

Main criterion for the success of the method was the percentage of stains which were successfully amplified and not the amount of DNA isolated because this does not always correlate with the amplification rate if inhibitors are present.

RESULTS

DNA from more than 200 stains was extracted with phenol/chloroform as well as with Chelex. Generally DNA from all kinds of stains investigated could be amplified with both methods. Table 1 summarizes the results:

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Table 1: Comparison between the conventional phenol/chloroform and the Chelex DNA extraction method. The table shows the percentage of stains which could be amplified and analyzed in ethidium-bromide stained agarose gels (left) or after blotting and hybridizing with a digoxygenin labeled Apo B probe (right).

	ethidiumbromide-stained gel		blot	
DNA extracted with	phenol	Chelex	phenol	Chelex
stamps	45 %	59 %	97 %	97 %
cigarette butts	63 %	57 %	97 %	100 %
saliva samples	97 %	97 %	97 %	93 %
sperm	79 %	97 %	100 %	100 %
vaginal secretion	97 %	57 %*	100 %	100 %*
blood	75 %	65 %*	100 %	85 %*

*: without further purification

Saliva stains:
While incubating the stain in PBS and washing the cells once seemed to be sufficient to remove potential inhibitory substances from stamps and saliva samples on filter paper, DNA from cigarette butts did not amplify without further purification such as ethanol precipitation or spin dialysis with centricon 100. With this treatment more than half of the amplification products could be typed in ethidiumbromide stained agarose gels, all resulted in a signal after blotting. From nearly all saliva samples enough DNA could be extracted to be typed in ethidiumbromide stained gels. These results show that the Chelex method works well for saliva stains and that there is no difference between Chelex and phenol/chloroform extracted DNA.

Post-coital samples, blood:
DNA from sperm isolated with the phenol/chloroform method amplified well and reliably. However, when DNA was extracted with Chelex without further purification the amplification was weak and bands were only seen in the blot at high dilutions. Ethanol precipitation improved the amplification rate, but only centrifugation through Centricon 100 lead to results comparable to or even better than those obtained with the phenol/chloroform method.

The same was true for DNA from vaginal epithelian and blood cells: without further purification the amplification was less effective and interpretable results were often only seen in the blot. In some cases DNA isolated from blood failed to amplify even after spin dialysis.

DISCUSSION

The most striking advantage of the Chelex method ist the simplicity and speed (DNA extraction and amplification within one day) especially for a lab where many DNA preparations have to be managed by a small number of assistants. However, there are also some disadvantages. Table 2 summarizes the main features of both methods:

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Table 2: Characteristics of the phenol/chloroform and the Chelex DNA extraction method.

Chelex 100	phenol/chloroform
rapid and simple extraction	long DNA isolation procedure
non hazardous	use of organic solvents
no loss of DNA	higher possibility to loose DNA during isolation
whole cell extract, may contain inhibitors, further time consuming and expensive purification may be necessary	more or less pure DNA, less inhibitors
DNA is single stranded, therefore not suitable for RFLP	DNA ist double stranded, RFLP can still be performed

Our results show that DNA extraction with Chelex from stamps, envelopes, saliva samples and cigarette butts was comparable to the conventional phenol/chloroform extraction method. It does not matter that the DNA is single stranded because ist is rare to get enough DNA for a RFLP-analysis from these stains. Therefore we think that the Chelex extraction method provides a good and fast alternative to the conventional DNA isolation method.

DNA extracted with Chelex from post coital samples and blood also amplified well, but required an additional purification and washing step through Centricon 100 to get a reliable amplification. Alternatively the gel had to be blotted. This reduces the differences in speed between the two extraction methods. Furthermore DNA from blood sometimes failed to amplify even after purification and post-coital samples require an extra treatment to separate the female from the male fraction which ist not practical if they are extracted together with saliva stains. Therefore, at the moment it seems to be the most practical way in our lab to continue to isolate all - even small - semen/vaginal secretion and also blood stains with phenol/chloroform. In any cases we use this conventional method for RFLP-analysis. An additional advantage of this procedure is the possibility to perform a RFLP analysis if more DNA than presumed was isolated.

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