TECHNICAL NOTE

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Evaluation of an Alkaline Lysis Method for the Extraction of DNA from Whole Blood and Forensic Stains for STR Analysis

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ABSTRACT: A modified alkaline lysis protocol for extracting DNA from forensically relevant specimens is evaluated and compared with the chelex 100 method. For whole blood, bloodstains and sperm stains, both methods yielded comparable results after amplification for a pentameric STR locus (HumCD4). The main advantages of the new method are: only approximately ten minutes and two pipetting steps are necessary and the expenses for the extraction are extremely low as only NaOH, TrisHC1 buffer and a single microcentrifuge tube are required. Alkaline lysis also proved to yield DNA suitable for typing longer STRs by using dye-labeled primers and capillary electrophoresis. These advantages should render this protocol especially interesting for high-throughput laboratories in combination with multiplex PCR and fluorescent dye technology, although the storability of the extracts proved to be problematic.

KEYWORDS: forensic sciences, DNA typing, extraction, alkaline lysis, short tandem repeats, chelex 100, HumCD4

The process of forensic stain typing using STR loci roughly consists of three steps: extraction of DNA, PCR amplification, and typing using native or denaturing polyacrylamide gel electrophoresis (PAGE) (1). Until recently these procedures were equally tedious. The advent of multiplex PCR and the application of fluorescence dyes in combination with capillary electrophoresis, however, have reduced the expenditure of time and work necessary for these procedures considerably (2). Yet the expenditure for the DNA extraction could not be reduced up to now, and commonly used protocols for forensic materials (phenol-chloroform extraction (3), "salting out" (4) and the chelex 100 protocol (5) require at least two hours and more. Therefore it seems to be worth evaluating new, less time-consuming approaches to this problem.

One possible alternative is alkaline lysis of cells (6). Strong alkaline solutions exert a strong denaturing and solubilizing effect on proteins due to ionization of aspartic, glutamic, cysteic, and tyrosine residues (7). Incubation at alkaline pH should therefore disrupt cell and nucleus membranes, denature nucleases and dissolve the DNA, whose primary structure is relatively stable to such treatment (8). This approach is widely used for isolation of plasmid DNA from bacteria (9) and genomic DNA from plant tissue (10), but only one author has reported the application of this protocol to forensically relevant specimens up to now (11). The goal of this study was to further evaluate the applicability of the alkaline lysis method in a forensic PCR laboratory, to modify the method, if necessary, and to compare the results obtained by this protocol with those of a widely used DNA extraction protocol, the chelex 100 method (5).

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Methods

The protocol for alkaline lysis published by Dissing et al. (11) is given in Fig. 1. Nevertheless, the yield of DNA using this protocol proved to be insufficient. Better results from both whole blood and stains were obtained when the initial incubation in distilled water was omitted. This slightly modified protocol gave satisfying results and was used for comparing the results for altogether 86 experimental stains (Tables 1 and 2) to those of the chelex 100 lysis (5) as well as for real casework (166 stains: Table 3).

Of all samples from both alkaline and chelex lysis, 5 μL (i.e., 2.5%) were used for amplifying a pentameric STR mapped to the CD4 locus followed by native PAGE and silver staining as described (12).

Quantification of the DNA content of the extracts was performed by using slot blotting as described (13).

For testing the suitability of the extracts yielded by the alkaline lysis for typing longer STRs, 50 blood samples were extracted using both alkaline lysis and a salting out procedure (3). These extracts were then coamplified for nine STRs ranging between approximately 100 and 340 bp using the AmpF1STR Profiler Plus Kit® (PE Applied Biosystems, San Jose, CA) and typed using capillary electrophoresis according to a slightly modified protocol (14). Subsequently it was judged whether all nine loci were successfully amplified or allelic drop out was observed for one or several loci.

Results and Discussion

Both chelex 100 (4) and the alkaline lysis are single-tube protocols. While this type of protocol cannot yield 100% pure DNA, the inevitable loss of DNA during additional purification steps can be

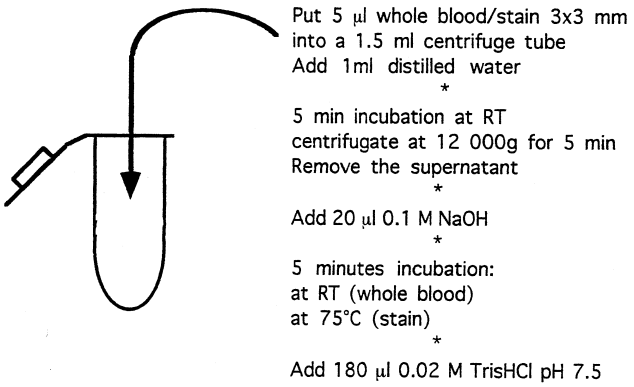

FIG. 1—*Protocol for the alkaline lysis of forensically relevant specimens proposed by Dissing et al. (11).*

TABLE 1—*Semiquantitative comparison of the sensitivity of alkaline lysis and chelex 100: different amounts of whole blood and dried blood on cotton were extracted using both techniques and amplified for the CD4 locus. The samples were then analyzed using native horizontal PAGE and the quantity of the PCR products was compared for both methods (+++ very strong band; ++ strong band; + faint but sufficient bands; - no band or no sufficient band). The electropherograms for some of these samples are given in Fig. 2.*

		Alkaline Lysis	Chelex
Whole blood (µL):	5	+++	+++
	3	+++	+++
	2	++	++
	1	++	++
	0.5	+	+
	0.25	+	+
	0.12	–	–
Blood on cotton thread (mm)	4	++	++
	3	++	++
	2	+	+
	1	+	+
	0.5	–	–

TABLE 2—*Semiquantitative comparison of the sensitivity of alkaline lysis and chelex 100 using six types of forensically relevant specimens.*

	Akaline Lysis				Chelex			
	+++	++	+	–	+++	++	+	–
10 Cigarette butts	2	4	4	0	3	3	4	0
10 Stamps	0	0	6	4	0	0	5	5
10 Buccal swabs	8	2	0	0	7	3	0	0
15 Hairs	0	0	7	8	0	2	10	3
10 Sperm stains	7	3	0	0	8	2	0	0
10 Bloodstains	8	2	0	0	7	3	0	0

NOTE: See Table 1 title for symbol identity.

TABLE 3—*Comparison of the results for alkaline lysis and chelex 100 in real casework (166 samples).*

		Alkaline Lysis		Chelex	
Type of Stain	No.	Pos.	Neg.	Pos.	Neg.
Bloodstains on:					
glass/leather/plastic/steel	20	18	2	17	3
rusty iron	3	3	0	3	0
colored wool	5	4	1	5	0
blue jeans	19	15	3	16	3
black jeans	9	7	2	5	4
linen	3	3	0	3	0
printed paper (voucher)	4	0	4	0	4
Sperm stain on:					
silk	3	3	0	3	0
cotton	4	4	0	4	0
black jeans	2	2	0	2	0
Saliva stain on:					
bottle	2	2	0	2	0
stamp	10	4	6	6	4
envelope	10	2	8	4	6
cigarette butt (fabricated)	63	49	14	43	20
cigarette butt (self-made)	3	0	3	0	3
Formaline fixed tissue embedded in paraffin:	3	0	3	0	3
Fresh muscle:	3	0	3	0	3

avoided. The question of whether impure DNA without loss or highly purified DNA of a smaller quantity is more suitable depends on the method applied for further analysis of the extracts. For DNA profiling using STRs, which are the most frequently used DNA polymorphisms for forensic purposes today (15), highly purified DNA does not seem to be necessary and chelex 100 has become a recommended routine protocol in a forensic DNA laboratory (16), whose performance is only slightly worse than that for highly purified extracts yielded by phenol-chloroform extraction followed by Centricon 100 concentration (17). If the results for the alkaline lysis should be comparable to those for the more tedious chelex 100 protocol, this new method might be an interesting alternative in a forensic DNA laboratory.

In the alkaline lysis protocol described by Dissing et al. (10), the initial incubation in double distilled water was performed in order to remove the heme, hematin and hemoglobine, potent inhibitors of PCR (18,19), from the tube. Surprisingly, the results for these samples were weak and improved considerably after having omitted this step. It seems possible that the minute amount of water, which inevitably escaped removal from the tube before adding NaOH, diluted the alkaline solution and thus interfered with the lysis process. The extracts obtained after omitting this incubation step were of a moderate to dark red color, depending on the amount of blood used. Nevertheless, this impurity obviously did not exert an adverse effect on the results of the PCR reaction. This phenomenon might be explained by the fact that the hemoglobin was found to precipitate during the amplification process and thus sedimented to the bottom of the tube, while most of the assay solution seemed to be free of any color.

As the results for this modified protocol were encouraging, different quantities of whole blood and experimental stains were extracted using both chelex and modified alkaline lysis (Tables 1 and 2). For both methods as little as 0.25 µL of whole blood were sufficient to yield enough DNA for successful typing of the CD4 locus (Fig. 2; Table 1). The results after PCR were also comparable for small fresh bloodstains on cotton twine (Table 1) and experimental blood-stains and sperm stains on white cotton, cigarette butts, stamps, and buccal swabs (Table 2). However, using the alkaline lysis approach, only 2 of 10 selected hair roots with sheath were positive with or without adding DDT, while 7 of 10 were positive using Chelex.

Nevertheless, on retesting these samples after storing at 4°C for

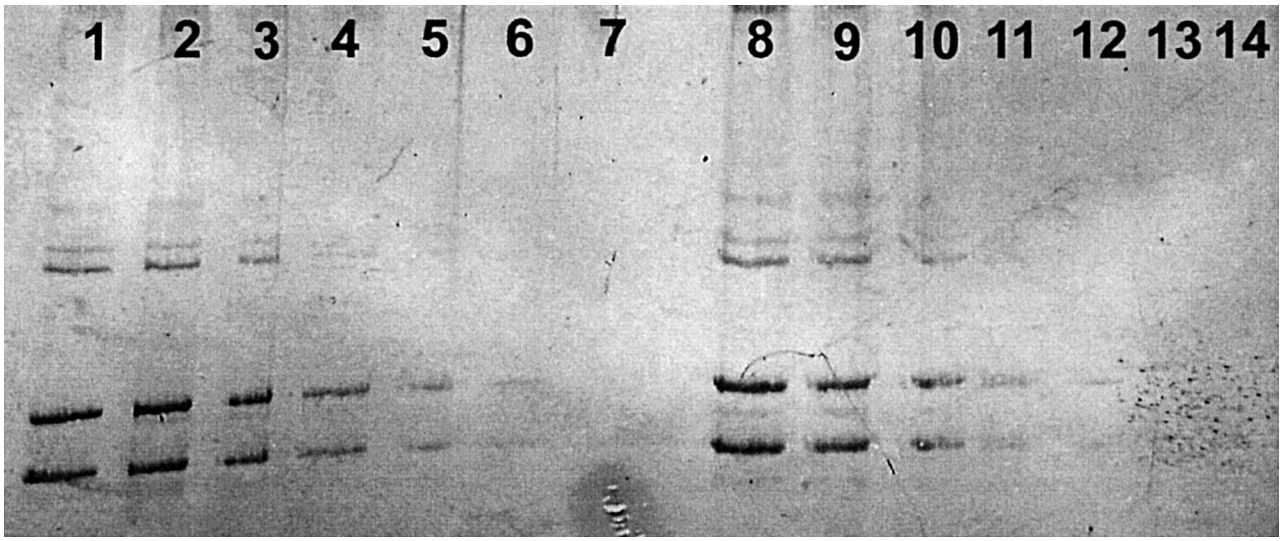

FIG. 2—*Electropherogram of the PCR products amplified for the locus CD4 (genotype 5,10) using the blood samples listed in Table 1. Samples 1 through 7 were extracted by alkaline lysis. Samples 8 through 14 were extracted by chelex 100. Lanes 1 and 8: 5 μL blood; lanes 2 and 9: 3 μL blood; lanes 3 and 10: 2 μL blood; lanes 4 and 11: 1 μL blood; lanes 5 and 12: 0.5 μL blood; lanes 6 and 13: 0.25 μL blood; lanes 7 and 14: 0.12 μL blood.*

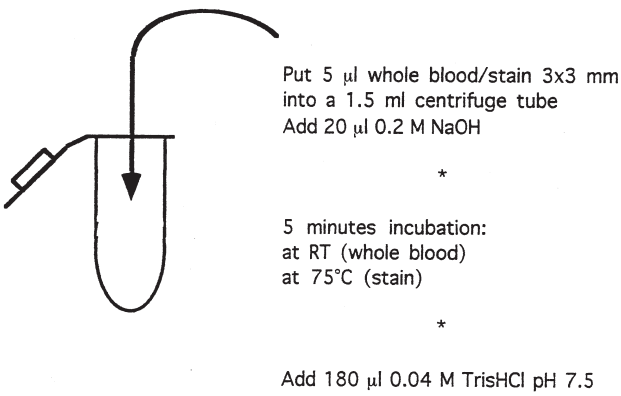

FIG. 3—*Final protocol for the alkaline lysis of forensically relevant specimens used in this study.*

several days, almost all alkaline lysis samples were negative. Repeated experiments revealed that some of the alkaline extracts were even unsuitable for PCR amplification after storage at 4°C for less than one day. To confirm this disappointing and unexpected finding, which is in contradiction to the results reported by Dissing et al. (11), the DNA quantities of the extracts were compared with those obtained by the chelex protocol by slot blotting and hybridization (13). According to this test most of these samples now only contained minute amounts of DNA or were even negative. Possible explanations for the insufficient storability of the alkaline extracts include the incomplete denaturation of nucleases and the formation of complexes between the denatured proteins and the DNA set free from the lysed nuclei. In order to overcome this obstacle, new extractions were performed by using the double concentrations for both NaOH and Tris buffer (pers. comm., J. Dissing, Copenhagen). The extracts obtained by this modified protocol, which are described in Fig. 3, yielded the same amount of PCR fragments after amplification for the CD4 locus as the original protocol, but the extracts were now considerably more stable. Although they were still not suitable for long-term storage at 4°C or higher, extracts which were stored at −20° for more than one year, during which they were monthly thawed and used for amplification, did not show any loss of template.

As the results for the alkaline lysis on experimental stains using this new protocol were encouraging, this time-saving method was implemented into real casework (Table 3). To that end 166 biological stains submitted to the DNA laboratory of the Institute of Legal Medicine in Graz for typing over a period six months were selected prospectively for double extraction using both chelex and alkaline lysis. The only requirement for inclusion into the study was that enough material was available for allowing double extraction forensic relevance. These results further confirmed the suitability of the alkaline lysis for forensic purposes.

CD4 is a STR with extremely short fragment lengths (85 to 125 bp). Nevertheless, a DNA extraction method would be suitable only for routine casework and especially DNA databasing if the extracts could also be used for the larger STRs implemented into commercially available multiplex kits. To investigate this question 50 blood samples were extracted by using this protocol and a method which yields high molecular DNA (salting out (3). The extracts were amplified using the Profiler Plus® kit. No dropout of a single locus was observed in any of the samples. Although generally a slight decrease in the peak height was observed for the larger loci, the same was also observed for the salting out samples (Fig. 4). Thus it might be concluded that DNA yielded by alkaline lysis is suitable also for amplifying larger STRs and both for dye primer PCR as well as for PCR using unlabeled primers.

There are two explanations for the similar success rates of PCR amplification with chelex and alkaline lysis extracts:

1. Both methods yield the same amount of DNA and both are of comparable quality (i.e., they are equally prone to amplification).
2. One of these methods yields more DNA, yet of poorer quality, while the DNA extracted by the other method is smaller in quantity, but more suitable for PCR amplification. Theoretical considerations supporting the latter hypothesis would be, on the one hand, the presence of inhibitory heme in the alkaline lysis extracts or, on the other hand, the fact that a recent study found NaOH treat-

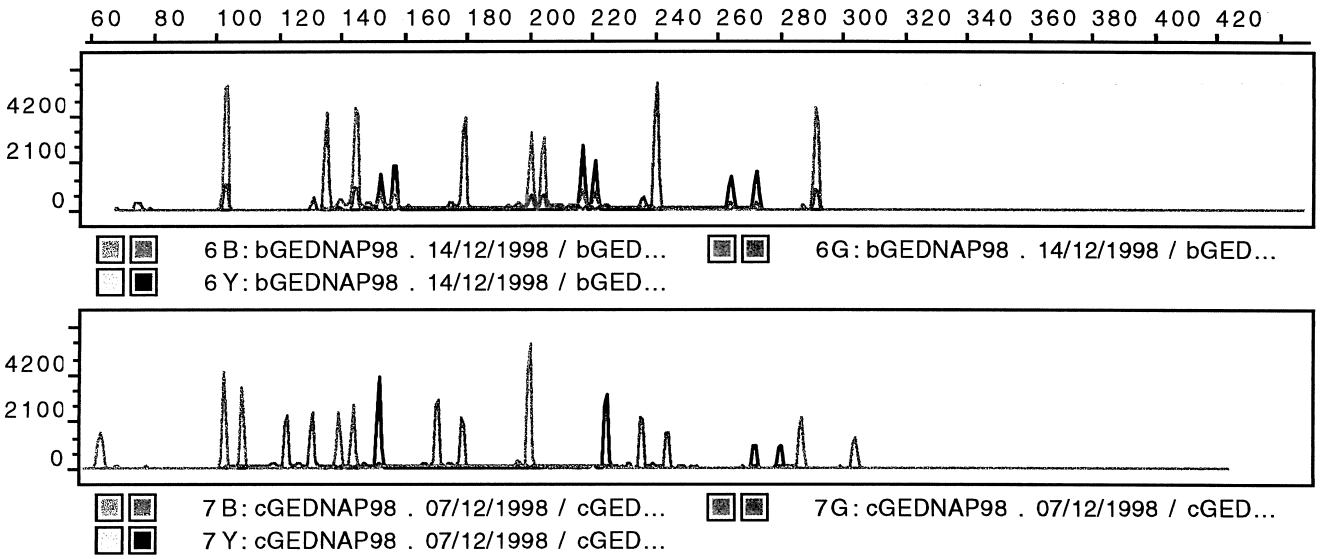

FIG. 4—*Typical electropherogram for DNA extracted from fresh blood samples amplified for the Profiler Plus® kit: Above: blood sample extracted using a method yielding high-quality DNA (proteinase K incubation followed by a salting out protocol). Below: blood sample extracted using alkaline lysis as described.*

ment of extracted DNA helpful for overcoming Taq polymerase inhibition (20).

To investigate this question, DNA was extracted from ten bloodstains measuring 3 × 3 mm by alkaline lysis, and from ten stains of the same size by chelex 100. The results after PCR for CD4 were comparable for both groups of stains (Table 2). The extracts were quantified by slot blotting and hybridization (13). For the chelex extracts an average amount of 54.8 ng DNA was detected (20 to 76 ng); using the alkaline lysis an average of 49.1 ng DNA (20 to 82 ng) was extracted. As these amounts do not differ substantially, the assumption that both methods yield extracts of both similar quantity and quality is strongly favored.

In conclusion, the alkaline lysis proved to be a quick and inexpensive single-tube method for extracting DNA from forensic specimens such as whole blood or blood, saliva and semen stains. The results for these types of specimens were comparable to those of the more tedious chelex extraction. Other forensic specimens, e.g., hair roots or tissue, proved to be unsuitable for alkaline lysis. One major limitation, nevertheless, is the problematic storability of the extracts, especially at temperatures higher than 0°C. When using the modified protocol proposed in this paper, however, the extracts proved to be stable at long-term storage at −20°C, which should be sufficient for paternity testing and for most forensic purposes.

Acknowledgments

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