Int J Legal Med (2000) 113 : 114–116

TECHNICAL NOTE

S. Banaschak · B. Rolf · B. Brinkmann

Influence of different staining techniques on the DNA analysis of histological sections

Received: 4 December 1998 / Received in revised form: 10 March 1999

Abstract The use of stained histological sections as a source for DNA may be necessary in forensic case work if confusion of tissue is suspected, for identification or paternity cases. To elucidate the influence of different staining techniques on the PCR amplification of DNA, histological sections of liver tissue were prepared using eight different staining techniques and two histochemical methods. The DNA of the sections was extracted by a modified Chelex extraction and amplified using a commercial triplex kit. Staining with hematoxylin-eosin, hemalum-eosin, azan, periodic acid-schiff and prussian blue showed no adverse effect on the amplification of DNA while the extracts of tissue stained by Masson-Goldner, Ladewig and elastica-van Gieson methods had to be purified before amplification was possible. Staining with phosphoric tungsten acid hematoxylin and Gomori led to a degradation of DNA probably due to the use of potassium permanganate solution.

Key words Histological sections · Staining technique · DNA identification

Introduction

DNA technology can be applied to the individualisation of different biological materials. If confusion of histopathology tissue samples is suspected, the DNA identification of histological slides may become necessary to assign a sample to a patient. Further more, there exist cases where tissue sections are the only source of DNA for personal identification and for the establishment of biological relationships.

In a crime case, only histological slides (liver) obtained from one paraffin block were available which had been stained with a variety of dyes. In some sections we obtained positive microsatellite results while others were negative. This observation let us suspect that artefacts could occur depending on the staining procedure used. For this reason we have performed the subsequent study to investigate the influence of different staining procedures on microsatellite results.

S. Banaschak · B. Rolf · B. Brinkmann (✉)
Institute of Legal Medicine, Westfälische Wilhelms-Universität, Von-Esmarch-Straße 62, D-48149 Münster, Germany
Fax +49-251-8355158

Material and methods

Liver samples with a maximum size of 1 cm^2 taken from five individuals were fixed in 10% buffered formalin for 24 h to simulate the maximal duration of the fixation in clinical pathology and to be sure that all tissue was fixed. The histological diagnoses are shown in Table 1. After fixing, the tissue was embedded in paraffin and histological sections were stained using eight different techniques and two histochemical methods: hemaleum-eosin (HemE), hematoxylin-eosin (HE), elastica-van-Gieson (EvG), azan, Masson-Goldner (MG), phosphoric tungsten acid hematoxylin (PTHA), prussian blue and periodic acid-Schiff (Böck 1989), Ladewig (modified Mallory-Heidenhain, Romeis 1968) and Gomori's silver impregnation (Gomori) (Smith and Bruton 1979).

The histological slides were placed in xylol until the cover glass could be easily removed. The slides were placed in ethanol solutions with decreasing concentrations and finally in distilled water. The tissue was removed with a cotton swab and placed in a tube containing 1 ml distilled water. 950 µl of the distilled water was removed and DNA was extracted by incubating with 200 µl protease K- Chelex-solution (leading to a 250 µl extraction vol-

Table 1 Personal data and histological diagnosis of the liver samples

No. and personal data	Histological diagnosis of the liver
1. 40-year-old female	acute congestion, beginning fibrosis
2. 21-year-old male	acute congestion, no hepatits
3. 64-year-old male	acute and chronic congestion
4. 57-year-old male	signs of shock
5. 76-year-old female	micronodular cirrhosis

ume) over night at 37 °C before boiling for 8 min (Walsh et al. 1991). For comparison, buccal smears were taken and unstained sections of each sample were processed in parallel. Of the extract 10 µl was amplified using a commercial triplex kit (AmpFLSTR Blue, ABI, Weiterstadt, Germany) including the STR loci D3S1358, vWA and FGA. If the amplification gave no product, the extract was further purified from 450 µl distilled water and 50 µl of the Chelex extraction by concentration (Microcon Amicon, Beverly, MA, USA). This step was repeated once, the extraction was transferred to a new tube and distilled water added up to a final volume of 50 µl. In order to distinguish between inhibition and degradation in cases of negative amplification, 200 pg of control DNA were added to the extract. All samples were analysed with a capillary electrophoresis system (ABI CE 310, Perkin-Elmer Applied Biosystem, Weiterstadt, Germany).

Results and discussion

The amplification of DNA from the Chelex extraction of the unstained, paraffin-embedded tissue was successful in all three STR systems indicating that the fixation with formalin did not affect the results. If such tissue is available this should be the first choice. Formalin is a cross-linking fixative which does not affect PCR if an adequate examination protocol is used (Nuovo 1996). If the paraffin-embedded material gives a negative result this could be due to degradation resulting from long lasting formalin fixation (Wiegand et al. 1996; Romero et al. 1997). Because formalin is widely used in clinical practice and a fixation time of about 24 h simulates the condition of daily work we have chosen these conditions for our experiments. The results obtained from the different stainings could be divided into three groups (Table 2).

The DNA analysis of the tissue samples stained with HE, PAS, Azan and Prussian Blue was always successful. The DNA patterns from both buccal and tissue samples were identical. The successful DNA amplification of tissue stained with hematoxylin-eosin is in contrast to Burton et al. (1998) who concluded that hematoxylin staining should be avoided. This difference could be due to all steps involved: different staining procedures (staining time by Burton 90 min, 20 min in our protocol, other dye), removal and extraction procedures (Burton: extraction with proteinase K, Tris-HCl buffer), DNA purification and PCR.

The first analysis of the MG and Ladewig stained sections was negative and also the control DNA added gave no product indicating an inhibition. After further purification the loci D3S1358 and vWA gave positive results while FGA was positive in one sample only. Sections stained with EvG were positive for two loci (vWA and D3S1358) in two sections only. After further purification, all loci in all sections gave positive results, except for one sample with two loci only. These results indicate the presence of PCR inhibitors, for example residual staining components which could be removed by dialysis in a microcon concentrator. The reaction pattern that only two loci out of three were affected can be explained by differential inhibition. The same observation was made by Wallin et al. (1998) who showed that from the three loci included in the Blue Kit, the FGA locus has the highest sensitivity to inhibition. The amplification of FGA, the system with the longest fragments (219–267 bp), was not possible in MG and Ladewig stained sections even after further purification. This could either be due to impurities still disturbing the PCR of the longest fragments or to partial degradation of the DNA due to the staining procedure.

The amplification of DNA extracted from the tissue samples stained with PTAH and Gomori remained negative even after using the Microcon protocol. The amplification of control DNA added to the extract was always positive in the PTAH stained samples. The Gomori stained samples showed positive results for control DNA added for locus D3S1358 alleles while locus vWA was positive in 3 out of 5 sections. This indicates degradation of the tissue DNA during the staining process and an inhibition of the amplification of the control DNA. Both staining protocols include the incubation in potassium permanganate solution (Gomori: 2 min oxidation in 1% potassium permanganate; PTAH: 1 min acidic potassium permanganate). Potassium permanganate is a strong oxidative agent suitable for the degradation of DNA even after short periods of exposure.

It can be concluded that many of the commonly used staining protocols allow investigation of DNA extracted from histological slides. If the amplification fails, inhibition of the PCR by soluble inhibitors has to be ruled out. Harsh chemicals in the staining process, i.e. oxidative agents, could be a reason for degradation of the DNA.

Table 2 Amplification results after staining

Staining method	Successful amplification	Successful amplificaton only after purification	Negative amplification
HE	×		
HemE	×		
PAS	×		
Azan	×		
Prussian Blue	×		
MG		×	
Ladewig		×	
EvG		×	
Gomori			×
PTHA			×

References

Böck P (ed) (1989) Romeis-Mikroskopische Technik, 17th edn. Urban & Schwarzenberg, Munich Vienna Baltimore

Burton MP, Schneider BG, Brown R, Escamilla-Ponce N, Gulley CE (1998) Comparison of histologic stains for use in PCR analysis of microdissected, paraffin-embedded tissues. Biotechniques 24:86–92

Nuovo GJ (1996) Preparation of samples for polymerase chain reaction in situ. Scanning Microsc Suppl 10:49–55

Romeis B (1968) Mikroskopische Technik, 16th edn. R. Oldenbourg, Munich Berlin

Romero RL, Juston AC, Ballantyne J, Henry BE (1997) The applicability of formalin-fixed and formalin fixed paraffin embedded tissues in forensic DNA analysis. J Forensic Sci 42(4): 708–714

Smith A, Bruton J (1979) Farbatlas histologischer Färbemethoden. F.K. Schattauer, Stuttgart New York

Wallin JM, Buonchristiani MR, Lazuruk KD, Fildes N, Holt CL, Walsh SP (1998) TWGDAM validation of the AmpFLISTR™ Blue PCR Amplification Kit for forensic casework analysis. J Forensic Sci 43:854–870

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 4:506–513

Wiegand P, Domhöver J, Brinkmann B (1996) DNA-Degradation in formalinfixiertem Gewebe. Pathologe 17:451–454