International Congress Series 1261 (2004) 574–576

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Evaluation and comparison of deoxyribonucleic acid typing methods on human tissue fixed with different fixatives

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Abstract. Commonly employed fixing fluids result in very satisfactory histomorphologic analysis; however, they don't allow deoxyribonucleic acid (DNA) typing methods to provide good results. This study investigated the effect of eight fixatives on DNA extracted from placenta samples. Fresh tissue (placenta) specimens were fixed by immersion in different fixing fluids for different periods (from 24 h to 60 days). Different DNA extraction methods were assayed. Gene Amp 2400 and 9700 Thermal Cyclers coupled to AmpFLSTR Identifier kit (Applied Biosystems), that allows the simultaneous amplification of 15 STRs loci, was used for quantification. Amplified products were analyzed by capillary electrophoresis on ABI PRISM 310 and 3100 Genetic Analyzers (Applied Biosystems).

Keywords: Tissue; Formalin; IQ; Fixative solution

1. Introduction

The possibility of typing DNA has been of great interest in personal identification, particularly for organs and tissues taken from autopsies and preserved for very long periods of time. In these cases, it is necessary to obtain very good fixing, preserving the molecular structure of tissues during the exam. The aim of the present work is to study and search for fixatives, which permit excellent utilization of tissues in forensic science.

2. Materials and methods

Fresh tissue (placenta) specimens were fixed by immersion in different fixing fluids for different periods (from 24 h to 60 days). DNA extractions were performed according to two different procedures: one incubation in PK with deproteinization in phenol–chloro-

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0531-5131/
doi:10.1016/S0531-5131(03)01705-9

form, and the use of paramagnetic particles covered with silice (DNA IQ™ System, Promega) [1]. Prior to DNA extraction with phenol–chloroform, samples of placenta were washed several times with phosphate buffered saline (PBS) and homogenized in the same solution. Tissues fixed by Bouin fixative were washed several times with 50% ethanol to eliminate yellow colour of the picric acid. Cytoscreen™ was purchased by Ddk Italia. Specimens were then incubated overnight at 40 °C in a solution of lysis buffer (10 mM Tris–HCl, pH 8; 10 mM EDTA, pH 8; 100 mM NaCl, 2% SDS) containing Proteinasi K (100 mg/ml). The mixture was extracted twice with phenol–chloroform, in according to Ref. [2]. Prior to DNA typing, all samples were purified using DNA and Gel Band Purification Kit (Amersham Pharmacia Biotech) in accordance with the manufacturer's instructions. The evaluation of quality and approximate yield of DNA extracted was performed by 1% agarose gel electrophoresis and ethidium-bromide staining. Another extraction method used was DNA IQ™ System; this extraction was applied in accordance with the manufacturer's instructions [1]. STR amplification was carried out by Gene Amp 9700 and 2400 thermal cyclers (Applied Biosystems), with the ''AmpFLSTR Identifier'' kit (Applied Biosystems) [3]. Amplified products were analyzed by capillary electrophoresis on two ABI PRISM 310 and 3100 Genetic Analyzer (Applied Biosystems) employing ABI software (DATA Collection, GeneScan Analysis, Genotyper Fragment Analysis).

3. Results

Our results indicate that in specimens fixed with formaldehyde or with other fixative containing different concentrations of formaldehyde, there is a significant reduction in DNA extraction, both quantitatively and qualitatively, as compared to specimens treated with alcoholic fixatives; this reduction is less pronounced when using an IQ System for the extraction. Results from typing of DNA extracted with phenol–chloroform from samples fixed in 10% formaldehyde were less significant after 24 h due to the lack of amplification in a few polymorphisms or to the presence of particular phenomes such as ''allelic drop-outs''. These results can appear less significant if compared with DNA extraction by IQ System, which permitted good typing using samples treated with other fixatives containing formaldehyde.

Similar results were obtained with specimens fixed with 2,5 glutaraldehyde in SSPE at 4 °C and 4% paraformaldehyde in SSPE at 4 °C; in these samples, a small amount of degraded DNA could be detected in 24 h. In samples fixed with a Bouin liquid, a certain amount of highly degraded DNA can be detected after 24 h. This data might be caused by picric acid.

DNA typing detected on samples treated with alcohol fixatives, using extraction with phenol–choloroform and with IQ System, has provided good and reliable results, also after two mounts and four mounts for Citoscreen™.

4. Discussion

Our data on quantitative and qualitative yield, on typing DNA extracted with fixing fluids containing formaldehyde, are confirmed by international scientific literature [4–6]. DNA structure is damaged by formaldehyde, which produces an interaction with hydrogen links, tissue nuclease, and with fixed histonic proteins, causing damage to polymerase

chain reaction (PCR). In our experience, and according to others, fixatives containing formaldehyde cause more damage on less marked DNA structures. The authenticity of the typing results must be based on the use of fixing fluid and on the DNA extraction method: IQ System seems quite convincing for samples treated with formaldehyde [7]. The series of result obtained suggest that fixatives with alcohol, and in particular with Citoscreen™, permit a better qualitative and quantitative conservation of DNA and an optimum typing with forensic genetic methods.

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