

## STR typing of fixed human tissue: formalin vs. an alcohol-based method

Sarah Gino\*, Simona Varacalli, Carlo Robino, Carlo Torre

*Laboratorio di Scienze Criminalistiche Dipartimento di Anatomia, Farmacologia, Medicina Legale,  
Università degli Studi di Torino C.so Massimo D'Azeglio 52, 10126 Turin, Italy*

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**Abstract.** In this study, we evaluated the effect on DNA typing of storage of human tissue in formalin versus an alcohol-based method (Complucad®). Samples of tissue were collected at different time from fixation. DNA was isolated and, after quantitation, was amplified by quadruplex PCR including four STR loci (LPL, F13B, FESFPS and F13A01). For alcohol-fixed tissue, quantitation showed that DNA yield remained unchanged regardless of storage time and complete profiles were obtained. Instead, the DNA amount recovered from those fixed with formalin was lower and DNA looked very degraded. Actually, for these samples, we observed a lack of amplification of >200 bp loci. © 2003 Elsevier B.V. All rights reserved.

*Keywords:* Tissue fixation; STR; Forensic casework; Paternity testing

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### 1. Introduction

DNA analysis of human tissues fixed for histopathological studies is not infrequently performed in forensic casework and paternity testing. However, it is well known that the most widely used fixative, formaldehyde, heavily interferes with PCR-based STR typing because of time-dependent degradation and cross-linking of DNA often leading to no results after fixation longer than 72 h [1], whereas it was observed that ethanol fixation is a most suitable method for preserving DNA integrity over time [2].

Considering the above, we evaluated the effect on DNA typing of storage ( $\geq 72$  h) of human tissue in formalin, in comparison with a commercially available fixative containing organic peroxides and alcohol (Complucad®) [3].

### 2. Materials and methods

Samples of brain, lung, heart, kidney and liver tissue were collected from five individuals at post-mortem examination. Before fixation, DNA was isolated from fresh brain tissue in order to obtain reference genetic profiles. Twenty-five milligrams of each

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\* Corresponding author. Tel.: +39-0-116707731; fax: +39-0-116707732.

*E-mail address:* sarah.gino@unito.it (S. Gino).

fixed sample were collected after 3, 7, 30 and 90 days of storage at room temperature for tissues in Complucad® and after 3 and 7 days for those in formaldehyde. DNA was extracted using spin columns (Macherey-Nagel), quantified spectrophotometrically and with the Quantiblot Human Quantitation Kit (Applied Biosystem) and then amplified by quadruplex PCR including the STR loci LPL, F13B, FESFPS and F13A01 (Promega). Amplification products were separated by capillary electrophoresis on the ABI PRISM™ 310 Genetic Analyzer (Applied Biosystems). Electrophoretic data were analysed using GeneScan™ software (Applied Biosystems).

### 3. Results and discussion

The results of quantitation showed that the DNA yield of Complucad® fixed tissues, though ranging from 5 to 35 µg according to the type of tissue, remained unchanged in each sample, regardless of storage time, up to 90 days. The total amount of DNA recovered from formalin-fixed samples was decidedly lower, varying between 6.25 and 25 ng after a fixation time of 7 days, with agarose gel electrophoresis showing clear signs of DNA degradation.

As for STR typing, complete profiles corresponding to fresh brain standards were obtained from tissues fixed with Complucad for 90 days. Profiles from tissues fixed with formalin for 7 days, though consistent with those from controls, showed lack of amplification of >200 bp loci (FESFPS and F13A01).

This preliminary study showed that Complucad®, an alternative alcohol based fixation method, allowed to isolate high molecular weight DNA—even after a long time—useful for genetic analysis in forensic casework and paternity testing.

### References

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