



DNA extraction assays from formalin fixed tissues: paternity testing from a 3-week-old embryo

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DNA forensic samples are generally critical in terms of quantity and quality. In a recent criminal case, we had to fingerprint a very early embryo (less than 4 weeks old) included in the chorion issued from the dead pregnant victim, to test paternity of two alleged fathers. Multiple difficulties appeared: (1) the selective dissection of the embryo (1 mm length) among the chorionic material; (2) the 6 months formalin treatment of the autopsic tissue; (3) the necessity to have most informative DNA profile (many loci) with minimum biological material. In this way, use of multiplex amplification kits was greatly recommended instead of several singleplex PCR.

Prior to the investigations on the critical sample, some assays were realised using autopsic test tissues as liver and cardiac muscle, formalin fixed for a long term, and on the chorionic tissue around embryo. Two extraction protocols were tested: Qiagen Tissues Protocol and an organic phenol-chloroform like technique after treatment of the samples in a GTE buffer to neutralise formalin excess. DNA was then quantified (Quantiblot) and typed using SGM+ and/or Identifiler multiplex PCR kits revealed on Capillary Electrophoresis with ABI 310 genetic analyser (Applied Biosystems).

Although significative DNA amounts have been detected from liver and cardiac tissue, no profile was obtained in STR fluorescence detection. However a complete profile was obtained from the chorion, providing mother fingerprint. So a tricky dissection of the chorion was attempted in order to remove the 1 mm long embryo. Several “suspected samples” were extracted, quantified and STR typed: some of them exhibited a partial profile mixture (10/15 STR markers) with a clear Y peak, the others showed a partial profile compatible with the mother DNA fingerprint.

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In conclusion, suitable DNA amplified fragments (<250 pb) were obtained in multiplex PCR from formalin fixed samples. In further assays, we try to type specifically Y STR on the samples issued from the “embryo-chorion” fragments. We also look forward to perform, on the questioned sample, singleplex PCR of the largest discriminant markers between the two alleged fathers.