



# Comparison of DNA preparation results from autopsy tissue specimens with or without previous postmortal CT angiography



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## ABSTRACT

During postmortal CT angiography the body is perfused with lipophilic contrast medium soluted in liquid paraffin. The present study investigated, whether molecular pathology investigations on autopsy tissues following this procedure are impaired or not. Heart tissue from fifteen cases following this type of CT scan as well as the same number of control cases matched for age, gender, pathological findings in the heart, time between death and autopsy, as well as fixation time of the samples were analyzed. DNA preparation was carried out employing a commercially available silica-column based system. The extraction results were quantified and real-time PCR was carried out using an assay for a point mutation frequently assessed in clinical pathology. Low and highly variable recovery rates of DNA were observed both in the study group as well as in the control group. The presence of liquid paraffin and lipophilic contrast medium did not interact with tissue fixation and the DNA preparation technique. Further, no differences were seen between the PCR results from the material with or without previous postmortal CT angiography. Hence, molecular pathology applications can be carried out successfully on autopsy tissues regardless of previous CT angiography.

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## 1. Introduction

Postmortal CT angiography is among the most rapidly developing fields in current forensic radiology [1]. Carrying out the procedures, lipophilic contrast medium soluted in liquid paraffin is administered through the arterial and venous branch of the vascular system. After completion of the CT scan, the contrast medium remains in the body leading to tissues completely saturated with liquid paraffin. It is commonly acknowledged, that the macroscopic results of forensic autopsy are not affected by this [2,3]. However, it has still not been finally excluded, that histological assessment as well as molecular pathology procedures on material from these autopsies might be impaired. In particular, interactions between the contrast medium and formalin solution used as a fixative for these samples has not yet been investigated.

## 2. Material and methods

Formalin-fixed, paraffin embedded tissue specimens from the heart of 15 autopsy cases carried out in the Institute of Forensic

Medicine Munich following postmortal CT angiography were selected for the study group. As a control group, 15 corresponding cases matched for (a.) the pathological conditions in the myocardium, (b.) the age and gender of the deceased, (c.) the time between death and autopsy, and (d.) the period of tissue fixation in formalin solution between autopsy and DNA preparation were retrieved from the archives of the Institute. A silica column-based extraction system especially designed for formalin fixed tissues (QIAmp DNA FFPE Tissue Kit, Qiagen, Hilden, Germany) was applied for the preparation of DNA. The effectiveness of DNA recovery was evaluated using a fluorescence based quantification technique (Qubit dsDNA HS Kit, Invitrogen, Darmstadt, Germany). The applicability of the extracted DNA for molecular pathology investigations was investigated employing a PCR assay for the BRAF V600E-mutation [4]. (Fig. 1).

## 3. Results

Successful preparation results, albeit with crucially lower recovery rates of DNA compared to extraction procedures from blood or frozen tissue samples, were seen in all specimens. Juxtapositioning the yields of DNA from material with or without previous perfusion of the body with contrast medium demonstrated no tendency towards lower or higher DNA concentrations

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**Fig. 1.** View of the opened heart in a case of postmortal CT angiography prior to forensic autopsy. Note the presence of reflective fluid on the surface which is liquid paraffin containing the contrast medium.

obtained from the material in which contrast medium had been applied (Table 1). Investigating the quality of the extracted DNAs using real-time PCR for the BRAF c.1799T>A p.V600E mutation showed positive amplifications using the wild-type primers and probe along with negative results when employing the mutant-type primers and probe system.

#### 4. Discussion

Low and highly variable recovery rates of DNA were seen in the preparations from tissues with or without previous contact to lipophilic contrast medium solution. The formation of secondary structures leading to impaired yields of DNA after the fixation of material in formalin solution is generally acknowledged [5]. Our findings indicated, that this process is not affected by the saturation of tissues with liquid paraffin prior to their exposure to the fixative. We further showed, that the lipophilic contrast medium itself also does not interact with tissue fixation and DNA recovery as well as with PCR amplification as a downstream application. Employing an assay for a point mutation frequently investigated in clinical casework, e.g., in the decision for particular cancer therapies [6–8], we were able to confirm that DNA extractions after forensic postmortal CT angiography is of

**Table 1**

Concentrations of extracted DNA in [ng/ml] from formalin-fixed paraffin embedded heart tissue sections from cases with (CM+) or without (CM–) previous postmortal CT angiography with contrast medium.

Case no.	CM+	CM–
1.)	84	33.6
2.)	44.7	11
3.)	40.1	15
4.)	52	29.5
5.)	20.6	12
6.)	24.9	20.3
7.)	19	17
8.)	15	17
9.)	20	22.9
10.)	15	35
11.)	14	39.9
12.)	34.4	61.7
13.)	14	48.4
14.)	33.4	78.9
15.)	19	69

similar quality compared to material in clinical molecular pathology.

#### 5. Conclusion

DNA prepared from archived tissues from autopsies following postmortal CT angiography is equally suitable for molecular pathology compared to samples without previous contact to contrast medium.

#### Conflict of interest

The authors disclose any financial or personal relationships with other people or organizations that could inappropriately influence their work.

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