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Review Article

A review of the optimisation of the use of formalin fixed paraffin embedded tissue for molecular analysis in a forensic post-mortem setting

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ABSTRACT

Molecular analyses in a post-mortem setting are becoming increasingly common, particularly in cases of sudden unexplained death, with the aim of identifying genetic mutations which may be responsible for causing death. In retrospective investigations, the access to suitable autopsy biological samples may be limited, and often formalin fixed paraffin embedded (FFPE) tissue is the only sample available. The preservation of tissue in formalin is known to damage DNA through crosslinking activity. This results in the extraction of severely fragmented DNA of variable yields, which subsequently reduces the ability to perform downstream molecular analyses. Numerous studies have investigated possible improvements to various aspects of the DNA extraction and amplification procedures from FFPE tissue and this review aims to collate these optimization steps in a cohesive manner. A systematic review was performed of three major databases, which identified 111 articles meeting the inclusion criteria. Five main areas for optimization and improvements were identified in the workflow: (1) tissue type, (2) fixation process, (3) post-fixation, (4) DNA extraction procedure and (5) amplification. It was found that some factors identified, for example tissue type and fixation process, could not be controlled by the researcher when conducting retrospective analyses. For this reason, optimization should be performed in other areas, within the financial means of the laboratories, and in accordance with the purposes of the investigation. Implementation of one or more of the optimization measures described here is anticipated to assist in the extraction of higher quality DNA. Despite the challenges posed by FFPE tissue, it remains a valuable source of DNA in retrospective molecular forensic investigations.

Contents

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1. Introduction

The accessibility to the genetic information of an individual is rapidly becoming easier and more affordable [1]. Due to these advances, the role of genetics in public health and medicine is becoming a focal area in research and amongst clinical service providers. One such emerging field is the role of genetic variants in sudden unexpected deaths of infants and adults, whereby the identification of mutations in decedents has been able to resolve the cause of death [2–5]. While many molecular autopsy studies recommend the use of blood samples [2,6], a recent study conducted by Bagnall et al. [7], identified that formalin fixed paraffin embedded tissue as a suitable biological sample for the performance of a molecular autopsy [7]. In light of recent advances of massively parallel sequencing methods, the authors also motivate for the use of exome-based sequencing analysis [7,8]. The concept of a 'molecular autopsy' has been investigated and has shown a potential resolution in up to 35% of cases which previously did not have a cause of death established by conventional autopsy [6].

The potential value of a molecular autopsy is that it can be implemented retrospectively, when cause of death could not be established at autopsy. However, the access and availability of suitable biological samples may be a limitation. In retrospective investigations, preserved tissue is often the only accessible biological sample. Tissue samples are typically collected for the purposes of histopathological analyses and thus undergo preservation processes, commonly formalin fixation and paraffin embedding, which are known to damage nucleic acids [9–11]. This poses complications for the type and number of downstream molecular analyses that can be performed [12].

An abundance of literature is available that discusses various mechanisms to overcome the effects of fixation. Two review articles have been published in 2011, which discuss the various DNA extraction and purification protocols that have been patented regardless of sample type [13], and also the effect formalin has on nucleic acids (DNA and RNA) and proteins [14]. In light of the improved resolution, cost and availability of high throughput molecular technologies, and the vast resources represented in archived preserved tissue, there is an urgent need to ensure that optimum methodologies to process such preserved tissue are at hand. The aim of this review article is therefore to collate the reported optimization amendments to improve DNA yield and quality extracted from FFPE tissue, with special attention on the processes of deparaffinization, DNA extraction and DNA amplification.

1.1. Legislation

In most countries, legislation governs the scope of medico-legal death investigation. For example, in South Africa, the Inquests Act 58 of 1959 mandates circumstances under which a death is to be investigated further [15], with particular attention to an unnatural manner of death. Unnatural deaths can be classified into four categories: (1) death due to the application of external force, (2) the act of omission or co-mission, (3) procedure related deaths, and (4) sudden unexpected death [16]. The type of investigation performed depends on the nature of the unnatural death.

Investigation to determine cause of death involves the performance of an autopsy. This may consist of an external and/or internal examination as well as ancillary examinations [17]. Such investigation entails the assessment of a selection, or all, of the internal organs, depending on the circumstances surrounding death and the availability of clinical history. At this time, various tissue samples, or whole organs, may be collected and submitted for further examination.

Ancillary examinations investigate the involvement of non-physical factors in death, including, but not limited to: infection, substances and aberrant molecular pathways. Ancillary examinations typically vary between countries and even different mortuaries [18], but often include histology, toxicology, virology and microbiological screening.

1.2. Molecular autopsies: concept and implications

A molecular autopsy usually refers to the performance of molecular tests in a forensic setting, to determine cause of death [19]. Molecular tests concentrate on evaluating the biological and more specifically, genetic contribution towards death and disease.

The use of a molecular autopsy has been shown to be especially valuable in cases of sudden, unexplained deaths (SUD) [2,19–21], where previously unresolved deaths have been attributed to a genetic, and specifically a pathogenic mutation. For example, Long QT Syndrome (LQTS) which can be difficult to detect macroscopically or microscopically during autopsy, has been diagnosed through genetic analysis, and been able to resolve the cause of death in numerous cases [22–25]. For example, Tester and Ackerman [6] identified genetic variants in 17 out of 49 SUD cases which were previously unexplained, thereby potentially resolving 35% of these cases [6].

The methods used in molecular autopsies can be applied to other types of deaths to provide information regarding cause and/or manner of death, which may have value for living relatives [20]. For example, Koren et al. [3] investigated the death of a healthy 13 day old male infant who presented with periods of lethargy and difficulty with breastfeeding. Due to the lack of visible anatomical anomalies, ancillary examinations were performed. Results indicated that the infant had an elevated blood level of morphine [3]. The mother of the infant reported that she was on a prescribed dose of codeine, a precursor drug of morphine. Genetic analyses performed on the mother found that she had a drug metabolism mutation that produced elevated levels of morphine, elucidating the true cause of death as 'opioid-toxicity due to breastfeeding', likely in an accidental manner [3].

Molecular autopsy methods therefore vary depending on what clinical information is available and what genetic information is required. If a genetic variant of known pathogenic function is investigated, targeted Sanger sequencing is preferred [26]. However, whole exome sequencing is a valuable genomic tool in understanding and characterizing sudden, unexpected deaths, where the candidate gene is unknown [20,22]. This allows for genetic variants of unknown pathogenicity to be identified as well