Long-term DNA survival in ethanol-preserved archival material

Mark Spigelman1*, Ian Barnes1,2*, John Holton1, Dino Vaira3, Mark G Thomas2

1Department of Medical Microbiology, The Windeyer Institute of Medical Sciences, London, UK
2The Centre for Genetic Anthropology, Department of Biology, University College London, London, UK
3First Medical Clinic, University of Bologna, Bologna, Italy

We have examined a number of ethanol-preserved specimens for the presence of both bacterial and human mitochondrial DNA (mtDNA). The samples were kindly provided by the trustees of the Hunterian Museum at The Royal College of Surgeons of England and were taken from John Hunter’s original specimens, having been stored in alcohol since removal in the 1750s. They consisted of specimens of gastric ulcers and cancers as well as specimens of carcinoma of the cervix. The museum has over 5000 such specimens and they were considered a potential source of informative DNA sequences, particularly from bacterial and viral DNA.

Preliminary work on the specimens showed the presence of recognizable micro-organisms in both touch preparations and histological sections. We identified by staining bacteria including diplococci suggestive of Neisseria gonorrhoea and believe we have found putative Helicobacter pylori on the gastric ulcer specimens under electron microscopy. The finding of whole bacteria in such uniquely preserved specimens encouraged us to consider the study of the genetic composition of a number of bacteria and to compare these with their modern day equivalents. A full report of this work can be found in Barnes et al.: we present here a summary.

Concentrating initially on H. pylori, the specimens were collected and treated with all precautions necessary to prevent contamination and control false positive and negative amplification. We developed 21 specific primers for H. pylori and first confirmed their specificity and efficacy on modern samples, obtained from patients who had been diagnosed as being positive for H. pylori infection. The H. pylori primers were designed to amplify a range of DNA fragment sizes. We also used two pairs of human mtDNA primers, PMT1 and PMT2, which amplify a 277 bp segment of the mtDNA control region, and RVM1 and RVM2 which amplify a 111 bp region between the COII and tRNA\(^{/w}\) genes.

Each of six Hunterian samples was extracted using four of the techniques commonly used in ancient DNA (aDNA) extraction, all of which have previously been used to recover DNA from archaeological and archival material. Each extraction was done on three separate occasions. PCR amplification of these DNA extracts was attempted using all the H. pylori primer pairs and the two human mtDNA primer pairs – a total of over 1000 amplifications. No amplification was observed with any of the H. pylori primers and no reproducible amplification was observed with any of the human mtDNA primer pairs. All PCR
amplifications were successful for a positive control of stomach biopsy DNA run concurrently in all PCRs. ‘Spiking’ of this positive control with the same volume of a museum extract did not lead to any reduction in amplification efficiency, suggesting that enzymatic inhibition did not occur.

As ethanol is widely used in molecular biology during DNA handling procedures, and since more recently ethanol-preserved material is routinely used as a source of DNA, these results seem surprising. There are several possible explanations for these results:

1. An insufficient number of samples have been tested to allow for the vagaries of preservation of biomolecules in this type of material. We would reject this suggestion on the basis that spirit storage, unlike the soil environment, is a fairly consistent and homogenous matrix for biomolecular preservation. Also, the fact that we were unable to consistently amplify human mtDNA indicates that H. pylori DNA would not be present, even under better preservation conditions.

2. The methodologies used were not suitable for the recovery of DNA from these specimens. We would refute this suggestion on the basis that: we have employed four different extraction protocols, all of which have been shown to recover DNA from archaeological and archival material. We have also employed a range of optimized PCR primers and conditions for both H. pylori and a range of amplicon sizes have been targeted.

3. DNA is present in the specimens, but cannot be PCR-amplified due to extensive base modification. Some chemical modification is likely over time periods but there is no evidence to suggest that this modification is more likely in ethanol-preservation than other environments and we would therefore reject this suggestion.

4. An absence of DNA in the specimens, caused by complete degradation of the DNA. Of the possible explanations for the lack of recovery of DNA, we would suggest this to be the most likely.

We believe that for these specimens several factors relating to long-term ethanol storage explain the apparent absence of DNA: (i) poor penetration of the tissue by ethanol would leave the interior of the specimen in an enzymatically active state, allowing nucleases enzymes to destroy DNA; (ii) cell wall disruption due to protein denaturation, allowing DNA to migrate from the specimen into the surrounding preservative medium (the ethanol preservative in these specimens is known to have been changed on a number of occasions, in order to dispose of lipid leached into the medium); and (iii) acid hydrolysis of the DNA due to the use (historically) of low quality ethanol. While purified DNA is extremely stable in ethanol, it appears that bulk specimens stored in ethanol for long time periods may show poor DNA preservation – the most likely explanations for this are chemically and biologically mediated degradation of the DNA, coupled with incomplete fixation of the component macromolecules.

There has been considerable interest in the recovery of pathogen DNA from specimens such as those in the Hunterian collection. Our data should not be taken as a suggestion that all of the samples held in the collection have poor DNA preservation. However, a knowledge of specimen history and curatorial practice are vital in determining the potential of museum-held material for genetic research. We would suggest that better samples for molecular research are likely to include ethanol preserved material which has been sealed since deposition, formalin-fixed material, dried soft tissues and skeletal material.

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References
