Extraction and amplification of DNA from archival haematoxylin and eosin sections and cervical cytology Papanicalau smears

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The clinical application of molecular biological techniques has been greatly facilitated by the use of archival pathological material. Extraction of DNA from paraffin-embedded blocks and fresh cervical cytology specimens has been described (1,2), however, these are not always available. We describe the use of archival haematoxylin and eosin (H&E) stained sections and Papanicalau (Pap) stained cervical smears for polymerase chain reaction (PCR) amplification.

The archival slide, a parallel newly prepared stained slide and an unstained 5 micron section from each of five stored paraffin-embedded blocks five, ten and twenty years old were used, as well as two 10 year old Pap stained, mounted cervical smear preparations. Coverslips were removed by soaking in warm xylene for two days, and DNA extracted as previously described (3). This was assayed quantitatively by fluorimetry, and qualitatively by 0.7% agarose gel electrophoresis (Fig. A). A standard PCR protocol was used to amplify a 120 bp fragment of the K-ras gene (4) from the extracted DNA. PCR products were analysed by 2% agarose gel electrophoresis (Fig. B).

Figure A DNA extracted from (2) archival Papanicalau smear (1969) (3)(6)(9) paraffin-embedded sections (3) 1969, (6) 1979, (9) 1984, (4)(7)(10) new H&E sections (4) 1969, (7) 1979, (10) 1984, (5)(8)(11) archival H&E sections (5) 1969, (8) 1979, (11) 1984. (1) λ DNA Hind III digest. Figure B PCR product. (1) and (7) 123 bp DNA standard (3)-(6) 120 bp fragment of K-ras gene amplified from DNA extracted from: (3) archival Papanicalau smear (1979), (4)(5)(6) archival H&E tissue section (4) 1969, (5) 1979, (6) 1984.

Better yields of DNA were extracted from archival H&E stained tissue sections (mean 1.71 μ g/section), than either a stained or unstained section newly cut from the block. Good yields were also extracted from Pap stained cervical smears (mean 5.17 μ g/section). DNA showed a range of molecular weight, the smears particularly produced larger fragments (> 20 kbp) (Fig. A), suitable for detection of a single copy gene by PCR (Fig. B). PCR of H&E sections widens the source of archival pathological material, whereas PCR of old Pap smears makes available genetic information on millions of individuals for molecular epidemiology, forensic PCR fingerprinting and study of the biology of cervical neoplasia.

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