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Extraction of DNA from formalin-fixed paraffin-embedded pathology specimens and its use in hybridization (histo-blot) assays. Application to the detection of human papillomavirus DNA

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To identify specific RNA or DNA targets in pathology specimens that have been formalin-fixed and embedded in paraffin, the only method available is that of in-situ hybridization (1). This is both time consuming and technically demanding. We investigated the possibility of extracting DNA from blocked tissue and then using the much more rapid and simple membrane dot-blot assay. Sections (3μm)were removed from paraffin-embedded specimens until approximately 0.3 - 0.4ml packed volume was collected in the bottom of a 1.5ml microcentrifuge tube. After two xylene extractions (1ml xylene, vortex 15 sec, centrifuge 5 min at 10,000xg), and two ethanol washes (1ml each), the preparations were dried (37°C, 2hr) and resuspended in 200μl of digestion buffer (50mM Tris-Cl, 5mM EDTA, 1mg/ml Proteinase K, pH 8.0). During incubation for 2 - 4 hr at 37°C, the tissue was dispersed by sonication (5 x 10s bursts). Samples (10μl) were spotted onto nylon (Zeta-probe, BioRad) membranes and the DNA covalently bound using acid and alkali treatments according to the manufacturers instructions.

The results of probing extracted tissue DNA with ^{32}P-labelled human papillomavirus DNA is shown in the figure below. Strong and type-specific signals were observed from tissue up to 13 years old. Such "histo-blots" are stable; can be successively probed many times and provide useful and rapid information for later in-situ hybridization studies. The method can be applied to large scale retrospective studies.

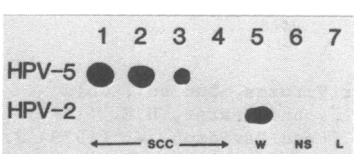

Fig. 1. Histoblot hybridized at high stringency with ^{32}P-labeled type 5 (top row) and type 2 (bottom row) HPV DNA. Specimens; 1-4; four different squamous cell carcinomas of the skin, 5; skin wart, 6; normal human skin, 7; normal human liver. All specimens had been fixed and embedded. Autoradiography was for 4 days.

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REFERENCES

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