tissues also. No proteolytic enzyme predigestion of sections is required. This can sometimes affect the quality of the sections. However, a brief period of processing in a microwave oven is needed. This is a critical and essential step which has to be carried out in the precise manner described. The results obtained with 1D5 were more intense, but otherwise comparable with those obtained with ER-ICA antibody when used on paraffin wax sections.

Although the staining results were less intense than those obtained with frozen sections, paraffin wax sections have the obvious advantage of using routinely processed tissue and they provide sections of higher quality which are easier to interpret.

The semiquantitative scoring system used is simple and reproducible. Its results correlate well with the quantitative estimates obtained with DCC. The scores can provide an estimate of the level of ER in a given tumour and whether it is absent, low, moderate, high or very high which may be of value to oncologists.

This study was partly supported by a grant from Dako Ltd.

1 Scottish Cancer Trials Breast Group and ICRF Breast Unit, Guy's Hospital, London. Adjuvant ovarian ablation versus CMF chemotherapy in premenopausal women with pathological stage II breast carcinoma: the Scottish trial. Lancet 1993;341:1293-8.

**Prevalence of Epstein–Barr virus in the cervix**

Y Taylor, W T Melvin, H F Sewell, G Flannelly, F Walker

**Abstract**

Cervical smears from 327 women were examined using the polymerase chain reaction (PCR) targeted to a sequence in the *Bam* H1 W region of the Epstein–Barr virus (EBV) to determine the prevalence of the virus in the cervix. EBV was detected in 131 (40%) of the 327 women. Of the 235 women with normal cytology, 98 (42%) were positive. Of the 92 women with dyskariotic smears, 33 (36%) were positive.

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Epstein-Barr virus (EBV) is a human herpes virus which is associated with a variety of neoplasias such as Burkitt's lymphoma and nasopharyngeal carcinoma. The reasons for this association are poorly understood, but the virus can transform cells bearing the EBV/C3d receptor and make them receptive to other oncogenic events.

In almost all healthy EBV antibody positive people the major site from which the virus is shed is the oropharynx, and viral replication seems to be restricted to squamous epithelial cells. With the recognition that the uterine cervix is another site where the virus is shed and can replicate, the role of EBV in cervical neoplasia requires elucidation. As an initial step it was decided to establish if EBV is commonly present in the cervix and whether there is any difference in its prevalence in cytologically normal and cytologically dyskariotic cervices.

**Methods**

The study population of 327 was drawn from the Grampian call/recall cervical screening programme. It comprised 235 women (mean age 33-4, range 17-67 years) with normal cervical smears attending family planning and general practitioner clinics, and 92 women (mean age 31-8, range 18-62 years) with dyskariotic smears attending colposcopy clinics. Ninety of the latter group had confirmation of squamous epithelial abnormalities in biopsy specimens taken subsequent to the smears.

Cervical smears collected with a spatula were collected into phosphate buffered saline and stored at −20°C until DNA extraction. All DNA samples were subjected to an amplification reaction directed to a portion of the β globin gene to validate the quality of the extracted DNA.

The polymerase chain reaction (PCR) was targeted to a sequence in the *Bam* H1 W region of the EBV genome. Reactions were carried out in 50 µl volumes containing 50 mM K Cl, 10 mM TRIS-HCl (pH 8·3), 1 mM MgCl2, 0-01% gelatine, 200 µM each dNTP (dATP, dGTP, dCTP and dTTP),
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1·0 μM each primer, 2·5 units Taq enzyme and 0·1 μg DNA. Reaction mixes were overlayed with mineral oil and amplifications performed in a Perkin Elmer thermal cycler. After an initial denaturation step of five minutes at 94°, 30 cycles were performed at 94° for one minute and 59° for 30 seconds (2nd stage only), with a final seven minute extension at 72°C. PCR products were visualised under ultraviolet light on 1·5% agarose gels containing ethidium bromide. The specificity of the reactions was confirmed by Southern blotting and, in some instances, by sequencing of cloned PCR products. Each sample was amplified in duplicate.

Results
Overall, 131 (40%) of the 327 women had EBV in their cervical smears. Of the 235 with normal smears, 98 (42%) were EBV positive. Of the 92 with dyskariotic smears, 33 (36%) were EBV positive.

Discussion
These results show that the cervix frequently harbours EBV. The actual prevalence figures compare with five (18%) EBV positive results, from 28 women, originally reported as of undetermined cytological status, and 12 (33%) from 36 women with cervical epithelial abnormalities. The former study used less sensitive techniques of cell transformation and in situ hybridisation: the latter study, corresponding to our own dyskariotic group, used PCR.

The source of the EBV is not determinable from our study but there are three main possibilities. The virus could be shedding in its cell free infectious form; the scrape might contain lymphocytes and those with the EBV/C3d receptor could well harbour the virus; and cervical squamous epithelial cells can replicate EBV infection. None of these possibilities is exclusive.

There is a compelling body of evidence that cervical neoplasia, especially cervical intraepithelial neoplasia (CIN), is related to viral infection. The questions of which virus is, or which viruses are, involved remain unanswered, but EBV is unlikely to act alone because its prevalence in normal and abnormal cervixes is similar. However, EBV is certainly a candidate in a multiviral hypothesis. In hairy cell leucoplasia of the tongue a lesion with a histological resemblance to cervical dysplasia, there is sound evidence that both EBV and human papilloma virus (HPV) are implicated. HPV of various types is very common in the cervix. Synergism between EBV and HPV is a possibility, perhaps by way of the former expressing the BCRF1 gene, which codes for an interleukin-10 (IL-10)-like molecule, which can modulate the immune reaction to both viruses. This merits further investigation.

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