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Human papilloma virus detection by in situ hybridisation signal amplification based on biotinylated tyramine deposition

The greatest advantage of non-isotopic in situ hybridisation (NISH) over PCR amplification, for the detection of human papilloma virus (HPV), is a combination of morphological preservation with cellular localisation. Hence, the description of a novel NISH signal amplification method, using catalysed reporter deposition (CARD-ISH) is especially pertinent for HPV DNA detection in paraffin wax embedded sections. This is particularly useful for the detection of low copy number HPV DNA below the threshold of conventional NISH methods. Using the CARD-ISH amplification system, Poddighe et al demonstrate 1–3 integrated copies of HPV DNA in SiHa cells producing distinct intranuclear punctate/dot signals. This is undoubtedly far more sensitive than the usual detection threshold of viral copies per cell with NISH.

However, Poddighe et al also used their amplification system for detecting about 500 integrated copies of HPV16 DNA in CaSkI cells and 20–50 integrated copies of HPV18 DNA in HeLa cells. The resultant amplified signal with CARD-ISH in both these human cervical carcinoma cell lines is a blotchy, lumpy intranuclear signal. This latter signal destroys the distinctive punctate/dot signal that can easily be demonstrated with conventional NISH methodology. The significance of the punctate/dot-like signal (type 2 NISH signal pattern) is that it denotes an integrated physical state of HPV DNA in cervical neoplasia; adding a third advantage of NISH over PCR, other than morphological preservation and cellular localisation. Hence, it would appear that CARD-ISH would destroy this valuable signal pattern in a cell containing more than 20–50 integrated viral copies. The type 2 NISH signal pattern, already validated by three independent laboratories, is a simple technique for the detection of integrated HPV DNA in cervical neoplasia. The use of the CARD-ISH system could therefore be useful only for producing and preserving this signal pattern in low copy number HPV DNA.

Another unusual phenomenon illustrated by Poddighe et al is a cervical lesion with HPV DNA demonstrated by NISH positive/PCR negative. Although the highly sensitive general primer (GP5+ and GP6+) mediated PCR directed towards the L1 open reading frame was used, it nevertheless failed to demonstrate HPV DNA. This phenomenon of NISH positive/PCR negative HPV DNA in clinical samples has been demonstrated previously. Conventional NISH requires approximately 12 viral copies per cell for detection of HPV DNA in paraffin sections. In contrast, it has been estimated that PCR requires at least 1000 viral copies per cell in a paraffin wax embedded clinical sample for amplification of the virus. This extends further the advantages of NISH over PCR. The alternative explanation for the NISH positive/PCR negative cervical lesion in CaSkI cell lines is that CARD-ISH study may be related to the integration of HPV16 DNA in this case with disruption of L1 ORF, preventing amplification with the GP5+/GP6+ PCR system that targets the L1 gene. Although the L2 gene is consistently disrupted with HPV DNA integration into host chromosome, the L1 gene may also occasionally be interrupted. In such instances, the use of PCR systems directed towards L6 may be more useful for the demonstration of the HPV DNA.

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