Malaysian J Pathol 1992; 14(2): 69 – 76

REVIEW

In *situ* hybridisation: principles and applications

Lai Meng LOOI MD, MRCPath and Phaik Leng CHEAH MPath, MRCPath

Department of Pathology, Faculty of Medicine, University of Malaya, Kuala Lumpur.

Abstract

In situ hybridisation (ISH) is based on the complementary pairing of labelled DNA or RNA probes with normal or abnormal nucleic acid sequences in intact chromosomes, cells or tissue sections. Compared with other molecular biology techniques applicable to anatomical pathology, ISH enjoys better rapport with histopathologists because of its similarity to immunohistochemistry. It has the unique advantage over other molecular biology techniques - largely based on probe hybridisation with nucleic acid extracted from homogenised tissue samples - of allowing localisation and visualisation of target nucleic acid sequences within morphologically identifiable cells or cellular structures.

Probes for ISH may bear radioactive or non-radioactive labels. Isotopic probes (^{3}H, ^{32}P, ^{35}S, ^{125}I) are generally more sensitive than non-isotopic ones but are less stable, require longer processing times and stringent disposal methods. Numerous non-isotopic labels have been used; of these biotin and digoxigenin are the reporters of choice. Optimised non-isotopic systems of equivalent sensitivity to those which use radioactwe-labelled probes have been described.

In ISH, finding the optimal balance between good morphological preservation of cells and strong hybridisation signals is crucial. Tissue fixation and retention of cytoskeletal structures, unfortunately, impede diffusion of probes into tissues. ISH sensitivity is also influenced by inherent properties of the probe and hybridisation conditions.

Although ISH is largely a research tool, it is already making strong inroads into diagnostic histopathology. It has been applied for the detection of various infective agents particularly CMV, HPV, HIV, JC virus, B19 parvovirus, HSV-1, EBV, HBV, hepatitis delta virus, *Chlamydia trachomatis*, salmonella and mycoplasma in tissue sections. Besides detection of infective agents, it also allows localisation of sites of infection, elucidation of mechanisms of virus transmission and dissemination and investigation of the link between viral agents and cancer. Sex typing, localisation of genes on chromosomes and detection of structural and numerical chromosomal changes in tumours are among the other applications of ISH.

Key words: In situ hybridisation, molecular probes, technology.

INTRODUCTION

The use of molecular probes in anatomical pathology (encompassing histopathology, cytopathology and electron microscopy) is based on the hybridisation of labelled DNA and RNA probes with nucleic acid sequences in samples of diseased tissues. The main techniques are (1) *in situ* hybridisation (ISH), where labelled probes are used to detect complementary DNA or RNA in tissue sections or smears, and (2) examination by Southern or other blotting techniques of nucleic acids extracted from homogenised biopsy material. Of these ISH enjoys better rapport with histopathologists because of its similarity to immunohistochemistry. First described in 1969,[1,2,3] its developmental stage was largely in the 1980s when recombinant DNA technology was applied to overcome the early problems of probe preparation. ISH has the unique advantage over other molecular biology techniques of allowing localisation and visualisation of target nucleic acid sequences within morphologically identifiable cells or cellular structures in a heterogenous cell population. Such specific localisation has a marked advantage over older molecular methods where detection of the nucleic acid target from a tissue homogenate precludes identification of the actual affected cell, appreciation of cellular detail and relations and subse-

Address forcorrespondence *and reprint* requests: Professor L M. Looi, Department of Pathology, Faculty of Medicine, University of Malaya, 59100 Kuala Lumpur, Malaysia.

quent reconstructive interpretation of the findings.

From the practical view point, the most important advantages of ISH to the histopathologist running a service laboratory are (1) the simplicity of its methodology, (2) the specificity of results obtained, (3) ease in interpretation of findings and (4) its applicability on tissue sections (frozen or formalin-fixed, paraffin-embedded) and smears without the need for special specimen collection or processing. ISH has the promising potential of becoming as "routine" as an immunohistochemical stain, especially when nucleic acid probes become commercially available and techniques for detection in formalin-fixed and paraffin-embedded tissue are perfected.

IN-SITU HYBRIDISATION

Probes and labels

Probes currently available for ISH include double-stranded DNA (dsDNA), single-stranded synthetic oligonucleotides, single-stranded DNA (ssDNA) and single-stranded complementary RNA (sscRNA).

Reporter molecules can be radioactive or non-radioactive. Radioactive reporters include ^{3}H, ^{32}P, ^{35}S, and ^{125}I. Of these, ^{3}H is the most well established for ISH work. Because of its short track length in photographic emulsions, it allows precise localisation of signals to cellular details and produces high resolution autoradiographs. However, its low beta energy dictates long exposure times. In recent times, ^{35}S has emerged as a good alternative. ^{32}P is a readily available label but is too penetrating for good ISH results.

The most frequently used non-isotopic reporters are biotin (vitamin H of B, complex) and more recently, digoxigenin (aglycone derivative of digoxin).[4] Radioactive probes are generally more sensitive than non-radioactive ones, but suffer from the distinct disadvantages of poorer stability, requirement of longer processing times and more stringent waste disposal procedures (Table 1). Marked improvement in the sensitivity of non-radioactive probes has been achieved in recent times and their use is fast gaining popularity in histopathology.[4,5] Nevertheless, radioactive labels are still the reporters of choice for detection of low-copy-number sequences and particularly in the case of cRNA probes for the detection of low-copy-number mRNA.

Problems unique to ISH

In ISH, finding the optimal balance between good morphological preservation of cells and strong hybridisation signals, is crucial. This poses a problem unique to ISH. Because cells and even cellular structures have to remain intact and identifiable morphologically, tissue preservation, usually through the use of a fixative such as formalin or paraformaldehyde, is necessary. Fixation, however, impedes diffusion of probes into cells and causes cross-linkages among nucleic acids and proteins, effectively reducing accessibility of probe to target. The use of frozen sections obviates the need for fixation but compromises morphological clarity. Notwithstanding the problems posed by fixation, the mere retention of cell membranes and cytoskeletal structures already impede diffusion of probes into tissues, so that the preservation of cellular integrity is a factor directly opposed to hybridisation efficiency. To promote penetration into tissues, short probes (e.g. about 50 to 300 bases) are more suitable.[6] In ISH, pretreatment of tissues with detergent or proteinase is also a standard procedure to unmask DNA/RNA and improve probe penetration.

In practice, formalin fixation and paraffin embedding will allow the detection of DNA and mRNA in high copy numbers and this is suitable for most clinical specimens. However, the detection of mRNA would require that tissue be fixed or frozen as soon after excision as possible since mRNA is in a more dynamic physiological state than DNA, is steadily synthesised and degraded more readily. The preferred probe for localising mRNA is an "antisense" cRNA because of the high thermal stability of RNA-RNA hybrids. In addition, background reaction can be readily reduced by RNAase digestion of unhybridized probes. Finally, the use of a labelled "sense" strand provides a negative control system.

TABLE 1: Comparison of radioactive and non-radioactive probes

	Radioactive	Non-radioactive
Stability:	Weeks	Months-years
Exposure:	Days-weeks	Hours
Sensitivity:	+++	++
Resolution:	++	+++
Washing procedure:	Hours	Minutes
Waste disposal:	Stringent	Simple

ISH sensitivity and specificity are also influenced by inherent properties of the probe and hybridisation conditions. **Properties of probes** to consider are: (1) *probe construct:* oligonucleotide probes are better than traditional probes based on cloned DNA segments because of high specificity, single-strandedness and short probe length (10-50 nucleotides), (2) *efficiency of labelling:* labelling by random priming (using the Klenow fragment of DNA polymerase I on heat-denatured DNA) has been reported to be more efficient than nick translation (nicking of DNA with DNAase I and incorporation of nucleotides by DNA polymerase I), although both methods can be adjusted to yield probes of suitably short lengths, (3) *percentage of* GC *base pairs:* the higher the content of GC pairs, the higher the Tm (melting temperature), (4) *RNA versus DNA probes:* the strength of the probe-target bond decreases in the order of RNA-RNA, DNA-RNA, DNA-DNA, *(5) probe length:* the shorter the probe, the better its penetration into cells, *(6) signal detection systems:* autoradiography for radioactive labels is reputed to be more sensitive than the immunoenzyme systems. **Hybridisation** reactions are influenced primarily by the following conditions: (1) pH, (2) temperature, (3) salt concentration, (4) concentration of formamide, and (4) stringency of post-hybridisation washes.

ISH technique

The technique of ISH is well documented in scientific literature[4,6,7] and readers are referred to them for details. Only the general principles will be considered here.

Pretreatment

Tissue sections must adhere well to specially treated glass slides to avoid loss of tissue during the hybridisation process. Various "adhesives" are available including poly-L-lysine, gelatin chrome alum, and aminopropyltriethoxysilane (TESPA). Following adhesion of the tissue section on to the slide, the target nucleic acid sequence must be made accessible to the molecular probe, this procedure being especially important with paraffin-embedded sections. "Unmasking" requires dewaxing the section and treatment with various enzymes and detergents. The potency of different enzymes varies from type to type and for a single enzyme, manufacturer to manufacturer. Hence the enzyme dilution must be individually optimised for any experimental system. In our laboratory we use hydrochloric acid, Triton X-100 and proteinase K to increase probe accessibility.[8]

Hybridisation

Molecular hybridisation is the process whereby a single-stranded target sequence is annealed to a complementary single-stranded probe to form a double-stranded hybrid. Prior to hybridisation, both the target and the probe, if double-stranded, must be denatured to render them single-stranded and this can be achieved by heat or alkali treatment. Following denaturation, the single-stranded target and probe sequences are incubated in a hybridisation mixture, which provides an optimal environment for re-annealing of single-stranded sequences.

In general, reactions carried out at low temperatures and in the presence of a high salt and low formamide content of the hybridisation mixture result in low specificity. At low stringency conditions, a probe may bind to a target sequence with only 70-90% homology and this results in non-specific hybridisation signals. The manipulation of salt and formamide concentration has a predictable effect on hybridisation of closely homologous sequences and optimal stringency for a particular system should be determined by experiment.

Specificity control is achieved by varying the conditions under which hybridisation is carried out. In addition, the following checks can be carried out:

1. Running the procedure without the labelled probe to check for non-specific reactions.
2. Hybridisation of probe to extracted defined DNA or RNA in a dot blot system.
3. Hybridisation of probe to positive control sections or cells (e.g. HeLa cells for HPV18, CaSki cells for HPV16, Raji cells for EBV) should yield positive results.
4. Prehybridisation ("preabsorption") of probes with specific cDNA or cRNA should yield negative results.
5. Hybridisation of sections with sense RNA should yield negative results in the detection of mRNA.
6. Pretreatment of sections with DNAase or RNAase, whichever is relevant, should yield negative results with the DNA or RNA probe respectively.

7. Hybridisation of the section with non-specific vector sequences and irrelevant probes should yield negative results.

Post-hybridisation washes

Stringency washes after hybridisation aims at decreasing non-specific binding. However, it is preferable to hybridize stringently rather than wash stringently.

Detection

Various methods are available for visualisation of the hybridisation reaction depending on the type of probe label used.

Reactions using radioactive labelled probes are detected by autoradiography. This is based on the emission of fast-electrons or beta particles from the probe. Beta particles release a large amount of energy when they collide with atoms of an emulsion added to the section on the slide. The excessive energy released reduces ionic silver present in the emulsion to metallic silver. When this happens, a faithful record of the location of the collision between an electron and the silver ions in the emulsion is produced in the form of a latent image. This image, when visualised is the indicator of the probe location in the tissue or cell.

In practice, the slide (bearing the probed tissue section) is dipped or coated in photographic emulsion (e.g. Ilford *G5*, K5, L4 or Kodak TB2). An emulsion thickness of less than 5 um is best for good resolution. The slide is stored in the dark for an optimal period of time (e.g. 6 days for ^{35}S-labeled RNA probes)[6] to allow the formation of a latent image, which is then developed and fixed by normal photographic procedures.

Biotin-labelled probes can be detected by (1) avidin-based systems which exploit the natural affinity of avidin for biotin or (2) specific antibodies to biotin. An antibody to digoxin is used to detect digoxigenin-labeled probes based on the cross-reaction between anti-digoxin antibodies and digoxigenin. Single-step and amplified detection systems are available. In single-step detection, an indicator enzyme (such as alkaline phosphatase or horseradish peroxidase) is linked directly to the primary antibody or avidin and reacted against its substrate (nitroblue tetrazolium (NBT)/ S-bromo-4-chloro-3-indolyl phosphate (BCIP) for alkaline phosphatase and diaminobenzidine for horseradish peroxidase) resulting in a coloured product. A variety of reactions can be used to amplify signals and will not be detailed here. A digoxigenin-labelled ISH system for HPV is illustrated in Figs. I and 2.

MULTIPLE ISH

More than one probe can be applied to the same tissue section to detect different nucleic acid targets. By using different detection systems with each probe, resulting in different colour end products, visualisation of the different nucleic acid targets can be achieved.[9]

POLYMERASE CHAIN REACTION ISH

The advent of the polymerase chain reaction (PCR) has revolutionised molecular biology. By amplifying low copy number nucleic acid sequences, it allows detection of targets which would otherwise be undetectable by conventional techniques. Although PCR is generally applied to tissue homogenates, *in situ* amplification of target sequences with subsequent ISH detection is feasible and is being carried out in some laboratories.[10] It is evident that the advantages offered by this method over amplification and detection in tissue homogenates are similar to that of ISH over the various blotting techniques.

APPLICATIONS

Although ISH is largely a research tool in histopathology, it is already making strong inroads into the routine histopathology laboratory, especially in the diagnosis and pathophysiology of viral diseases. It has been applied for the detection of cytomegalovirus, human papilloma virus, human immunodeficiency virus, JC virus, B19 parvovirus, HSV-1, Epstein-Barr virus, hepatitis B virus, hepatitis delta virus, measles virus, *Chlamydia trachomatis*, salmonella and mycoplasma. It has also been applied to karyotype preparations to study chromosomal changes in tumours and viral infections. Demonstration of mRNA transcripts in cells is indicative of active protein synthesis and is a useful adjunct in elucidating the pathophysiology of disease. In general, the applications of ISH in histopathology can be categorised as follows:

Infective agents

Detection (determination) of infective agent

This is based on the detection of the infective agent's genome in the tissues or cells studied. Besides the facilitation of clinical diagnosis,

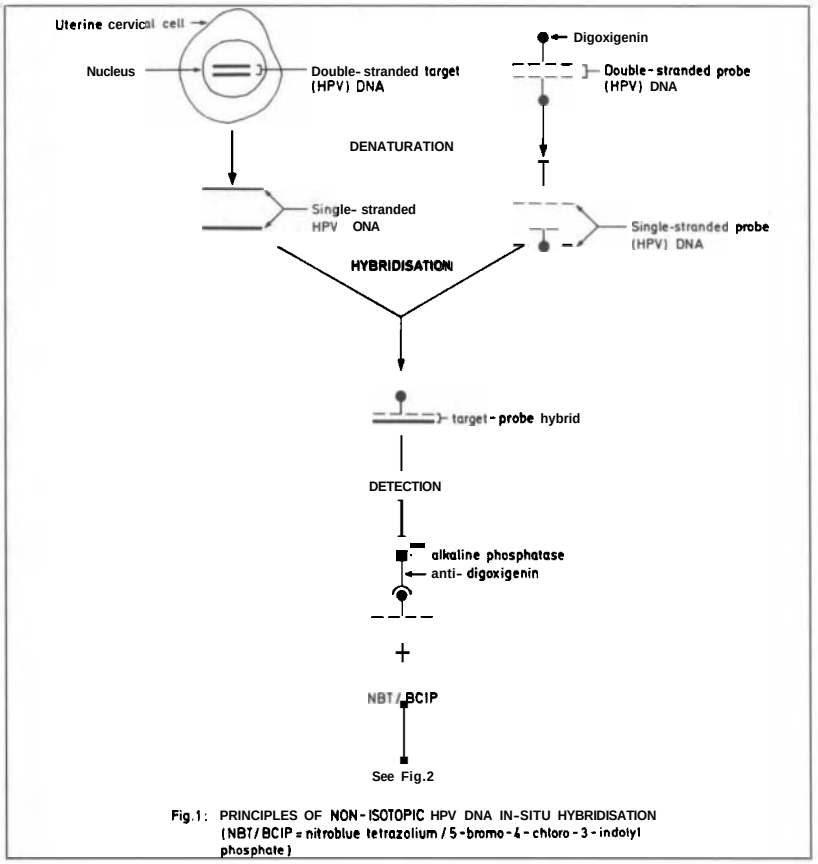

FIG. 1: Principles of non-isotopic HPV DNA *in situ* hybridisation. (NBT/BCIP = nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate)

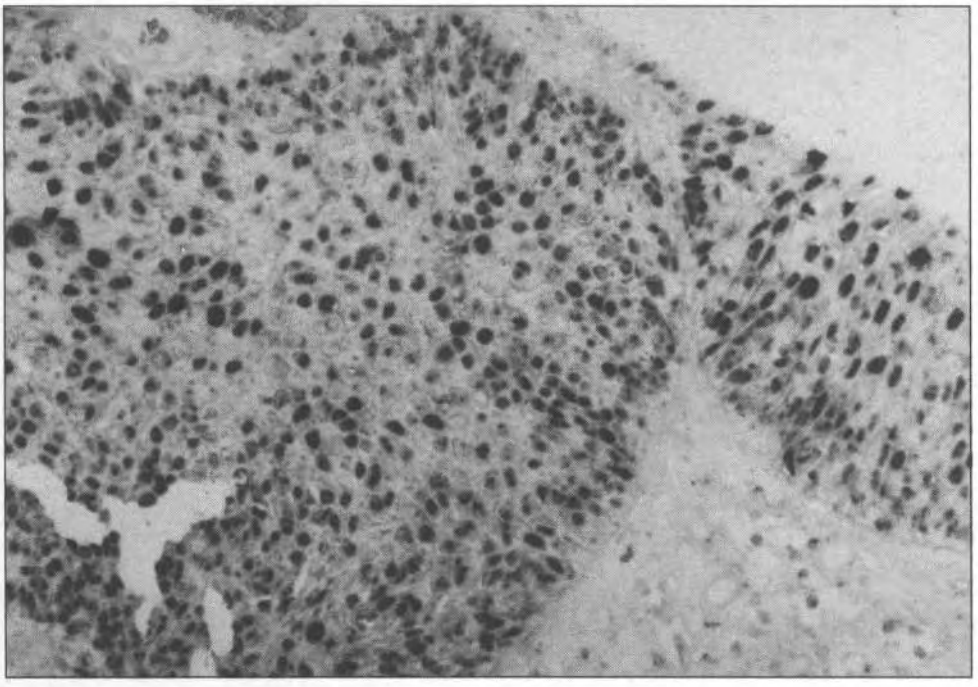

FIG. 2: Positive HPV nuclear signals in epithelial cells of a laryngeal papillorna. ISH using digoxigenin-labelled DNA probe against HPV6 and NBT/BCIP detection system. X300.

specific typing of infective agents also have important implications for epidemiological surveys and outbreak investigations."

Localisation of active infection

The actual cell or cell structures harbouring the infective genome can be elucidated by ISH e.g. HBV in hepatocytes, parvovirus in cells of the lung.[12] Tissue trophism exhibited by specific viruses can also be appreciated e.g. HBV for hepatocytes, measles virus for neurons.

Elucidaiion of mechanism of virus dissemination and transmission

Natural horizontal and vertical transmission routes of viruses can be studied. For example, the presence of EBV in epithelia1 cells of the oropharynx provides a means for transmission of the virus through saliva. ISH has been used to elucidate the mode of transmission of CMV from cell to cell, dissemination of JC virus from somatic cells to the brain and dissemination of Varicella Zoster virus through the central nervous system.[13]

Localisation of persistent virus infection

Examples are the persistence of JC virus in oligodendrocytes in progressive multifocal leukoencephalopathy and measles virus in neurons and glia cells in SSPE.[14,15]

Link between virus agents and carcinogenesis

This is a widely expanding area of active research and clinical interest, centred largely on the aetiological role of various viruses in cancers and the mechanisms of malignant transformation of cells. The better known associations are: EBV and nasopharyngeal carcinoma and B cell lymphomas,[16] HBV and hepatocellular carcinoma[17] and HPV and cervical carcinoma.[18]

Elucidation of peptide gene expression

ISH has been applied using cRNA probes for detection of mRNAs coding for regulatory peptides in the neuroendocrine system. These investigations have provided some insights into the functional status of tumours and endocrine cells.[19,20]

Study of cell development

ISH detection of cell-type specific RNA in cells which do not exhibit morphological differentiation can be applied to identify the cell type. In cell developmental studies, the identification of the same RNA in developmentally early and late cells implies the former is a progenitor of the latter. ISH has also been employed in the study of genes involved in body pattern formation of *Drosophilia*. In *Drosophilia*, it has been shown that the cell phenotype governed by some RNA expressions are not so much involved in determining cell differentiation but rather how cells interact with other gene products in body pattern formation.[21]

Sex determination

The Y chromosome can be detected through hybridisation with the labelled pHY2.1, a Y-chromosome-specific repeat. This provides the means for antenatal sex determination, designation of sex in the phenotypically indeterminate and monitoring of donor and recipient cells in bone marrow transplantations when donor and recipient are of opposite genders.[22,23]

Human gene mapping

Using the technique of ISH, hybridisation of labelled probe to metaphase spreads allows direct visualization of genes on human chromosome. In addition, hybridisation under low stringency conditions also allows identification of closely homologous nucleic acid sequences of gene families. The localization of genes on chromosomes has important implications in the study of how genes are affected in hereditary diseases and cancer.

Interphase cytogenetics

ISH can be used to detect numerical chromosomal abberations in interphase nuclei. Probes recognising highly repetitive sequences in chromosomes 1, 7, 8, 9, 10, 15, 16, 17, 18, X and Y are now available and these can be used in the study of numerical changes of those chromosome. Structural changes involving deletions and translocations may also be identified by selectively staining whole chromosomes. Such analyses have been carried out in breast cancer,[26] neuroectodermal tumours[27] and bladder tumours[28] and may be useful in correlation with prognosis and progression of neoplasia.

CONCLUSION

While ISH using radioactive probes and other molecular biology techniques which require nucleic acid extraction from tissues continue to be important research tools, ISH using non-

radioactive probes, is a technique which we foresee will become increasingly important as a diagnostic tool. It has the advantage of topographic preservation which permits a more rational reconstructive interpretation of the disease process but avoids the inconvenience of working with radioactive labels. In work dealing with HPV, optimisation of probe designs, labels, hybridisation procedures and amplification processes e.g. PCR have increased sensitivity such that detection of a single viral genomic copy is feasible.[9] However, the pathological significance of such low viral copy numbers can be questioned and underlines the irony of such sensitive technology.

Currently, ISH suffers from one major setback - the limited availablitity of probes, which hampers its use for routine purposes. However, in time to come, more probes should become commercially available and ISH may become as commonly used in daily diagnostic work as immunohistochemistry.

ACKNOWLEDGEMENT

We thank Professor I. Lauder, University of Leicester, United Kingdom for his advice.

REFERENCES

1. Buongiorno-Nardelli M, Amaldi F. Autoradiographic detection of molecular hybrids between rRNA and DNA in tissue sections. Nature 1969; 225: 946-7.
2. Gall G, Pardue ML. Formation and detection of RNA-DNA hybrid molecules in cytological preparations. Proc Natl Acad Sci 1969; 63: 378-81.
3. John HL, Birnstiel ML, Jones KW. RNA-DNA hybrids at the cytological level. Nature 1969; 223: 912-3.
4. Morris RG, Arends MJ, Bishop PE, Sizer K, Duvall E, Bird CC. Sensitivity of digoxigenin and biotin labelled probes for detection of viral genomes. J Clin Pathol 1990; 43: 800-5.
5. Syrjanen S, Partenen P, Mantyjarvi R, Syrjanen K. Sensitivity of *in situ* hybridisation techniques using biotin and ^{35}S-labelled human papillomavirus (HPV) DNA probes. J Virol Methods 1988; 19: 225-38.
6. Polak JM, McGee JOD. (Eds). *In situ* hybridization: Principles and Practice. Oxford: Blackwell Scientific 1990.
7. Warford A, Lauder I. In *situ* hybridisation in perspective. J Clin Pathol 1991; 44: 177-81.
8. Cheah PL, Looi LM, Tan KL. Non-isotopic *in situ* DNA hybridisation technique for detection of human papillomavirus. TMS-5. Technical Aid Report of the IRPA Tumour Marker Studies Project 3/ 31/01, University of Malaya 1991.
9. Nederlof PM, Van der Flier S, Wiegant J, *er al.* Multiple-fluorescence *in situ* hybridization. Cytometry 1990; 11: 126-31.
10. Nuovo GJ, MacConnell P, Forde A, Delvenne P. Detection of human papillomavirus DNA in formalin-fixed tissues by *in situ* hybridisation after amplification by polymerase chain reaction. Am J Pathol 1991; 139: 847-54.
11. Tompkins LS. Use of molecular methods in infectious diseases. N Engl J Med 1992; 327: 1290-7.
12. Morey AL, Porter HJ, Keeling JW, Fleming KA. Non-isotopic *in situ* hybridisation and immunophenotyping of infected cells in the investigation of human fetal parvovirus infection. J Clin Pathol 1992; 45: 673-8.
13. Rostad SW, Olson K, McDougall J, Shaw CM, Alvord EC. Transsynaptic spread of Varicella Zoster virus through the visual system: a mechanism of viral dissemination in the central nervous system. Hum Pathol 1989; 20: 174-9.
14. Ironside JW, Lewis FA, Blythe D, Wakefield EA. The identification of cells containing JC papovavirus DNA in progressive multifocal leukoencephalopathy by combined *in situ* hybridisation and immunocytochemistry. J Pathol 1989; 157: 291-7.
15. Shapsak P, Tourtellotte WW, Wolman M et al. Search for virus nucleic acid sequences in postmortem human brain tissue using *in situ* hybridisation technology with cloned probes: some solutions and results on progressive multifocal leukoencephalopathy and subacute sclerosing panencephalitis tissue. J Neurosci Res 1986; 16: 281-301.
16. Weiss LM, Movahed LA. *In situ* demonstration of Epstein-Barr viral genomes in viral-associated B cell lymphoproliferations. Am J Pathol 1989; 134: 651-9.
17. Simon D, Searls DB, Cao Y, Sun K, Knowles BB. Chromosomal site of hepatitis B virus (HBV) integration in a human hepatocellular carcinoma derived cell line. Cytogenet Cell Genet 1985; 39: 116-20.
18. Stoler MH. Rhodes CR, Whitbeck A, Wolinsky SM, Chow LT, Broker TR. Human papillomavirus type 16 and 18 gene expression in cervical neoplasias. Hum Pathol 1992; 23: 117-28.
19. Steel JH, Hamid Q, Van Noorden S, er *al.* Combined use of *in situ* hybridisation and immunocytochemistry for the investigation of prolactin gene expression in immature, pubertal, pregnant, lactating and ovariectomised rats. Histochemistry 1988; 89: 75-80.
20. Hamid QA, Bishop AE, Springall DR, *et al.* Detection of human pro-bornbesin mRNA in neuroendocrine (small cell) carcinoma of the lung: *in situ* hybridisation with cRNA probes. Cancer 1989; 63: 266-71.
21. Martinez-Arias A, Baker NE, Ingham PW. Role of segment polarity genes in the definition and maintenance of cell states in the *Drosophilia* embryo. Development 1988; 103: 157-70.
22. Bums J, Chan VTW, Jonasson JA, Fleming KA, Taylor S, McGee J. Sensitive system for visualising biotinylated DNA probes hybridised *in situ:* rapid sex determination of intact cells. J Clin Pathol 1985; 38: 1085-92.

23. Hutchinson RM. Pringle JH, Potter L, Patel I, Jeffreys AJ. Rapid identification of donor and receipient cells after allogeneic bone marrow transplantation using specific genetic markers. Br J Haematol 1989; 72: 133-40.
24. Bhan B, Bums J, Flannery D, McGee JOD. Direct visualisation of single copy genes on banded metaphase chromosomes by nonisotopic *in situ* hybridisation. Nucl Acids Res 1988; 16: 3951-61.
25. Poddighe PJ, Ramaekers FCS, Hopman AHN. Interphase cytogenetics of tumours. J Pathol 1992; 166: 215-24.
26. Devilee P, Thierry RF, Kievits T, Ko Kolluri R, Hopman AHN, Willard HF, Pearson PL, Cornelisse CJ. Detection of chromosome aneuploidy in interphase nuclei from human primary breast tumors using chromosome-specific repetitive DNA probes. Cancer Res 1988; 48: 5825-30.
27. Cremer T, Tessin D, Hopman AHN, Manuelidis L. Rapid interphase and metaphase assessment of specific chromosomal changes in neuroectodermal tumour cells by in *situ* hybridisation with chemically modified DNA probes. Exp Cell Res 1988; 176: 199-206.
28. Hopman AHN, Ramaekers FCS, Raap AK, Beck JLM, Devilee P, Van der Ploeg M, Vooijs GP. In *situ* hybridisation as a tool to study numerical chromosome aberrations in solid bladder tumours. Histochemistry 1988;89:307-16.