Reply to the letter from Poljak et al

Sir – In their letter Poljak et al. expressed their experience in processing of archival cervical smears for human papillomavirus (HPV) detection by polymerase chain reaction (PCR). In agreement with our observation, they found that a complete DNA extraction procedure is required for a successful amplification by PCR. However, they claim that DNA of sufficient molecular weight can easily be isolated to allow amplification of DNA fragments longer than 200 bp. This is in contrast to our findings, showing that it is far from easy to extract sufficient amounts of larger DNA fragments, particularly from older smears. It should be noted that this discrepancy may reflect differences in preparation of the Papanicolaou smear. In particular, the fixation procedure used might be of importance, as the preservation of nucleic acids is likely to be fixation dependent. It is well known that among the different cytological laboratories different fixatives are used to ensure an optimal morphology. This was discussed in our paper. Obviously, the fact that Poljak et al. were able to extract relatively large DNA fragments from their older smears reflects a better preservation of nucleic acids than in the long-stored smears analysed in our laboratory. However, our studies also revealed long-stored smears from which larger fragments could be amplified, indicating that the correlation between storage time and mean fragment size is not strictly linear. Because of the lack of proper documentation, we do not know if some of the long-stored smears analysed in our study were originally fixed in another way, which could be an alternative explanation for the observed differences.

Concerning the complexity of the GTC/silica beads method, we have the impression that, based on recent experiments, the use of diatoms rather than silica particles improves the yield after one extraction round. The use of diatoms has been described as one of the alternatives in the GTC/silica protocol (Boom et al., 1990). Currently, we have no explanation for this observation, but since diatoms are much larger than size-fractionated silica particles, it is conceivable that diatoms display less stringent requirements for nucleic acids binding.

Although we did not test the –30°C/37°C alternate incubation protocol for removal of coverslips, this suggestion is of interest and may save time.

In conclusion, before definitive conclusions can be drawn about the feasibility of different extraction methods, the effect of fixatives used for preparing Pap smears has to be analysed.

JMM Walboomers
PJJ Snijders
AM de Roda Husman
Unit of Molecular Pathology
Department of Pathology
Free University Hospital
De Boelelaan 1117
1081 HV Amsterdam
The Netherlands

References

BOOM R, SOL CIA, SALIMANS MMM, JANSEN CL, WERTHEIM-VAN DILLEN PME AND VAN DER NOORDAA J. (1990). Rapid and simple procedure for purification of nucleic acids. J. Clin. Microbiol., 28, 495–503.

Cancers related to contraceptive use

Sir – There is another hypothesis in addition to contraceptive use or oestrogen replacement therapy to account for the changing gender ratios of colorectal cancer reported by Silva and colleagues (1986). In their discussion of declining F/M incidence ratios, the authors overlooked the contribution that non-steroidal anti-inflammatory drugs (NSAIDs) may have. NSAIDs inhibit prostaglandin synthesis, which is elevated in human colorectal cancers, in venous blood therefrom and in peripheral blood in patients with metastases (Marnett, 1992; Narisawa et al., 1990). NSAID therapy reduced the incidence of carcinogen-induced large bowel neoplasms in rodents, unlike oral contraceptives or oestrogens. Aspirin and newer NSAIDs have delayed recurrence of polyps in hereditary polyposis of the colon and reduced by 30–50% colorectal cancer development in case–control and prospective clinical trials (Giardiello et al., 1995; Giovannucci et al., 1995).

Salicylates have been available since early in this century, and newer synthetic NSAIDs have been widely used for pain relief only during the past 30 years. The latter have been very profitable and hence heavily advertised. Not counting over-the-counter sales, well over 70 000 000 prescriptions for these drugs are filled annually in the US. In addition to premenstrual symptoms, women have about 60% more headaches, arthritis and back pains than men (Benson and Marano, 1994). Women also have more sluggish colonic peristalsis than men, perhaps increasing their cancer risk (Lampe et al., 1993).

Since 1940, colorectal cancer mortality rates (age adjusted to the 1970 US population) in US women has declined with little change in men; the F/M ratio fell from 1.0 to 0.68 (Wingo et al., 1995; Page and Asire, 1985; Riss et al., 1994). Since 1970, the Nebraska ratio has declined by over 40%, falling from 0.93 to 0.53, resulting in 200+ fewer deaths annually. From 1987 to 1993, there were no significant gender differences in staging or operability of these tumours in our state (Nebraska Dept. of Health, 1991).

Consumer surveys are needed to determine which of these therapies may be most important in contributing to this welcome decrease in the incidence and mortality of colorectal cancer in women, but not yet in men.

Bryan A Rettig,
Planning and Data Analysis Support Section,
Nebraska Department of Health,
PO Box 95007,
Lincoln,
NE 68509-5007.

Henry M Lemon,
Section of Oncology and Hematology,
Department of Internal Medicine,
University of Nebraska Medical Center,
Omaha,
NE 68198-3330.