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The assessment of DNA and RNA preservation in liquid based cytology media

The Papanicolaou cervical smear method has long been used for cervical cancer screening, but there is a trend to develop liquid based cytology media and monolayer techniques to reduce false negative rates. These last two types of technique seem to increase the sensitivity of cervical cancer detection,¹ and also provide cellular residues that are useful either for spotting additional slices or performing molecular techniques, such as hybrid capture or the polymerase chain reaction (PCR).² Different media exist and to date the PreservCyt™ medium used in the ThinPrep® Pap Test™ method (Cytoc, Boxborough, Massachusetts, USA) has been validated by the Food

and Drug Administration as an alternative method to the conventional smear. Different methods of human papillomavirus (HPV) detection have been compared and the PCR seems to have higher analytical sensitivity than hybrid capture, at least on the samples processed with the PreservCyt liquid.² A previous report, which tested serial dilutions of an HPV positive cell line, showed that the PreservCyt liquid is suitable for molecular techniques because of good preservation of total nucleic acids (RNA and DNA).³

We compared two different media—PreservCyt and Seroa® (Seroa, Monaco)⁴—used for cervical cytology for DNA and RNA based techniques; in particular, for cellular residues (that is, in routine practice). We randomly selected 10 cellular residues of cervical smears fixed in each medium and performed DNA and RNA extraction with protocols that have already been published.⁵ Briefly, after centrifugation, the pellets were split into two aliquots—one for DNA and one for RNA extraction. The extraction provided equal amounts of DNA in the two series (ranges, 200–1000 ng/µl). However, the amounts of RNA were low, with no clear differences between the two media, varying from 0 to 15 ng/µl. To compare the quality of the DNA and RNA samples, we performed PCR techniques with primers specific for the β actin gene β1/β2 (PCR products: DNA, 560 bp; RNA, 360 bp) after 15, 20, 25, and 35 cycles.⁵ PCR amplification of DNA was efficient in all cases (with primers specific for β actin or c-raf-1), and a visible band was obtained after 15 cycles, with slight differences of staining intensity by ethidium bromide. Regarding RNA preservation, six of the 20 samples gave rise to visible reverse transcription PCR (RT-PCR) products (PreservCyt, n = 3 and Seroa, n = 3). The best signal (plateau) was achieved after 35 cycles. RNA amplification failure is probably the result of poor RNA

preservation, rather than differences in the amounts of cell residue, which were similar in the two media. This is confirmed by the DNA amplification results where both media gave similar results. Our data indicate that in cellular residues of routinely processed samples both media are satisfactory and equivalent for DNA based techniques (PCR, hybrid capture), but the amount of RNA is frequently insufficient even for highly sensitive RT-PCR techniques. Because RNA degradation occurs frequently in routine conditions, the addition of RNase inhibitors to the media should be investigated as a means of circumventing this problem.

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