On the effectiveness of three DNA probes in hybridization experiments over HINF I blots containing aged/degraded samples

E. D'Aloja, V.L.Pascali, M.Dobosz, M. Pescarmona, and A. Fiori
Immunohematology laboratory, Istituto di Medicina Legale, Universitâ Cattolica del S. Cuore, largo F. Vito, 1 00168 Roma Italy

INTRODUCTION

Several DNA probes recognizing variable number of tandem repeats (VNTR) polymorphisms have been recently introduced in the analysis of forensic specimens. They include lambda MS1/31/43/G3 (Wong et al, 1987), alpha globin 3'HVR (Fowler et al, 1988) and YNH24 (Nakamura et al, 1987).
These probes belong to different though related families of tandem repeats with different core sequences, and recognize clusters of alleles with distinctive molecular sizes.
Little is presently known on the liability of these VNTR polymorphisms to aging/degradation damages.
A general notion is that on aging restriction fragments of a given digest undergo fragmentation. However, reduction in size of DNA is known not to be merely correlated to aging. Endogenous hydrolytic processes underlying size reduction largely depend on factors as sample hydratation, and the relative content in pirimidine bases (Paabo, 1989).
If this is true, then a different content in C and T bases within distinctive classes of VNTR could perhaps affect a selective resistance of VNTR alleles to hydrolytic degradation.
Even apart from general speculations, the hypothesis that some VNTR polymorpshisms could resist aging better than others merits to be verified as such.
In this study, we have reinvestigated filter blots pertaining to several criminal cases recently examined for DNA profile, by rehybridizing them to one/two additional single-locus probe systems.
Our purpose was to know further on the relative ability of three commonly used hypervariable probes to hybridize aged samples.

MATERIALS AND METHODS

Southern blot analysis of various forensic specimens (fifteen criminal cases) was performed according to standard procedures (Maniatis et al, 1982). HINF I digests were used

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throughout. Details on forensic specimens worked out have been described elsewhere (Pascali et al. 1989). When beginning this study, most filters had been generally hybridized once by 3'HVR under low/high stringency conditions, and at times reprobed with lambda MS1/31 at high stringency. For reprobing, they were washed in 0.1% SDS at 90°C for 30 min, then rinsed in excess 2 X SSC.
While hybridizing all our filters (referring to as much forensic cases) with 3'HVR, YNH24 and MS1/31 was in our aim, nine out of 15 filters were actually probed for these three systems. The remaining six filters were probed with only two. Labelling was done with a random exanucleotide priming method (Multiprime, Amersham). Hybridization (high stringency buffer, according to Church and Gilbert, 1984) was prolonged overnight (3 x 10^7 cpm/blot) with following high-stringency washes (3xSSC/5% SDS for 15'; 1xSSC/1% SDS for 15'; 0.1 x SSC/0.1% SDS for 15' at 65°C).
Time of exposure to Xray films varied according to the counts/sec over the filters.

RESULTS AND DISCUSSION

The outcome of multiple hybridizations over fifteen filters can be summarized as follows. Ten out of 23 forensic samples analyzed revealed coherent bands with all (either two or

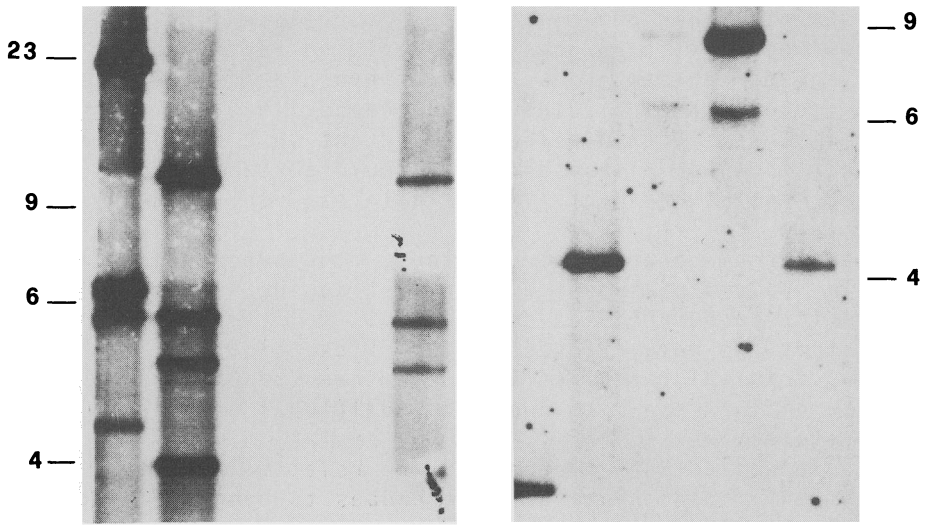

Fig. 1. Discrepant hybridization experiments with MS1/31 (left) and 3'HVR over the same filter. From left: a, reference; b, suspect; c,d, car seat bloodstains; e, shirt bloodstain.

three) probes used. Ten forensic specimens failed to detect any readable bands with all probes used.
In two cases only, discordant results were obtained with different probes. In the first case (a blood stain 7 months old absorbed over a cloth) readable pattern resulted from the use of 3'HVR under both low and high stringency; conversely lambda MS1 and YNH24 failed to detect readable patterns. In a further case, two seven months-old blood stains absorbed to a car seat acrylic cover yielded a single-locus pattern with 3'HVR, but not with lambda MS1/31 mix nor with YNH24.
These results are difficult to evaluate. While a marked resistance of 3'HVR/Hinf I polymorphism to degradation processes would be argued, the number of samples examined is too small to allow significant inferences.
If letting unanswered the question as to whether some VNTR systems are more refractory to aging than other ones, results reported here enphasize well that testing forensic DNA digests with several DNA probes is mandatory whenever they derive from very aged specimens.

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