Oligo-AP probes and chemiluminescence: sensitivity for stains analysis

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SUMMARY

The sensitivity of the oligo-AP probes (alkaline phosphatase labelled oligonucleotides) MS1 and YNH 24 (Cellmark Diagnostic) is studied on stains with respect to white cell counts for blood stains, sperm count for semen stains, and for volumes varying from 1 to 5o ul.

INTRODUCTION

DNA profiling with single locus probes (SLP) detects VNTR loci of high variability and can provide highly discriminating identification.
The NICE (Non Isotopic Chemiluminescent Enhanced) probes system from Cellmark Diagnostic presents a high sensitivity and is rapid, reliable and safe. It also enables the analysis of small or mixed samples.
In the case of blood or sperm stains, the sensitivity of the analysis can also depend upon the white cell count and the sperm count respectively.
BLOOD: the normal white cell values vary between 4.000 and 10.000 cells per mm3, but in the presence of illness or ethnical differences, it can be lower.
SEMEN: the normal sperm count is higher than $20x10^6$ sp/ml. In many cases it can be lower: alcohol, drugs, tobacco and medicaments abuses, infections, traumatisms, obstructions, hormonal deficits, illness, hereditary anomalies, exposition to toxics etc.
The aim of this study is to evaluate the sensitivity of the oligo-AP probes and chemiluminescence technique with respect to the volumes of stains and to determinate the influence of the cell count (white blood cells or sperms) on this sensitivity.

MATERIAL and METHOD

STAINS

BLOOD: blood samples were provided by the Central Laboratory of Haematology (Cantonal Hospital Geneva)
White cells counts: 2.000, 5.000 and 10.000 white cells/mm^3
Stains volumes: 5, 10, 20 and 50 ul.

SEMEN: semen samples were provided by the Andrology Laboratory (Geneva).
Sperm counts: $0,7x10^6$, $10x10^6$ and $40x10^6$ sp/ml.
Stains volumes: 1, 2, 5 and 10 ul.

Stains were made on white cotton, air dried, stored at room temperature and analysed after 5 days and after one month.

EXTRACTION

Stain material was incubated overnight at 37°C in 600 ul extraction buffer (0.01M TrisHCl pH 7.6, 0.01M EDTA pH8, 0.1M NaCl, 2% SDS, and for semen samples, 0.04M DTT) plus 40ul each of proteinase K(20 mg/ml) .
DNA was extracted from samples by a conventional phenol-chloroform method (phenol / phenol-chloroform / chloroform-isoamyl alcohol) followed by ethanol precipitation. The pellet was dissolved in 20 ul Tris-EDTA buffer.
A second series of stains was incubated under the same conditions and then extracted with the QIAamp Blood extraction Kit (QIAGEN) following the manufacturers' instructions (including a 10 minutes at 70°C step before the elution).

QUANTIFICATION

Extracted DNA were quantified by hybridisation with the probe D17Z1 using the ACES Human DNA Quantitation System and the Convertible slot-blot apparatus from Gibco-BRL (with modifications).
1 ul of each sample was denaturated in 500 ul alkaline solution, and then blotted onto a positively charged nylon membrane (Boehringer-Mannheim) using the slot-blot apparatus.
The membrane was neutralized and while it was still damp, the DNA was fixed by UV cross-linking (2 minutes at 302 nm). The membrane was then air dried during two hours (or alternatively baked for 15 minutes at 120°C) and hybridized to the D17Z1 probe (3 ul in 6 ml NICE hybridisation solution) following the same technique as for the NICE probes (see thereafter).

DIGESTION

All samples were subsequently digested with Hinf 1 (5u/ug) for 16 hours at 37°C and the quality of the digestion was checked by minigel electrophoresis.

SOUTHERN blot analysis

Fragments were separated by agarose gel (0,8% in TBE) electrophoresis (40V, 22 hours) and transferred onto a positively charged nylon membrane (Boehringer-Mannheim) by capillary blotting in 10x SSC for 16 hours. The DNA was then fixed by UV cross-linking (2 minutes at 302nm), followed by baking at 120°C for 15 minutes.

PREHYBRYDISATION

The membrane was placed in a plastic bag, for 30 minutes at 50°, in 30 ml hybridisation solution (0,5M Na_2HPO_4 pH 7,2, 0,1% SDS, and with 1% blocking reagent (Boehringer-Mannheim).

HYBRIDISATION

The blot was then probed with 10 ul MS1 in 16 ml hybridisation solution for 45 minutes at 50°C with agitation (or with 10 ul YNH24 after stripping). The blot was then washed twice for 10 minutes at 50°C in 0,01 M Na_2HPO_4pH 7,2, 0,1% SDS and twice for 5 minutes at room temperature in 1xSSC.

CHEMILUMINESCENT DETECTION

The membrane was equilibrated 5 minutes in a carbonate buffer (50 mM $NaHCO_3$ titrated to pH 9,6 with 50 mM Na_2CO_3, 1mM $MgCl_2$) and then incubated during 5 minutes in CSPD (Tropix) 1% in carbonate buffer. The membrane was then exposed for 6 hours and for 16 hours (or more) on X-ray film (Hyperfilm MP Amersham) at 37°C.

STRIPPING

Was performed in 0,1% SDS for 15 minutes at 80°C, followed by a rinse in 1xSSC.

SENSITIVITY

The sensitivity of this technique was tested using dilutions of K562 cell line DNA (1 ug to 7 ng).

RESULTS

SENSITIVITY

After an expoxure of 16 hours, it was possible to detect 7 ng of K562 DNA.

QUANTIFICATION

A better DNA yield is observed with the classical phenol-chloroform extraction. The DNA quantities obtained with the QIAamp Kit were lower; this was confirmed by subsequent Southern blot and hybridisation with MS1 (data not shown).
It is difficult to evalue with precision the exact quantities of DNA using the slot blot method. But by comparing these results and the results of the Southern blot to the theoretical quantities of DNA in each stain, we can evaluate that the phenol-chloroform extraction yields about 30% of the total DNA in semen stains, and 10 to 20% in blood stains. The quantities obtained by the QIAamp Kit are around 75% of those obtained by phenol-chloroform extraction.

HYBRIDISATION with MS1 and YNH24

(see DISCUSSION)

DISCUSSION

The oligo-AP probes (NICE probes Cellmark) and chemiluminescence detection are a powerful technique that enables the detection of a profile with DNA amounts down to about 7 ng.
The sensitivity of the test depends not only upon the volume of the stains but also upon the white cell count for blood stains and the sperm count for semen stains.
In the case of blood stains, it was possible to detect a profile with the probe MS1 for all 5 days old stains (2.000, 5.000, 10.000 wc/mm^3, and for all volumes (5, 10, 20, and 50 ul). For the one month old stains, the profile with the lower volumes (5 and 10 ul) and the lower white cells count (2.000 and 5.000 wc) was too faint to be useful.
In the case of semen stains, it was possible to detect a profile with MS1 for the 5 days old normospermic stains and for all of the volumes analysed (1, 2, 5, 10 ul). For the oligospermic stains ($10x10^6$ sp/ml) the profile was clear for the higher volumes (5 and 10 ul) after 16 h. of exposure. For the very low oligospermic stains ($0,7x10^6$ sp/ml) no profile was clear enough.
For the one month old stains, only the higher volumes (5 and 10 ul) of the normospermic stains are useful.
After stripping and reprobing with YNH24, and after an exposure of 16 hours, all profiles for all blood stains can be interpreted.

In conclusion, oligo-AP probes and chemiluminescence enable the analysis of small good quality stains with a high degree of sensitivity.With blood or semen stains of variable cell or sperm counts, and for various ages, there is good consistency of the results.

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