Studies on Sex Determination with Different Y-Specific Probes After Quantifying Human Genomic DNA with the Slot Blot Hybridization Method

Haas, H.; Hofmann, W.; Weiler, G.
Institute of Legal Medicine, University of Giessen, Frankfurter Str. 58, 35392 Giessen, Germany.

Introduction

Sex determination can be performed routinely by Southern blot analysis, by the PC Reaction or by dot/slot blot analysis. In forensic science the method for sex identification has to be fast and the amount of DNA available is sometimes limited. In case of vaginal swabs additionaly a good separation of female and male cells after differential lysis is required. At first the extracted DNA from forensic specimen has to be quantified. One possibility is the slot blot hybridization method with a human specific probe. It represents a method to detect small amounts of degraded DNA and to test its human specifity [1]. In this study a rehybridization of the membrane with different human Y-specific probes is investigated to identify the sex, to test human Y-specifity and to proof the quality of the differential lysis. Two alkaline phosphatase labelled human Y-specific probes are tested for their usefulness and one longer digoxigenin labelled human Y-specific probe. This method requires no additional DNA material for sex determination and quality control of DNA extracted from vaginal swabs.

Material and Methods

DNA from vaginal swabs was extracted according to the FBI protocol [2] with an organic extraction procedure. As female standard K562 DNA from Promega was used. Male standard DNA was human placenta DNA from SIGMA (Cat.No.3160). Bacterial DNA from E.coli was from Gibco BRL (Cat.No.220-4220). The vertebrate animal DNA from chicken, mouse, rat, dog, pig and bovine was from Promega (Cat.No. G3121, G3091, G3131, G3051, G3071, G3081). The bacterial and animal DNA were used to examine human specifity of the different probes. One µl from each fraction of the DNA extracted from the vaginal swab was used for slot blot quantification. All DNAs were diluted in distilled water up to 25 µl final volume. The different concentrations and the arrangement of the slot blot is visible in figure 1. All DNAs were denatured for 5 min. at r.t. with 500 µl 0.5M NaCl; 0.5M NaOH per sample. As a nylon membrane Biodyne A was used (Gibco BRL Cat.No.4866 SA). The membrane was prewetted for 5 min. in 2x SSC. After placing the membrane in the slot blot apparatus (Gibco BRL Convertible Slot Blot Vacuum Apparatus Cat.No.580-1055) the samples were loaded in the different slots. Each slot was rinsed with 500 µl of the denaturation solution. The membrane was removed from the apparatus and placed into a neutralization solution for 5 min.. Then the membrane was air dried and the DNA was fixed by UV crosslinking at a wavelength of 302 nm for 90 sec. and baked at 80°C for 30 minutes.

The first hybridization was carried out with the human specific probe D17Z1, an alkaline phosphatase labelled oligonuceotide probe (Gibco BRL Cat.No.4220 SA), analogous the PhotoProbe human DNA quantitation system protocol from Gibco BRL at 50°C. The exposition was carried out at 40°C overnight. Then the membrane was stripped with 0.1% SDS at 80°C for 30 minutes.

At first the membrane was rehybrized with the Gene Print Light human Y-specific probe from Promega in a concentration of 5 µl/10 ml hybridization solution of the PhotoProbe system. The hybridization protocol and exposition was the same as for the human specific probe D17Z1.

The second rehybridization was carried out with a self-constructed Y40 probe. This probe is 40 bp long and contains eight times the pentanucleotide sequence described in Nakahori et al. (1988) [3] as human and Y-specific. This probe was labelled with the Lightsmith luminescence engineering system for oligonucleotides (Promega Cat.No.F1280). A hybridization protocol analogous to the PhotoProbe system from Gibco BRL gave the best results. The final concentration of the probe per one ml hybridization solution was 1200 fmol.
At last the membrane was hybridized with the human Y-specific probe from Promega (Cat.No.DK5141). The probe was labelled with digoxigenin analogous the Feinberg and Vogelstein method [4]. The concentration of the probe was 5 ng/ml hybridization solution. The hybridization was carried out analogous to the protocol of Boehringer Mannheim at 72°C. The membrane was exposed for 3 hours at 40°C.
To validate the results the extracted DNA from vaginal swabs was amplified by PCR. Different loci such as D1S80, TC11, SE33 and YNZ22 were amplified.

Results and Discussion

Figure 1 shows the slot blot arrangement and figure 2-5 four different hybridization results. In figure 2 the hybridization result of the human specific probe D17Z1 is visible. The results indicate the human specifity of the probe. No signal is visible for bacterial and vertebrate DNA. Human DNA up to 1 ng can be detected. A difference in intensity for the signal of the female and male fraction after differential lysis of the same vaginal swab is visible. If the signal of the female fraction of the vaginal swab is stronger more female epithel cells are detected in the cytological investigation. If more sperm cells are detected in the cytological investigation, a corresponding stronger signal of quantified DNA is visible in the male fraction.
The first rehybridization of the same membrane with the alkaline phosphatase labelled GPL Y-specific probe indicates that this probe does hybridize to animal DNA as well. The strongest non-human signal is visible for chicken DNA. The signal intensity is increased for both, female and male standard DNA, so that amounts up to 0.1 ng DNA can be detected. With this probe no sex-determination is possible because both female and male DNA give a strong hybridization signal.
A second rehybridization with the self-constructed Y40 probe another alkaline phosphatase labelled oligonucleotide probe, indicates human-specifity. The signal for human DNA is less intense than the signal after hybridization with the human specific probe. In case of male standard DNA amounts up to 1 ng can be detected. In case of female standard DNA the limit is 2ng. No sex-identification is possible with this probe, because there is no signal difference for female and male DNA.
The digoxigenin-labelled probe indicates no human specifity because even for bacterial and animal DNA a hybridization signal is detected. The signal intensity is the same for different amounts of bacterial DNA and for the animal DNAs. This probe gives the best results with respect to Y-specifity. The hybridization signals of the human male standard show a higher signal intensity in comparison to the hybridization with the human specific probe. In case of male DNA amounts up to 0.2ng can be detected. The signals of the human female DNA standard are less intense for DNA amounts from 60-100ng. No increase of signal intensity is visible for DNA amounts less than 40ng. For the DNA extracted from vaginal swabs an increase in signal intensity can be observed for the male and the female fraction. This indicates that the female fraction still contains male DNA, meaning no good separation of female and male fraction through differential lysis. This is confirmed by the following PCR reaction (data not shown).

Conclusion

After determination of quantity and human specifity of DNA by slot-blot hybridization with a human specific probe the information can be used for a further investigation. The results indicate that the same DNA can be sex-determined by a further hybridization with a human Y-specific probe. In case of vaginal swabs an additional information can be obtained about the quality of the differential lysis.

Literature

[1] Waye, J.S.; Presley, L.A.; Budowle, B.; Shutler, G.G.; Fourney, R.M. (1989): A Simple and Sensitive Method for Quantifying Human Genomic DNA in Forensic Specimen Extracts. BioTechniques 7 (8), 852-855.

[2] Easteal, S., McLeod, N.; Reed, K. (1991): DNA Profiling. Harwood Academic Publishers, Philadelphia, 152-153.

[3] Nakahori, Y.; Mitani, K.M.; Yamada, M.; Nakagome, Y.(1988): A Human Y-Chromosome Specific Repeated Family (DYZ1) Consists of a Tandem Array of Pentanucleotides. Nucl.Acids Res. 14, 7569-7580.

[4] Feinberg, A.P.; Vogelstein, B. (1983): A Technique for Radiolabelling DNA Restriction Endonuclease Fragments to High Specific Activity. Anal.Biochem. 132, 6-13.

	1	2	3	4	5	6
A	♂ 100 ng	Vag.Swab	♂ 1.0 ng	Vag.Swab	E.coli 20 ng	♀ 2 ng
B	♂ 80 ng	Vag.Swab	♂ 0.4 ng	Vag.Swab	E.coli 20 ng	♀ 4 ng
C	♂ 60 ng	Vag.Swab	♂ 0.2 ng	Vag.Swab	Bovine 40 ng	♀ 10 ng
D	♂ 40 ng	Vag.Swab	♂ 0.1 ng	Vag.Swab	Dog 40 ng	♀ 20 ng
E	♂ 20 ng	Vag.Swab	♀ 0.1 ng	Vag.Swab	Pig 40 ng	♀ 40 ng
F	♂ 10 ng	Vag.Swab	♀ 0.2 ng	Vag.Swab	Rat 40 ng	♀ 60 ng
G	♂ 4 ng	Vag.Swab	♀ 0.4 ng	Vag.Swab	Mouse 40 ng	♀ 80 ng
H	♂ 2 ng	Vag.Swab	♀ 1.0 ng	Vag.Swab	Chicken 40 ng	♀ 100 ng

Figure 1:
Slot Blot Arrangement of the different DNAs: Standard female and male DNA, bacterial DNA and animal DNA, DNA extracted from vaginal swabs (V.S.) after differential lysis.

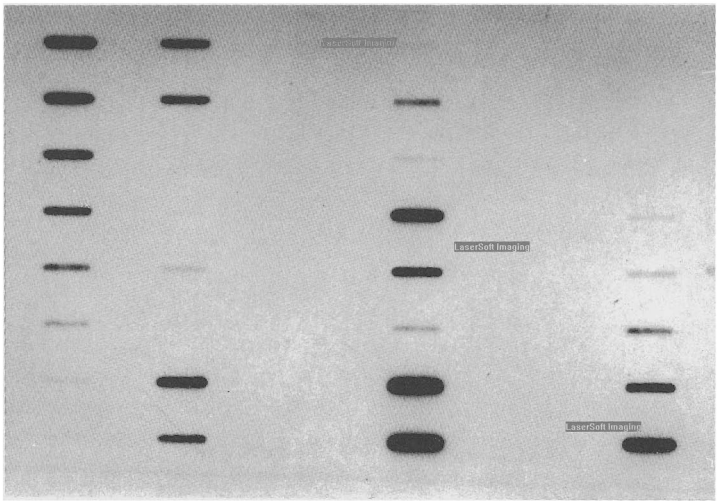

Figure 2:
Hybridization result after hybridization with the human specific probe (D17Z1)

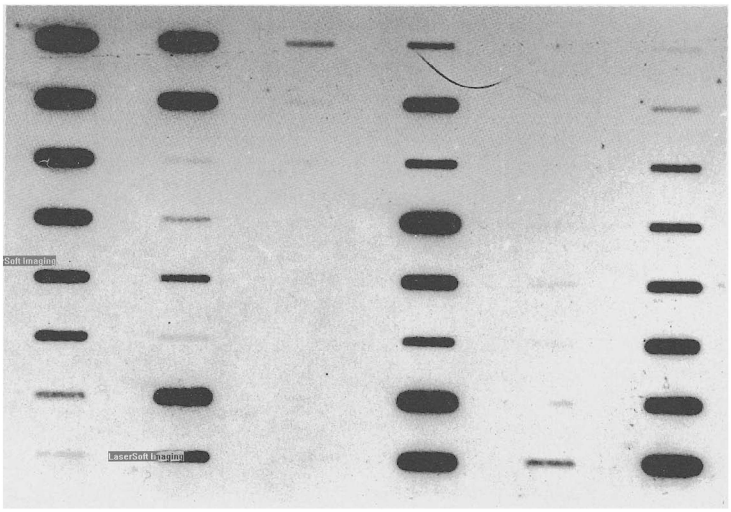

Figure 3:
Hybridization result after rehybrization with the GPL human Y-specific probe.

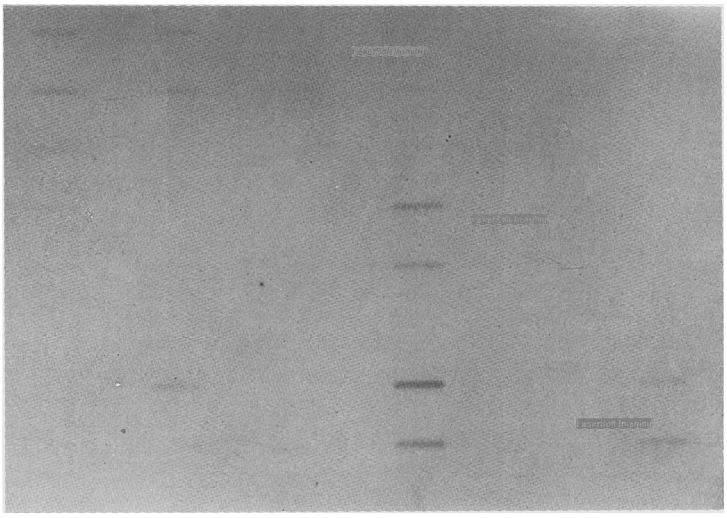

Figure 4:
Hybridization result after rehybridization with the self-construted, alkaline phosphatase labelled Y40 oligonucleotide probe.