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A Study of Sex Identification of Trace, Dried Bloodstains Using a Y-Chromosome-Specific Deoxyribonucleic Acid (DNA) Probe

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ABSTRACT: A new method is discussed which examines trace, dried bloodstains by gel in situ hybridization using a Y-chromosome-specific deoxyribonucleic acid (DNA) probe to determine the sex of the bloodstain for forensic medicine application. The complete DNA is transferred directly by electrophoresis onto the gel intact, bypassing the possibilities of impurities contaminating the sample and of DNA degradation. The method has proven accurate for small (2.5-mm-diameter) samples aged up to eight years and is quick, simple, and easily read.

KEYWORDS: forensic science, deoxyribonucleic acid, (DNA), electrophoresis, human identification, in situ hybridization, Y-chromosome, sex identification, bloodstains, agrose gel, extraction, fluorescence, ultraviolet

In homicide cases, a bloodstain from the victim found on the clothing of the suspect is important evidence. If the victim and the suspect are of the same blood group, however, establishing merely the blood type of both obviously does not provide any information helpful to the case. In cases in which the blood group is the same but the sex of the suspect and victim is different, the atabrine-stained Y-body method normally would be used to determine the sex of the bloodstain [*1*].

Because of the development and application of molecular genetics and the deoxyribonucleic acid (DNA) recombinant technique in recent years, the identification of human sex has reached the molecular level, permitting new physical evidence for forensic medicine [*2*]. The study described in this paper uses a Y-chromosome-specific DNA probe to identify the sex of trace, dried bloodstains, thus bringing DNA molecular hybridization technique to the identification of sex of bloodstains.

The process described herein does not require that DNA from dried bloodstains be digested with restriction endonuclease; instead, the complete DNA is directly transferred by

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electrophoresis onto the gel and gel hybridization is done in situ [3]. Advantages of the process are that only a small sample is required, the sample is not influenced by impurities, and the process is simple, quick, accurate, and easily read. It also provides forensic medicine with a new technical method for the determination of the sex of bloodstains.

Procedure

1. Blood from the elbow veins of 25 healthy male and 25 healthy female adults were dotted onto a piece of white cotton cloth. The samples were stored at room temperature and allowed to dry naturally.
2. Blood from the elbow vein of a healthy male adult in 2, 4, 8, and 16-μL quantities was dabbed onto a piece of white cotton cloth to yield 2.5, 3.5, 5.5, and 9-mm-diameter stains, respectively. The samples were stored at room temperature and allowed to dry naturally.
3. Two of each of the four sizes of bloodstains on white cotton from the known male adult (No. 2 above) were stored in our laboratory for one, three, five, and eight years and then prepared for use in the study.

The method used is shown in Fig. 1.

Results

Quality and Quantity of DNA in Samples

All DNA extracts of bloodstains from the different sexes (No. 1 above) were examined by agrose gel electrophoresis containing 0.5 g/mL ethidium bromide (EB) and were shown to be

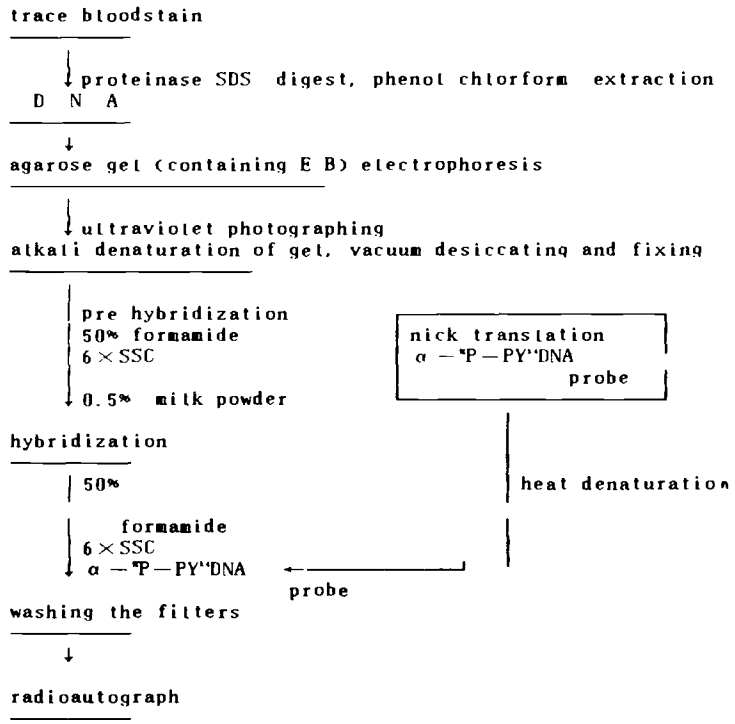

FIG. 1—*Flow chart of method for determining sex of bloodstains.*

large molecular DNA without the contamination of RNA. There was no degradation or damage in the extraction process, the quality of which was shown to be reliable (Fig. 2). Comparison of the given fluorescent intensity of the ultraviolet (UV) photograph and of the different quantities of DNA electrophoresis permitted the quantity of DNA in the bloodstains to be determined. About 0.01 μg of DNA can be extracted from the 2-μL bloodstain sample (Fig. 3).

In Situ Hybridization of Gel

Sex Identification by DNA—The Y-specific hybridization band of the male bloodstain was observed in its radioautograph pattern, which was examined by gel in situ hybridization using a Y-chromosome-specific DNA probe. The male identifier yielded a positive result (presence of the Y-specific hybridization band), the female identifier a negative result (no Y-specific hybridization band) (see Fig. 2*b*).

Minimum Amount of DNA—Sex detection of trace, dried bloodstains by gel hybridization in situ using a Y-specific DNA probe requires, on cotton cloth, a minimum bloodstain diameter of about 2.5 mm (approximately 2 μL of fresh blood) to yield accurate results (see Fig. 4).

Aged Bloodstains

With the passage of time, the extraction amount of DNA decreases and the molecular degradation of DNA increases. The radioautograph intensity of molecular hybridization which the Y-specific DNA probe uses to identify the sex of the bloodstain also weakens with

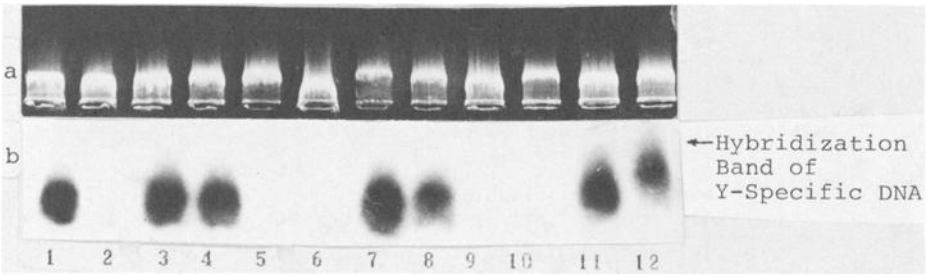

FIG. 2—(a) *UV photograph of DNA from different sex bloodstains on gel electrophoresis.* (b) *Pattern of gel in-situ hybridization band of* α^{-32} *PY-chromosome-specific DNA probe. No. 1 is male control; 2 is female control; 3, 4, 7, 8, 11, and 12 are DNA from male bloodstains; 5, 6, 9, and 10 are DNA from female bloodstains.*

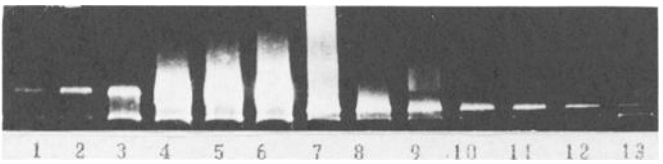

FIG. 3—*UV photograph of quantitative comparison between DNA in different amounts of bloodstains and DNA of known fresh blood was made after gel electrophoresis. Nos. 1 to 6 are DNA in 2, 4, 8, 16, 32, and 64-*μ*L bloodstains, respectively: 7 to 13 are 0.05, 0.25, 0.125, 0.075, 0.0375, 0.0187, and 0.0096-*μ*g DNA in flesh blood, respectively.*

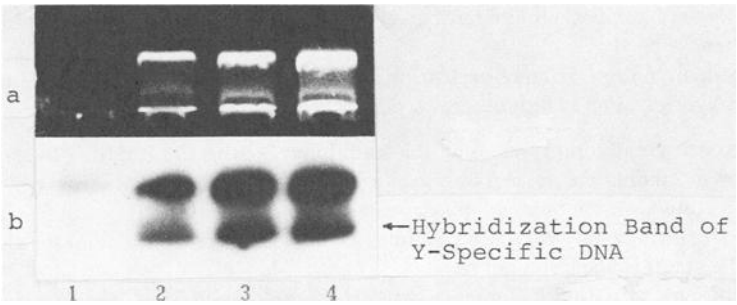

FIG. 4—(a) *UV photograph of DNA from different amounts of male bloodstains on gel electrophoresis.* (b) *Pattern of gel in-situ hybridization of* α^{-32} *PY-chromosome-specific DNA probe. Nos. 1 to 4 are, respectively. DNA from 2, 4, 8, and 16-*μL male bloodstains.

time. However, if a greater amount of bloodstain is examined, clear bands of hybridization can still be seen. This is shown clearly in Fig. 5: the hybridization band intensity of Y-specific DNA from 1.0-cm^2 bloodstains aged one, three, and five years weakened gradually, but bloodstains twice that size (2.0 cm^2) showed clear hybridization bands even after eight years.

Discussion

The experimental results of human trace bloodstains identified by gel, in situ hybridization using a Y-chromosome-specific DNA probe show that the Y-specific hybridizing band of DNA from a male bloodstain is observed in the pattern of DNA hybridization without the DNA digesting restriction endonuclease, but not from a female bloodstain. This indicates that this method is reliable to identify the sex of human bloodstain. The Y-specific DNA hybridization band, which is very sensitive and which reaches a trace level, can be detected (male) or ruled out (female) in bloodstains of 2.5-mm diameter (equivalent to 2 μL of fresh blood). This method can identify the sex of bloodstains aged for eight years. For bloodstains aged longer than eight years, it is absolutely possible to determine sex correctly as long as the bloodstain is large enough that 0.01 μg or more of DNA can be extracted.

The method of the detection of human sex in dried bloodstain by gel hybridization in situ using a Y-chromosome-specific DNA probe includes the following:

1. The quality and quantity of DNA in the bloodstains are detected by UV photograph of agarose gel electrophoresis containing EB.
2. DNA is electrophoresed directly on agarose gel without digesting of restriction endonuclease.

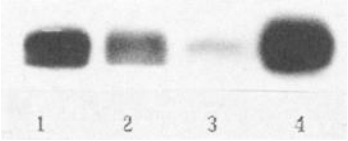

FIG. 5—*Pattern of gel in-situ hybridization band of* α^{-32}*Y-chromosome-specific DNA probe of DNA from different years of male bloodstains. Nos. 1 to 3 are 1-cm*^{2} *bloodstains aged one, three, and five years, respectively; No. 4 is a 2-cm*^{2} *bloodstain aged eight years.*

3. Directly drying the gel eliminates the need to transfer the DNA from the gel to a nitrocellulose filter.
4. The system and operation of prehybridization and hybridization are simplified.
5. Direct hybridization in situ obtains a perfect pattern of Y-specific DNA.

This approach greatly improves upon the traditional Southern translating hybridization technique by shortening the process by one or even two days and by eliminating the need for nitrocellulose filters, restriction endonuclease, and some proteinase. It is simple, fast, and economical, requiring only a minimum sample (2.5-mm-diameter bloodstain) for a sensitive reaction. The result is accurate and reliable.

This method uses existing DNA molecular hybridization and also employs a new technique in which human chromosome DNA extracted from trace, dried bloodstain is hybridized with a Y-chromosome-specific $PY^{3.4}$ fragment DNA probe. It allows the sex of human trace, dried bloodstains to be identified at the DNA molecular level. The Y-chromosome gene DNA which is examined is the more plentiful DNA fragments, which have a high sensitivity to hybridization. If the minimum amount of bloodstain is used (2.5-mm diameter), the appearance rate of Y-body of less than 6% is used to identify female bloodstain. Thus, the method has advantages other than that it uses Y-chromosome atabrine to observe the appearance rate of Y-body and thus to identify the sex of bloodstains.

Sex identification is often impeded by impurities in the bloodstain and by unskilled operators interpreting the impurities as Y-bodies, resulting in a sham positive result. This research overcomes the problems of previous methods. The impurities in bloodstains extracted by DNA and isolated by electrophoresis are sufficiently removed and do not affect the result of molecular hybridization. The molecular hybridization of more copied DNA fragment is very sensitive, and the amount of sample needed is decreased and is available to examine trace bloodstain. As long as the minimum amount of DNA is obtained, the molecular hybridization is specific and cannot yield sham positive results through human misinterpretation or error. The band patterns obtained after a certain qualitative DNA is hybridized with a Y-specific probe are all definite results for both positive ($>6\%$) and negative presence ($<6\%$) of Y-body.

On the other hand, the method described herein does require considerable care and skill in its execution, as well as a highly specific staining instrument. Other problem areas include the following:

1. The electrophoresis band is accurate only for DNA extracted from bloodstain which has been sufficiently dissolved.
2. The DNA density of the bloodstain sample should be no greater than 0.25 to 0.5 μg.
3. For a clear hybridization band to be obtained, it is necessary to raise the voltage and to shorten the electrophoresis time needed to degrade the DNA sample.
4. A sample stored for a long period of time is likely to have a decreased background, thus prolonging the time of prehybridization.

The method described in this paper of sex identification of trace bloodstain using a Y-chromosome-specific DNA probe is simple and easy to perform. The result is reliable and has a practical value and application in the forensic medical examination of physical evidence.

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