TECHNICAL NOTE

Mark J. Pettenati,[1] Ph.D.; P. Nagesh Rao,[1] Ph.D.; Sheryl Schnell,[2] B.S.; Rosa Hayworth-Hodge,[2] B.S.; Patrick E. Lantz,[3] M.D.; and Kim R. Geisinger,[4] M.D.

Gender Identification of Dried Human Bloodstains Using Fluorescence In Situ Hybridization

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ABSTRACT: Identification of the gender of an individual(s) from whom a bloodstain is derived represents important evidence in medicolegal cases. The efficacy of fluorescence in situ hybridization (FISH) using chromosome X and Y centromeric probes was tested to determine its ability to identify correctly the gender of extracted dried bloodstains. In this preliminary study, FISH correctly identified the gender of 2-week-old dried bloodstains in prepared mixtures of male-to-female blood as low as 1%. The technique is accurate, rapid, sensitive, easily performed and readily available. This application of FISH as a forensic laboratory technique holds great promise.

KEYWORDS: pathology and biology, gender determination, DNA, fluorescence in situ hybridization (FISH), blood, dried bloodstains

Identification of the genetic makeup of a bloodstain from a victim transferred onto a suspect can represent important evidence in homicide cases. Routinely, blood typing is used in these investigations. In some circumstances, molecular genetic laboratory procedures, such as DNA fingerprinting, are being used to provide further evidence. However, such molecular genetic techniques can be costly, time consuming and not readily available.

A new advance in molecular genetic tests is fluorescence in situ hybridization (FISH). This technique has successfully been applied in the clinical genetic testing of prenatal, newborn, leukemia, and solid tumors samples. It uses non-radioactive fluorescently labeled DNA probes to identify rapidly the presence of a chromosome, chromosome region or gene in cells. We recently evaluated the ability of FISH to identify correctly the gender of dried bloodstains as well as to detect male cells mixed in female blood. The results of this study are presented and the incorporation of FISH evidence into forensic laboratory investigations is discussed.

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[1]Associate and Assistant Professors, respectively, and Directors of the Cytogenetic and Molecular Cytogenetic Laboratory, Department of Pediatrics, Section on Medical Genetics, Bowman Gray School of Medicine of Wake Forest University.

[2]Molecular Cytogenetic Technologists, Molecular Cytogenetic Laboratory, Department of Pediatrics, Section on Medical Genetics, Bowman Gray School of Medicine of Wake Forest University.

[3]Assistant Professor and Forensic Pathologist, Department of Pathology, Bowman Gray School of Medicine of Wake Forest University.

[4]Professor and Director of Surgical Pathology and Cytopathology, Department of Pathology, Bowman Gray School of Medicine of Wake Forest University.

Material and Methods

Samples

Peripheral blood samples were obtained from chromosomally normal male (46,XY) and female (46,XX) volunteers. Seven individual blood samples were prepared consisting of 1) 100% male blood, 2) 100% female blood and mixtures of male to female blood in the following ratios: 3) 1:1; 4) 1:4; 5) 1:10; 6) 1:20; 7) 1:100. Two mL of each preparation were deposited onto 5.1 cm × 5.1 cm non-sterile rayon/polyester fabric gauze sponges (Nu gauze, Johnson & Johnson) and allowed to air dry for two weeks.

Extraction of the white blood cells was performed by placing the gauze squares into 10 mL of isotonic buffered saline, pH 6.8, vortexed for 10 s and allowed to remain at room temperature for 1 h. The gauze was removed and 1 mL of fixative (3:1 methanol: glacial acetic acid) was added and the samples spun at 1000 rpm for 8 min. The supernatant was removed and two changes of the fixative were added after which the cell pellet was dropped onto microscope slides over a 65°C steam bath and allowed to dry over the steam bath for 1 min. The samples and slides were coded and blinded to the technologist.

TABLE 1—*Percent of male and female cells identified by FISH in the blood mixtures.*

	Sample number						
	1	2	3	4	5	6	7
(% male cells)	(0)	(100)	(50)	(25)	(10)	(5)	(1)
FISH results							
% Male	0	100	47	22	11	4	1
% Female	100	0	53	78	89	96	99

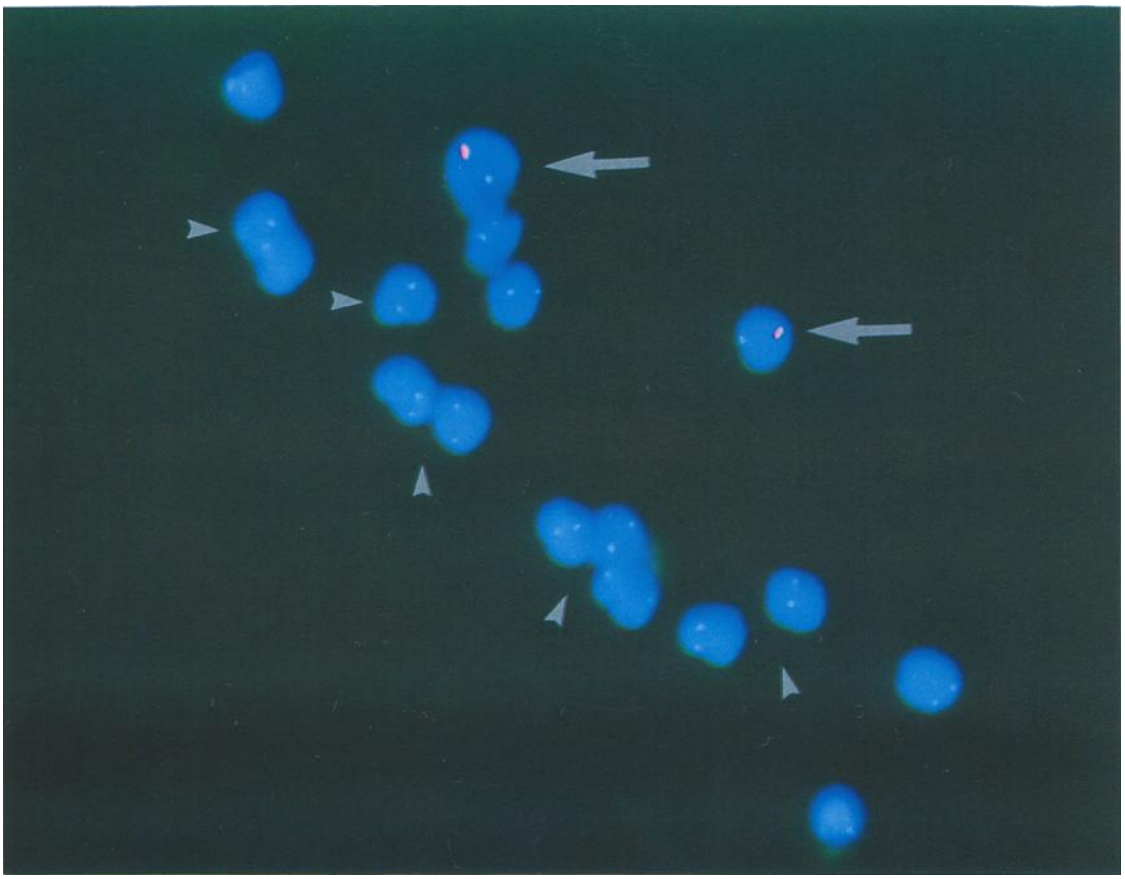

FIG. 1—*Dried blood smear showing the presence of male and female white blood cells. The arrow heads indicate female (XX) nuclei with 2 green (X chromosome) signals. The large arrows indicate male (XY) nuclei with 1 green (X chromosome) and 1 red (Y chromosome) signal.*

Fluorescence In Situ Hybridization

The Spectrum CEP Direct Chromosome Enumeration System (VYSIS [formerly Imagenetics]; Framingham, MA) using the X chromosome probe (alpha satellite DNA) labeled with Spectrum green and the Y chromosome probe (satellite III DNA) labeled with Spectrum orange. The Y chromosome probe is highly specific for the repeated alphoid DNA located at the centromere of human chromosome Y while X DNA probe hybridizes to the short repeats related to the AATGG in the pericentric heterochromatin of the X chromosome. The hybridization efficiency of the Y probe using FISH has been examined in our laboratory on cytogenetically normal males (46,XY) and females (46,XX) uncultured tissue samples (n = 180). In each instance, 100 interphase cells per case were counted for the presence or absence of a Y signal. These studies demonstrated the Y probe to be present in 99.97% of cells in males and 0.001% in females.

Slides were dehydrated through a series of ethanol dilutions (70%/80%/90%/100%) then denatured in 70% formamide/2XSSC at 75°C for 5 minutes followed by dehydration through an ethanol series and the slides were air dried. The DNA probes were denatured at 75°C for 5 min. Slides were hybridized simultaneously with the chromosome X and Y DNA hybridization mixture being added to each slide, then covered with a glass coverslip and sealed with rubber cement. Slides were incubated at 42°C for 1 h in a humidified chamber. Post-washing was done in 2XSSC at 75°C for 2 min, followed by a washing in 2XSSC/0.1% NP40 at room temperature for 1 min.

Cells were counterstained with DAPI (4,6-diamino-2-phenylindole) and covered with a glass coverslip prior to microscopic analysis using a fluorescent microscope equipped with a triple band pass (Omega, N.H.). Cells were easily visualized and only non-overlapping cells were counted. Cells were scored as positive and of male origin if an orange fluorescent signal indicating the presence of the Y chromosome was identified within a nucleus. One slide was examined per sample with analysis performed on 100 cells.

Results

In those samples in which the blood solely originated from either a male or a female, FISH detected only the presence of male or female cells, respectively (Table 1). FISH correctly identified the presence of male cells in all mixtures in which male blood was diluted in female blood (Fig. 1, Table 1). The percentage of male cells detected by FISH was comparable to its corresponding dilution factor.

Discussion

Identification of a suspected stain as human blood is one of the most important steps in the process of evidence examination. The presence and identification of blood is often crucial to the medicolegal process. It is therefore essential to obtain as much information as possible from bloodstains at the scene or from the clothes of a suspect. A number of techniques are used in determining and identifying various characteristics of bloodstains. These include:

histological, chemical, immunological, species determination, sex determination, age of stain, race and whether it is menstrual blood.

Simply establishing the blood type/group of a victim and a suspect is not useful if they are the same. In those instances, identification of gender may play an important role. Forensic gender identification has been achieved by testing for Barr Bodies, X and Y chromosomes and testosterone/estradiol ratios [*1*]. Sex identification of bloodstains has also been done by using fluorescent dyes that bind to the heterochromatic region of the Y chromosome within cells [*2,3*]. However, interpreting this test can be difficult. Molecular genetics techniques using Y-specific DNA probes have been utilized for gender determination of dried bloodstains for further evidence [*4–7*]. As with any molecular genetic technique however, they can be timely, costly and nearly always use radioactive probes. Use of non-radioactive labeling methods has helped eliminate some of these problems [*4,6*].

FISH is a new molecular cytogenetic genetic technique that uses a non-radioactive fluorescent labeled DNA probe(s) specific for a chromosome or chromosomal region that are hybridized to their homologous regions in cells. Identification of a chromosome(s) in cells is done by the observation of a fluorescent signal(s) under a fluorescent microscope. FISH is a relatively simple, rapid laboratory procedure with a high sensitivity and specificity in detecting human chromosomes and/or chromosomal regions in both metaphase and interphase cells. This technique has already found important roles in clinical and research areas of cytogenetics, prenatal diagnosis, tumor biology, and gene mapping [*8–10*].

Our laboratory has recently and successfully used FISH to provide forensic laboratory evidence for sexual assault cases by identifying non-sperm male epithelial cells in cervicovaginal smears [*11,12*]. As a natural extension of the application of this technique in forensic laboratory investigations, we tested the efficacy of FISH to identify correctly the gender of dried bloodstains. The results of the current study demonstrated that FISH could not only correctly identify the gender of an individual on 2 week old dried blood samples obtained from fabric but also identify low levels of cross-contaminating blood from an opposite sex.

An inherent obstacle with forensic molecular genetic testing is the possibility of contamination of DNA from another individual that can result in a false positive, especially when the polymerase chain reaction (PCR) is used. In the current test, only white blood cells are examined thus reducing the possibility of a false positive due to the contamination of an epithelial cell as can occur with PCR. In addition, the use of multicolor FISH can clearly demonstrate the presence of an X and Y chromosome (hence male) in the same nucleus reducing the likelihood of a false positive result due to non-specific hybridization of a Y probe.

FISH is a simple, rapid laboratory procedure that can be performed using commercially available kits. The technique is highly sensitive and specific, providing convincing additional evidence not otherwise obtainable through current methods. We have thus far demonstrated the application of FISH to identify correctly the gender of dried bloodstains and identify the presence of male epithelial cells in sexual abuse cases [*11,12*]. We believe that FISH in forensic science will see increased utilization and applications. In addition, adaptations of this technique to identify a specific individual will undoubtedly be of great value. Further studies in our laboratory are currently underway.

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Address requests for reprints or additional information to
Mark J. Pettenati, Ph.D.
Department of Pediatrics, Section on Medical Genetics
Bowman Gray School of Medicine
Medical Center Drive
Winston-Salem, NC 27157