TECHNICAL NOTE

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Restriction Fragment Length Polymorphism (RFLP) Typing of DNA Extracted from Nasal Secretions

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ABSTRACT: The restriction fragment length polymorphism (RFLP) analysis of blood, semen, and other body fluids, has become increasingly important in violent criminal cases. The identification of additional tissues suitable for comparison with suspected donors has obvious potential benefit. One type of tissue, that found in nasal secretions, has previously received little attention with regards to genetic analysis. We collected blood and nasal secretion samples from eight individuals, subjected them to traditional RFLP typing methods, and analyzed the results using probes for loci D2S44, D1S7, D10S28, D4S139, and D17S79. All nasal samples provided high DNA yields and hybridization results that matched the corresponding blood standards. Thus, nasal secretions are shown to have potentially significant evidentiary value.

KEYWORDS: RFLP, DNA typing, nasal secretions, mucus

The restriction fragment length polymorphism (RFLP) analysis of blood, semen, and other body fluids has become increasingly important in violent criminal cases [*1–4*]. The identification of additional tissues suitable for comparison with suspected donors has obvious potential benefit and has been pursued [*5,6*]. One type of tissue, that found in nasal secretions, has previously received little attention with regards to genetic analysis. In sexual assault cases, nasal secretions collected from a victim following fellatio may be important evidence. In other criminal cases, items such as the clothing of missing children or handkerchiefs and tissues used by a suspect and left at a crime scene may provide valuable information [*7*].

In this paper, the authors present RFLP results for nasal secretion stains and compare those patterns with corresponding blood standards' results. We demonstrate that deoxyribonucleic acid (DNA) can be isolated, with identifiable banding patterns obtained, from nasal secretion stains.

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Materials and Methods

Sample Collection

Eight individuals were given clean facial tissue and asked to blow their nose. The facial tissues were collected and each was placed into a paper envelope and stored at 4°C until analysis. Reference blood standards were collected from the same individuals, either by drawing into a tube with EDTA preservative and subsequently drying onto clean cotton cloth, or by fingerprick with immediate placement onto cloth. After drying at room temperature, the reference blood stains were each placed into a paper envelope and stored at −80°C until analysis.

Extraction of DNA from Nasal Secretions and Bloodstains

The bloodstains were extracted using the FBI standard RFLP protocol for the extraction of body fluid stains [*8*]. The facial tissues were first visually inspected for the nasal secretion stains, then tactually examined for crusty areas, and finally, visually assessed under ultraviolet light, circling the stained areas with a pencil. An approximately 3 cm^2 cutting of each stain was placed into a 1.5 mL microcentrifuge tube.

A slightly modified version of the FBI standard RFLP protocol for the differential extraction of DNA was then performed [*9*]. While the samples tested did not include any sperm mixtures, the differential extraction method was utilized in anticipation of future casework, which may. The procedural modifications involved an increase in the volume of extraction buffer (TNE) to accommodate the size of the tissue cutting, and an omission of the differential separation step, since there were no mixed samples.

The method was as follows: (1) 600 μL tris/EDTA/NaCl (TNE), 25 μL 20% sarkosyl, 75 μL distilled H_2O and 5 μL proteinase K were added to each sample. (2) Contents were mixed and incubated in a 37°C waterbath for two hours. (3) Sample substrates were then placed into the tube cap and spun for five minutes, with supernatant then removed to a new microcentrifuge tube [*10*]. The remaining fraction was not processed any further. The nasal extracts were then subjected to the remainder of the FBI standard RFLP protocol, beginning with a phenol/chloroform/iso-amyl alcohol cleanup to ensure complete removal of any contaminants [*8*].

Analysis of DNA

Following extraction, the DNA was quantified on a 1% agarose minigel, again following the FBI RFLP protocol [*8*]. The band of

TABLE 1—*Relative quantities of DNA extracted from blood and nasal secretions from the same individual.*

Sample number	Blood standards (ng)	Nasal secretions (ng)
1	504	504
2	1000	>4000
3	800	>4000
4	1000	4000
5	800	4000
6	504	248
7	504	1000
8	504	800

high molecular weight DNA recovered from each sample was visualized on an ultraviolet light box and quantity was estimated through comparison with Lambda phage DNA standards ranging from 500 ng to 15 ng. Table 1 includes the approximate DNA extraction yield for each sample.

The DNA was digested with Hae III (Gibco BRL, Gaithersburg, MD) at 37°C overnight, after which another minigel was run, this time to qualitatively assess the restriction success. Two analytical gels, each containing four blood standard and four nasal secretion samples, were then run. Per the protocol, the gels were 1% agarose (Ultrapure, Gibco BRL) and contained 0.01% ethidium bromide. The 11 × 20 cm gels included a 23 kb ladder (Gibco BRL) as a molecular weight standard, cell line K562 (Gibco BRL) as an allelic control, and Kpn 1 digested adenovirus (Gibco BRL) as a visual marker.

After the 17 hour, 26 volt run in TAE buffer supplemented with 0.01% ethidium bromide, the gels were checked under ultraviolet light, assessing the migration of the visual marker to ensure complete fragment separation. Then, the gels were Southern blotted onto nylon membranes (Pall Biodyne, Charlotte, NC).

Probes and Autoradiography

The probes (loci) used for this study were as follows: YNH24 for locus D2S44 (Promega Corporation, Madison, WI), MS1 for locus D1S7 (Lifecodes Corporation, Valhalla, NY), PH30 for locus D4S139 (Gibco BRL), TBQ7 for locus D10S28 (Promega), and V1 for locus D17S79 (Lifecodes). These probes are the five currently used for casework analysis in the Indianapolis-Marion County Forensic Services Agency (IMCFSA) laboratory.

The probes were labelled in-house using the random primer labelling method (Gibco BRL) followed by either nick column (Sephadex G-50, Pharmacia, Piscataway, NJ) or spermine precipitation cleanup. The autoradiogram films (Kodak XAR) were developed after a variety of time periods, ranging from two to fourteen days exposure time at −80°C.

After visual analysis, the autoradiograms produced for each probe were subjected to computer assisted sizing using the FBI Image Analysis Software [*11*]. Approximate base pair (molecular weight) sizing estimates were obtained for each sample. These values were then compared for each nasal secretion/blood standard pair. The results are shown in Table 2.

Results and Discussion

Table 1 lists the estimated quantity of DNA extracted from each sample. It is clear from these results that nasal secretions contain sufficient cellular material to yield high quantities of DNA.

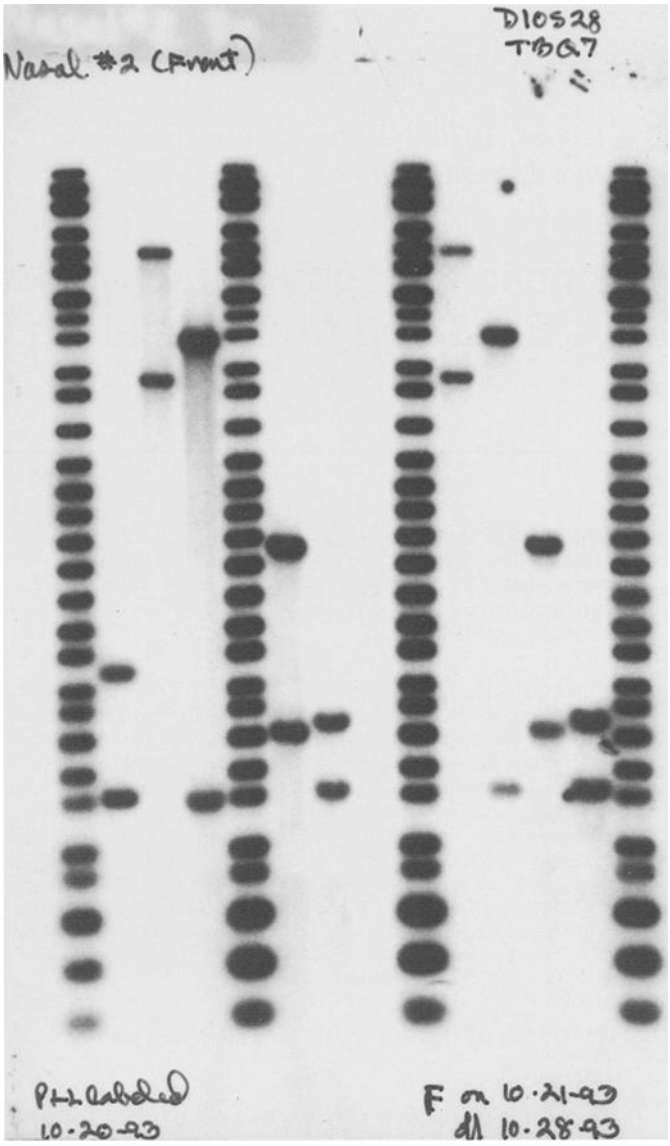

FIG. 1—*DNA profile results for the genetic locus D10S28 for nasal secretions (Lanes 10, 11, 12, and 13) and blood standards (Lanes 3, 4, 6, and 7). Lanes 1, 5, 9, and 14 contain size markers. Lane 2 is the K562 human cell line control. Lane 8 is an empty lane.*

Figure 1 shows autoradiogram results from hybridization with TBQ7 (D10S28). It can easily be seen that all of the blood samples visually match the nasal secretion sample of the corresponding individual. This is representative of the results for all probes.

Tables 2 and 3 list the molecular weight sizes and the percent difference between the blood and nasal secretion samples for each individual. All samples compared fell within the 6% match criteria established at IMCFSA.

Figure 2*A–E* graphically compare the sizing results for the blood standards to those for the nasal samples. Results were as expected, with the greatest variation found in samples above 10 000 base pairs.

The blood and nasal secretion samples from all eight individuals that were subjected to traditional RFLP typing methods and analyzed using probes for loci D2S44, D1S7, D10S28, D4S139, and D17S79, provided results as expected. All nasal samples provided

TABLE 2—*Sizing results for nasal and blood samples #1–4. All samples fall within the IMCFSA 6% match window. Samples >11 919 are inconclusive.*

		Blood sizing		Nasal sizing		% Difference Nasal to blood	
	Probe	Band 1	Band 2	Band 1	Band 2	Band 1	Band 2
Sample # 1	D1S7	7411	3647	7554	3740	1.93%	2.55%
	D2S44	2275	1798	2346	1852	3.12%	3.00%
	D4S139	8166	6658	8247	6842	0.99%	2.76%
	D10S28	1495	1034	1559	1059	4.28%	2.42%
	D17S79	1482	1255	1543	1321	4.12%	5.26%
Sample # 2	D1S7	7311	1869	7201	1963	−1.50%	5.03%
	D2S44	6001	1264	5921	1295	−1.33%	2.45%
	D4S139	6727		6678		−0.73%	
	D10S28	4970	3647	4994	3692	0.48%	1.23%
	D17S79	1775	1430	1814	1458	2.20%	1.96%
Sample # 3	D1S7	7587	984	7420	941	−2.20%	−4.37%
	D2S44	2330	1836	2295	1803	−1.50%	−1.80%
	D4S139	8343	5531	8332	5384	−0.13%	−2.66%
	D10S28	3987	1058	3906	1027	−2.03%	−2.93%
	D17S79	1535	1310	1515	1283	−1.30%	−2.06%
Sample # 4	D1S7	3924	3824	3894	3813	−0.76%	−0.29%
	D2S44	2314	1611	2363	1635	2.12%	1.49%
	D4S139	7655	5713	7738	5737	1.08%	0.42%
	D10S28	5831	985	5843	991	0.21%	0.61%
	D17S79	1377	1306	1394	1317	1.23%	0.84%

TABLE 3—*Sizing results for nasal and blood samples #5–8. All samples fall within the IMCFSA 6% match window. Samples >11 919 are inconclusive.*

		Blood sizing		Nasal sizing		% Difference Nasal to blood	
	Probe	Band 1	Band 2	Band 1	Band 2	Band 1	Band 2
Sample # 5	D1S7	8 563	2889	8 657	2898	1.10%	0.31%
	D2S44	5 125	1078	5 149	1096	0.47%	1.67%
	D4S139	9 325	5417	9 485	5443	1.72%	0.48%
	D10S28	8 133	4522	8 177	4560	0.54%	0.84%
	D17S79	2 902	1000	2 921	1005	0.65%	0.50%
Sample # 6	D1S7	11 261	3990	11 457	4088	1.74%	2.46%
	D2S44	1 903	1524	1 979	1571	3.99%	3.08%
	D4S139	11 895	4898	12 446	5029	inc.	2.67%
	D10S28	5 383	1171	5 378	1191	−0.09%	1.71%
	D17S79	1 501	1279	1 550	1316	3.26%	2.89%
Sample # 7	D1S7	9 791	9255	9 800	9170	0.09%	−0.92%
	D2S44	6 384	3459	6 366	3488	−0.28%	0.84%
	D4S139	9 772	5743	9 737	5760	−0.36%	0.30%
	D10S28	2 593	1452	2 600	1455	0.27%	0.21%
	D17S79	1 525	1376	1 533	1381	0.52%	0.36%
Sample # 8	D1S7	9 278	2937	9 381	2923	1.11%	−0.48%
	D2S44	4 273	3472	4 317	3496	1.03%	0.69%
	D4S139	17 661	7705	16 256	7863	inc.	2.05%
	D10S28	1 488	1191	1 497	1205	0.60%	1.18%
	D17S79	1 496	1314	1 510	1321	0.94%	0.53%

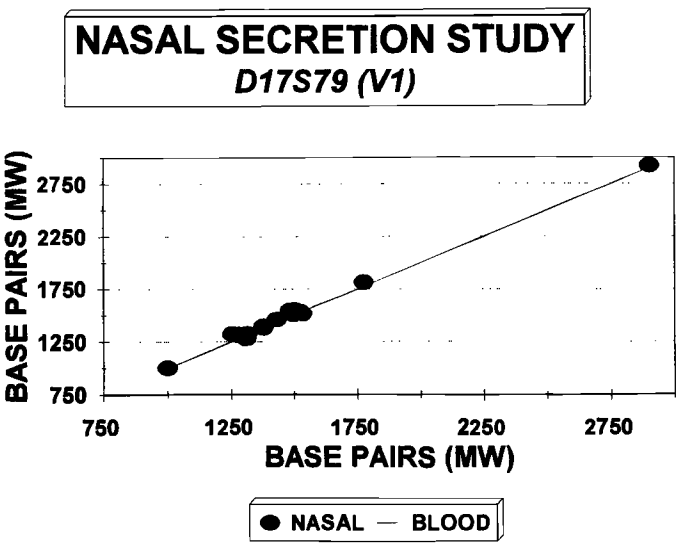

FIG. 2*a*—*D17S79 results. Blood standard results are depicted by the solid line, with the nasal samples band size indicated by the dots. Each probe exhibits very comparable blood and nasal secretion sizing data.*

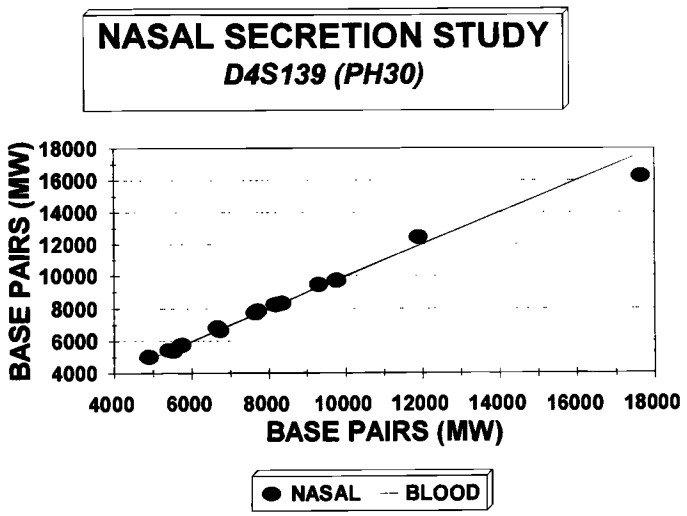

FIG. 2*b*—*D4S139 results.*

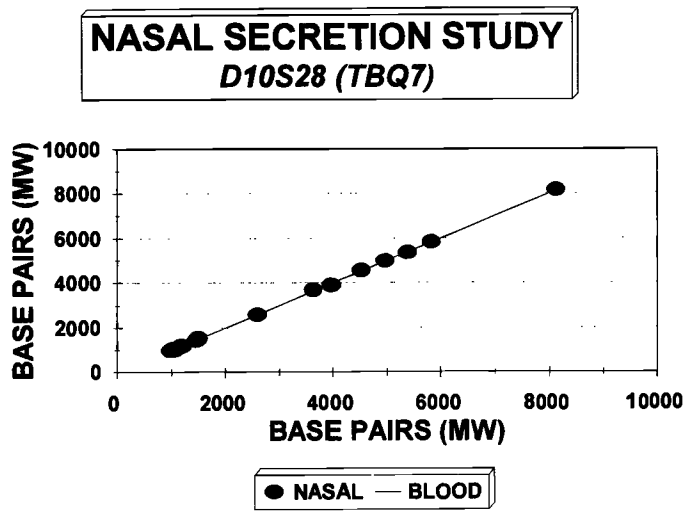

FIG. 2*c*—*D10S28 results.*

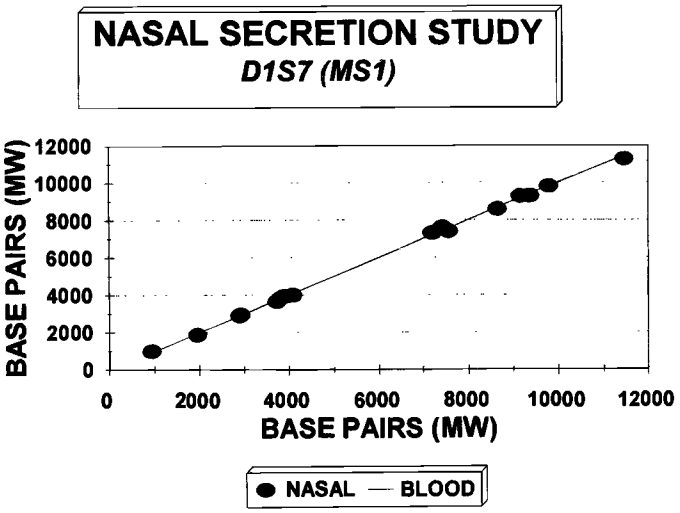

FIG. 2*d*—*D1S7 results.*

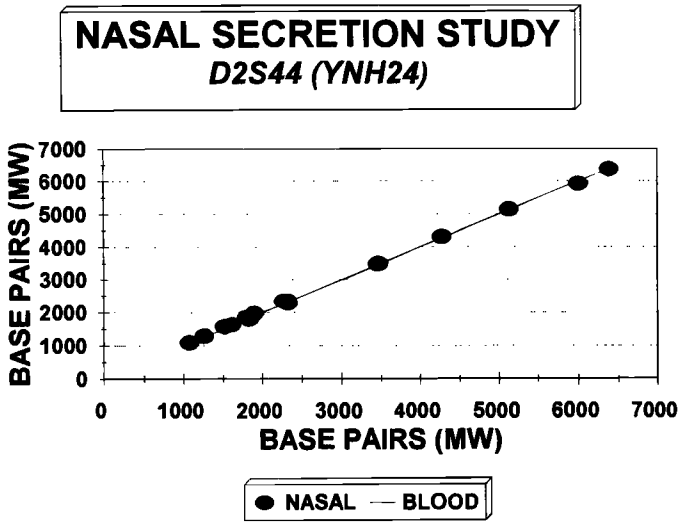

FIG. 2*e*—*D2S44 results.*

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Acknowledgments

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