TECHNICAL NOTE

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ABO Blood Grouping on Dental Tissue

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ABSTRACT: Twenty-five permanent teeth, including eight carious ones whose pulp cavities had been exposed, were used for this research 3–5 weeks after extraction. Phosphate-buffered saline (PBS, at pH 7.2) was employed to extract ABO blood group substance from tooth powder. ABO grouping was performed on blood-stained compresses from the extraction wound (as controls), tooth fragment, tooth powder, and cotton fibers immersed in PBS extract by absorption-elution (AE) technique and on the PBS extracts by the two-dimensional absorption-inhibition (2-D AI) technique. It was found that blood grouping in PBS extracts by 2-D AI yielded reliable results: no false positive results, and a high rate of correct grouping, (24/25), while blood grouping on other dental materials, such as tooth fragments, tooth powders, immersed fibers, by AE gave an unacceptable rate of false positive/negative results.

KEYWORDS: forensic science human identification, tooth grouping, ABO blood grouping, absorption-elution technique, two-dimensional absorption-inhibition technique, odontology

Blood grouping has been one of the cornerstones for identification of biological materials in forensic investigations, and ABO blood grouping is a widely used technique in forensic laboratories. The presence of ABO blood-group antigens in soft and hard dental tissues [*1–12*] makes it possible to assist in the identification of highly decomposed bodies, or of body parts where teeth and bone are the only significant tissues remaining. However, the low concentration of blood group antigens in tooth material, as well as the potential for oral and environmental bacterial contamination, may affect the blood grouping results [*11*].

For several decades, forensic scientists have been searching for a reliable method for the blood typing of teeth. Conventional absorption inhibition (AI) was employed for hard dental tissue by Shimura [*1*], and Suzuki et al. [*2*]. However, their methods required complicated pretreatments such as decacification, and the results were less than ideal. Using the mixed agglutination technique preceded by decalcification, Kramer [*13*] concluded that it had not been possible to demonstrate A and B blood group antigens in

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human dentine. About one decade later, the absorption elution (AE) technique was substituted for the AI technique almost exclusively for this purpose in forensic laboratories. Since 1971, a number of papers on blood typing of teeth under various conditions have been published in forensic journals [*3–12*]. However, the percentage of correct groupings varied from 64 to 100. The rate of false positive was found to be unacceptable. Therefore, the AE technique is still regarded as an unreliable method for the blood typing of tooth tissue [*10,11*].

In 1988, Lee et al. reported a novel inhibition procedure, called two-dimensional absorption-inhibition (2-D AI), whose sensitivity is 28 times that of conventional inhibition-titration tests [*14*]. This procedure has been successfully applied to the ABH grouping of urine, urine stains, perspiration stains [*14*] and bone tissue [*15,16*]. In this communication, we report the results of studies on the application of 2-D AI and AE to ABO grouping of dental tissue.

Materials and Methods

Twenty-five permanent teeth, including eight carious ones whose pulp cavities had been exposed, were used for this research. These teeth were extracted at the time of treatment at the Oral Department, Zhongshan Hospital, Shanghai Medical University and kept for 3 to 5 weeks after extraction at room temperature. Together with each tooth, a sterile cotton compress containing blood from the extraction wound was obtained as a reference sample to determine the donor's blood group, since this was more readily available than a liquid blood sample.

The pretreatment procedure of a tooth is shown in Fig. 1. The AE blood grouping

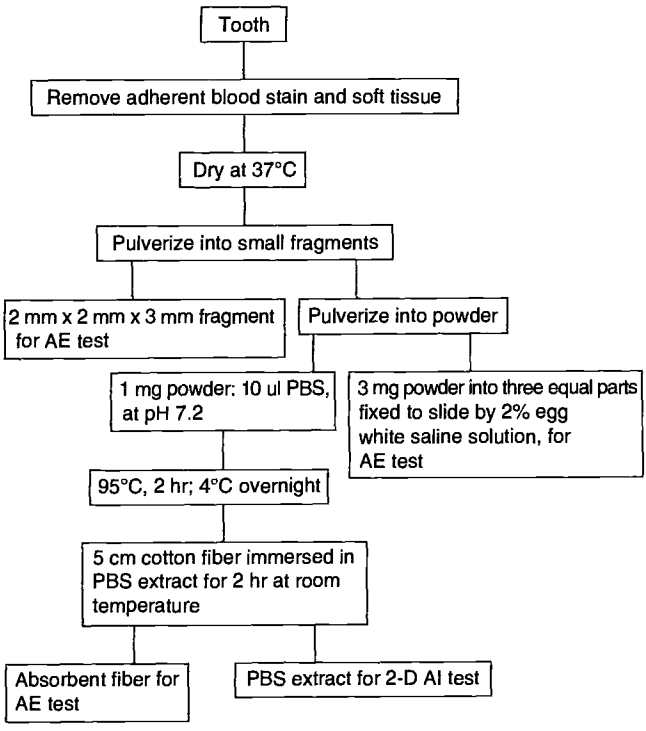

FIG. 1—*Pretreatment procedure of a tooth.*

technique as described by Smeet et al. [*11*] was performed on the bloodstained compress, tooth fragment, tooth powder and adsorbent cotton fiber of tooth extract. The 2-D AI procedure as described by Lee et al. [*14*] was performed on the PBS extracts. Polyclonal anti-A and anti-B antisera and anti-H lectin were obtained from Shanghai Central Blood Bank. Initial titers were all 128.

Results

Results obtained from the 25 permanent teeth are shown in Table 1. The rate of agreement between bloodstained compresses and tooth fragments, tooth powders, cotton fibers in PBS extract, and PBS extracts was 12/25, 16/25, 16/25, and 24/25, respectively. The rate of false positives was 4/25, 0/25, 2/25 and 0/25, respectively; and the rate of false negatives was 10/25, 9/25, 6/25, and 1/25, respectively.

Discussion

The presence of ABO blood group substances in blood [*17*] and body fluids (saliva, semen, liver bile, and other fluids) [*18*] was first recognized in 1900 and the 1920s, respectively. Blood in tooth pulp blood vessels provides abundant group antigens whereby its blood group may be determined. Suzuki attributed the origin of blood group substance in hard dental tissue to infusion-sedimentation of the blood group substance contained in saliva over a long period of time [*2*]. Smeets presumed that the blood group substance in dentine was located in dentine tubuli [*12*]. The distribution of ABH substances from

TABLE 1—*Blood grouping results of tooth materials.*

No.	Compress	Tooth fragment	Tooth powder	Adsorbent fiber	PBS extract
	(AE)	(AE)	(AE)	(AE)	(2-D AI)
1[a]	A	B	A	A	A
2	B	B	B	B	B
3[a]	A	AB	A	O	A
4	A	AB	O	A	A
5[a]	O	O	O	B	O
6	O	NAD	NAD	O	O
7	B	B	NAD	NAD	B
8[a]	A	A	A	A	A
9[a]	A	A	A	A	A
10	AB	B	AB	AB	AB
11	B	NAD	NAD	B	B
12	O	O	O	O	O
13[a]	AB	AB	AB	AB	NAD
14	A	A	A	A	A
15	A	B	A	A	A
16[a]	AB	AB	AB	AB	AB
17	O	O	O	O	O
18	B	B	B	B	B
19	AB	O	AB	A	AB
20[a]	A	NAD	NAD	AB	A
21	AB	NAD	NAD	AB	AB
22	A	NAD	NAD	A	A
23	A	NAD	NAD	NAD	A
24	AB	AB	AB	O	AB
25	AB	B	NAD	B	AB

[a]Carious tooth.
NAD: No antigen detected.

the pulp cavity wall to the dentine edge and to the enamel decreases gradually because of fewer possibilities for diffusion of antigens from both blood and saliva.

Although earlier work by others has shown that hard dental tissue contains blood group substances, their concentration is comparatively low, and blood grouping at the time by AI yielded unsatisfactory results [*1,2,13*]. Since the 1970s, the AE technique originally devised by Siracusa [*19*], and refined by Kind [*20*], has been employed almost exclusively for the blood typing of teeth in forensic laboratories. Although the AE technique is sensitive, we, as well as Smeets et al. [*11*], have found that it is not a reliable method because it gives an unacceptable high rate of ABO mistypings, producing not only false negative results but also false positive ones. As to the false positive results, gram-negative aerobic oral bacteria yield acquired antigen activity, especially acquired B antigen [*21*].

More recently, Lee et al. [*14*] reported a novel absorption inhibition procedure, called 2-D AI, which is characterized by high sensitivity and high specificity. Using the 2-D AI technique on tooth samples from 25 individuals, no false positives were observed and in only one sample did we fail to detect antigen. We conclude, therefore, that the 2-D AI technique is a reliable method for blood typing of teeth. Further research on blood-typing of teeth under various conditions by 2-D AI technique is being investigated.

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