The Typing of ABH Antigens in Human Bone

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INTRODUCTION

Forensic serologists are occasionally called upon to perform ABH grouping tests on human bones or bone fragments, usually to aid in the identification of human remains. The antigenic receptors which characterize the ABO system occur not only on the surfaces of blood cells but are widely distributed throughout human tissues (Schiff 1931; Hartmann 1941). Over the years, a number of reports on ABH grouping of various tissues have appeared (Gaensslen 1983; Slavik and Meluzin 1970; Troger et al. 1976) primarily using procedures which were devised originally for grouping ABH antigens in bloodstains. However, the application of blood grouping techniques to bone tissue has yielded mixed results, particularly when aged bones or bones subjected to putrefactive processes have been analyzed. Earlier investigators utilized absorption-inhibition procedures (Boyd and Boyd 1934; Candela 1936; Thieme and Otten 1956, 1957), while absorption-elution techniques have been utilized almost exclusively in more recent attempts to type ABH antigens in bone tissue (Borgonini 1968; Yada et al. 1972; Beyer 1982; Berg et al. 1983; Hauser et al. 1984). The percentage of incorrect results in these studies has been so high, however, that the procedures could not be regarded as reliable. Greater but still limited success has recently been reported by Hauser (1986) using a semiquantitative elution technique which we (Gaensslen and Lee 1984; Gaensslen et al. 1985a, 1985b) and others (Lincoln 1973; Lincoln and Dodd, 1973) have previously described and recommended for the evaluation of antisera for bloodstain grouping and for the grouping of blood group antigens other than ABH in bloodstains. Recently, we have explored a combination procedure for ABH typing of bone involving extractive absorption-inhibition and direct absorption-elution procedures (Lee et al. 1987). Because of the sensitivity required, we have found that the only extractive inhibition component of any value in this overall procedure is our recently described two-dimensional method (Lee et al. 1986; and see R.E. Gaensslen & H.C. Lee in these Proceedings).

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METHODS

Bone samples for analysis are first examined visually and microscopically for possible blood or other trace materials, and the type, size and weight of the sample are recorded. Samples are then washed successively in tap and distilled water, and in several organic solvents to remove fatty tissues. The sample is then minced or crushed into small fragments, which may be subjected to direct elution testing, or to further preparation for extractive inhibition testing (Figs. 1 and 2).

RESULTS and DISCUSSION

Results of testing 21 fresh and 37 older bone samples from persons of known ABO type by extractive two dimensional absorption inhibition are shown in Table 1. "Older" bone samples are those six months old or older.

Table 1. Absorption Inhibition Results on Bones from Persons of Known ABO Blood Group

Type of Bone	ABO Type	n	Result[a] A	B	H	AB	NAD	INC
Fresh	A	6	6	-	-	-	-	-
	B	4	-	4	-	-	-	-
	O	11	-	-	11	-	-	-
	AB	-	-	-	-	-	-	-
Older	A	10	9	-	-	-	1	-
	B	10	-	9	-	1	-	-
	O	15	-	-	12	-	3	-
	AB	2	-	-	-	2	-	-

[a] NAD = no antigen detected; INC = inconclusive

Of 58 samples tested, 54 yielded conclusive results. No antigens were detected in the remaining four. All fresh bones and all except one older bone giving conclusive results were correctly typed. One sample from a group B person yielded an A + B antigen result.

Results of testing 71 fresh and 41 older bone samples from persons of known ABO blood group are shown in Table 2. Of 71 fresh bone samples tested, all but one yielded conclusive results. However, one group B sample yielded H antigen, and 5 group O samples yielded A antigen results. With 41 older bone samples, 25 yielded conclusive results and no antigen was detected in 16 others. Five of the 25 samples giving conclusive results, however, gave incorrect results.

Table 2. Absorption Elution Results on Bones from Persons of Known ABO Blood Group

Type of Bone	ABO Type	n	Result[a] A	B	H	AB	NAD	INC
Fresh	A	19	19	-	-	-	-	-
	B	19	-	18	1	-	-	-
	O	33	5	-	27	-	-	1
	AB	-	-	-	-	-	-	-
Older	A	10	4	-	-	1	5	-
	B	10	-	5	-	1	4	-
	O	19	-	3	10	-	6	-
	AB	2	-	-	-	1	1	-

[a] NAD = no antigen detected; INC = inconclusive

Our results to date are consistent with those of previous investigators in indicating that neither elution nor inhibition (including the two dimensional) procedures alone yield uniformly correct typing results from bone samples.

Figure 3 shows a representation of our current overall approach to bone grouping as developed to the present time. If a blood group is assigned only in cases where the sample yields conclusive results with both elution and inhibition, and where the results are consistent with each other, no erroneous typing results have been observed in our studies thus far.

Studies on bone grouping are continuing in our laboratories to test additional samples for ABO antigens, and to determine whether the Gm/Km antigens can be determined in bone tissues. Inhibition procedures as well as ELISA techniques can be used for Gm/Km antigen typing. In addition, DNA can be isolated from fresh human bone samples, and its nature and quantity in this tissue are also being studied.

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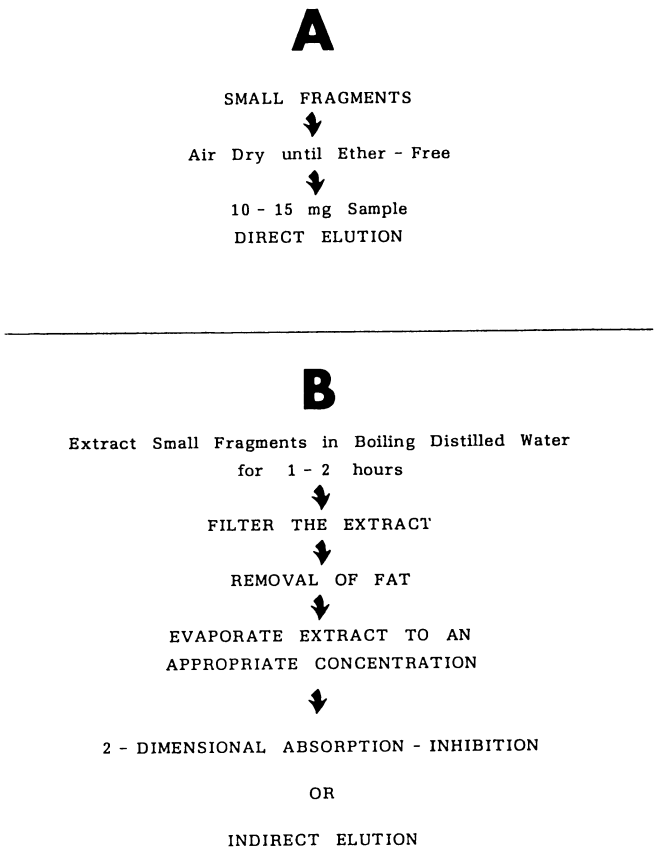

Fig. 1. Preparation of Bone Samples for Grouping Procedures

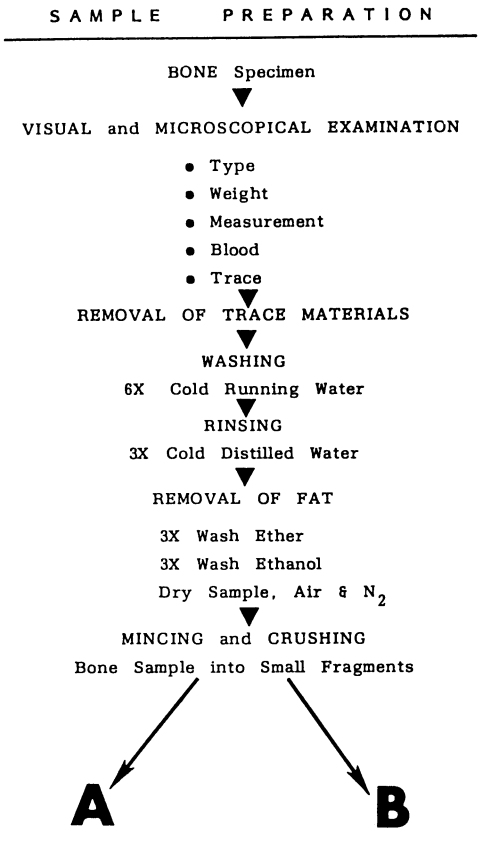

Fig. 2. Preparation of Bone Samples for Direct Grouping or Extraction for Indirect Grouping

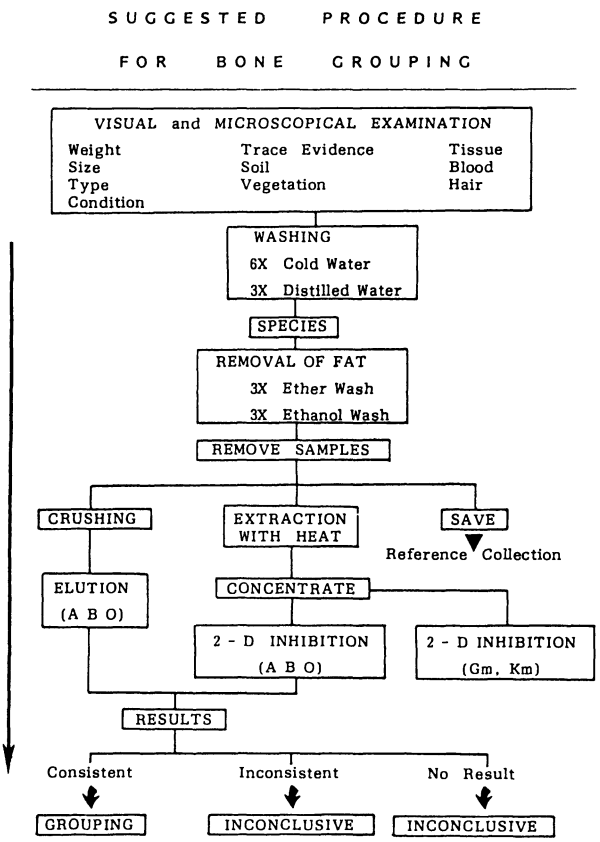

Fig. 3. Overall Procedure for Bone Grouping and Interpretation of the Results