US ARMY MEDICAL RESEARCH LABORATORY

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THE FORENSIC TESTING LABORATORY, 1971-- PROBLEMS, PROGRESS, AND PEOPLE

(Progress Report)

by

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30 June 1971

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13. ABSTRACT

Current technics of blood group serology are presented as useful tools of forensic identification. These procedures cover human and animal sources of blood, bone, hair, saliva, semen, and nails. Various problems and pitfalls are discussed as they relate to validity of testing and reporting medicolegal materials. Special consideration is given to the problem of staffing a forensic laboratory and deciding who is qualified to perform tests. A criterion of qualifications is suggested as representative of the minimum requirement.

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ABSTRACT

THE FORENSIC TESTING LABORATORY, 1971-- PROBLEMS, PROGRESS, AND PEOPLE

OBJECTIVE

Current serologic technics employed in the Reference and Forensic Testing Laboratory, Blood Transfusion Division, US Army Medical Research Laboratory, Fort Knox, Kentucky, are presented for use in forensic identification of specimens. Various problems of methods, personnel, controls, and the pitfalls encountered are discussed.

METHODS

Preservation and collection of controls were accomplished on the various specimens submitted. Technics for origin determination and the resulting ABO grouping of blood crusts, bloodstains, saliva, semen, hair, and bone were evaluated and employed by a reference and blood grouping laboratory. A review of forensic literature was made and references are quoted for further investigation.

RESULTS

Establishment of protocol and forms for identifying, receiving, and reporting results were among the chief considerations in establishing a forensic testing laboratory. Controls are also of major importance and must be representative of the material submitted for identification. Special consideration is given to the problem of staffing a forensic laboratory and deciding who is qualified to perform the tests. A criterion of qualifications is suggested as representative of the minimum requirements.

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INTRODUCTION

In the practice of modern medicine the Forensic Testing Laboratory is becoming increasingly more important in resolving medicolegal problems. Much of this work may be performed in a reference and blood grouping laboratory by trained, competent personnel.

Specimens received for testing include: whole blood, blood crusts, bloodstains, seminal fluid and stains, hair, nails, saliva, and bone. The specimens may be human, animal, or both; this must be taken into account in any test procedure. Tests performed should include the detection of the human blood group systems and subgroups, antihuman precipitin tests, inhibition technics, and special assays including use of the AutoAnalyzer for quantitation and Gm typing.

IDENTIFICATION OF SAMPLES AND PROTOCOL FORMS

Since most testing will eventually have medicolegal application, it is essential to establish and document positive identification of all samples received in the laboratory. Protocol and forms for this procedure must be carefully designed by the laboratory to support and validate all handling, testing, and reporting. A copy of the protocol form used in reporting paternity testing by the Reference and Forensic Testing Laboratory, Blood Transfusion Division, US Army Medical Research Laboratory, Fort Knox, Kentucky, is reproduced in Figure 1. Tests are performed independently by two individuals, using different lots of reagents, and reliable controls.

PRELIMINARY AND ORIGIN TESTING

There are several chemical tests used to determine the presence of blood in a stain. The benzidine test is commonly used; it is very sensitive and will eliminate those stains which are not blood. Next, to establish the origin of a bloodstain, a precipitin test is performed. For example, precipitin serum is prepared by injecting a rabbit with human serum (antihuman rabbit serum). This neat rabbit serum is specific for human protein present in a bloodstain. A dilute extract of the stain is overlayed onto antihuman precipitin serum. A white precipitate present in the interface between the extract and the antihuman precipitin serum indicates that the stain is of human, or primate, origin and not lower animal blood. Antisera for most of the common domestic animals and some of the more common wild animals are available commercially.

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*Presented in part at the 23d Annual Meeting of the American Association of Blood Banks, 25-30 October 1970, San Francisco, California.

IDENTIFICATION OF A BLOODSTAIN

A relatively simple and reliable procedure for the determination of the A-B-O groups in a bloodstain is that of Dr. A. S. Wiener (1). This is a direct method to determine the presence of isoagglutinins in a saline extract of the stain. A control, using an unstained portion of cloth, must be tested concurrently. An indirect method, that of Coombs and Dodd, employs an inhibition technic whereby a small portion of the stain is exposed to a dilution of antisera and titered to demonstrate a reduction in antibody reactivity (2). Successful identification of the MN blood groups in dried bloodstains was reported by Margaret Pereira in 1963 (3). Using an inhibition procedure described by Jones and Diamond, the Kell factor may be identified from a bloodstain (4). The determination of the Rh factors in bloodstains has been partially successful using an absorption-elution method (5). Determination of the Rh phenotype varies with the selection of antisera used and the condition of the stain. Experimental studies have shown it is possible to determine Gm groups (6) and haptoglobin types in dried bloodstains (7).

IDENTIFICATION OF A BLOOD CRUST

A blood crust results when a stain is present on a nonabsorbent material such as glass, metal, stone, etc. Either a direct method or an inhibition technic may be employed in determining the blood type. In the direct method, equal portions of the unknown blood crust are deposited on three separate areas of a glass slide (Fig. 2). The particles of crust are overlayed separately with fresh A_1, B, and O cells and a cover slip. A similar preparation is made with control crusts. The tests are incubated approximately 30 minutes in a moist chamber at room temperature. During this incubation phase they are removed periodically and observed microscopically for the presence or lack of agglutination. An example of a group A crust is seen in Figures 3, 4, and 5 (8).

In the inhibition procedure the blood crust is reduced to a rough powder. Small amounts of powder (10 mg) are added separately to diluted anti-A and anti-B antisera in small test tubes. The antisera should be titered and diluted so as to contain about 4 to 7 agglutination doses. In general, the dilution having a 1+ reaction is used. The mixtures are kept for 30 to 60 minutes at room temperature, then centrifuged and the supernatant fluid tested for its agglutinin content. The absence of the isoagglutinin from either test serum, or a significant reduction in the titer of the test serum, indicates the presence of the corresponding agglutinogen in the blood crust (8).

Known blood crust controls are necessary for concurrent testing with the unknown sample. These controls are preserved in parallel on glass slides stored in wooden boxes and as chipped crusts sealed in glass ampoules, at room and at cooler temperatures. Crusts are maintained on the major blood groups, their quantitative variants, and as Group O crusts classified according to other blood group systems. Genetic coding includes sex, age, race, and geographical location if indicated. The blood crusts are prepared from *fresh shed blood* rather than *anticoagulated blood* (9).

BLOOD GROUP DETERMINATION FROM HAIR AND NAILS

Blood group determination from hair and/or nails can be of considerable importance in the medicolegal investigation in cases of violent death. Much investigation in this area of forensic medicine has been done by Dr. Shoichi Yada in Japan. A technic developed by Dr. Yada employs an elution method after first carefully washing and completely crushing the hair specimen (10). Figure 6 shows an excellent result obtained by this method.

Lincoln and Dodd at the London Hospital Medical College in London, England, have used mixed agglutination as a method for the determination of A, B, and H blood groups of hair (11). The most successful results were obtained using shavings rather than head hair.

Few experiments have been reported using nail fragments in determining the A-B-O group for identification purposes. However, R. A. Outteridge has reported successful results using an absorption-elution technic similar to that employed in grouping bloodstains on fabrics (12).

A simple method for blood grouping fingernails has also been reported by Dr. Shoichi Yada (13).

It is worth noting that the hair and nail, in contrast to the red blood cell, possess low amounts of the A-B-H antigens; nevertheless the elution method has been used successfully with these substances. Because hair and nails are simple to collect and can be preserved almost indefinitely without any protective measures, it can be anticipated that they will be used extensively in forensic medicine.

BLOOD GROUP DETERMINATION FROM BONE

The success in the grouping of skeletal remains by the absorption technic probably depends on the presence or absence of A-B-H substance in the dried marrow lining the cancellous bone used for the examination.

In 1936 P. B. Candela reported the successful blood type determination of Egyptian mummies using an absorption procedure (14-18). Since that time testing procedures have advanced little, if at all.

Bone, of itself, makes a difficult working specimen, since it has a high degree of nonspecific adsorptive power. This makes it essential to start with A and B test sera of exactly equal strength; if the sera are not balanced or if they become unequal through deterioration, the nonspecific reduction in titer of the weaker one may lead to a false A or B interpretation. Therefore, the preliminary titration of the test sera is of the utmost importance.

The condition of the bone specimen submitted for blood group determination is most important. It must be free of any preservative or chemicals. Only a small section of the cancellous bone is necessary for the absorption-inhibition procedure. As shown in Figure 7, the specimens are freeze-dried 24 to 48 hours, depending on the amount of moisture present. This lyophilization procedure also breaks down the undesirable fat residue which may interfere with the serologic reactions.

Next, as seen in Figures 8, 9, and 10, the cancellous interior is scraped, pulverized, and the previously determined dilution of antisera added. Incubation at 4 C for 24 to 48 hours follows. Two technics are employed in this laboratory. Using the microtiter technic, titers demonstrating group A and group O bone are shown in Figure 11.

Bone controls of known blood groups are a prerequisite for testing. These may be obtained from autopsy or amputation, along with a blood sample necessary to determine blood type. They are freeze-dried, pulverized, and stored in sealed glass ampoules.

PATERNITY TESTING INCLUDING Gm TYPING

Paternity testing is generally avoided by the general hospital blood bank, and rightly so. However, with personnel trained in forensic testing procedures, specific antisera and proper controls, the reference laboratory need not shun the paternity case.

Most important is the blood specimen. It *must* be properly collected and labeled. Of no less importance is the verification and identification of the individuals bled by the phlebotomist.

Antisera for the blood group systems, A-B-O--including subgroups of A, Rh, and M-N-S, are commonly used in paternity testing. Tests should be performed independently by two individuals, using different lots of reagents and reliable positive and negative controls. Most investigations are limited to three antisera in the A-B-O system, including absorbed human anti-A serum or anti-A_1 lectin, three antisera in the M-N-S system, and four antisera in the Rh system. Thus, in the A-B-O system, it is usually said that 17 percent of falsely accused men could be excluded; with the M-N-S system it approaches 27 percent and with the Rh system, 25 percent. The chance of exoneration by use of all three systems is not exactly their sum, because of the possibility of exclusion by more than one system. Thus, the chance of exoneration with the three commonly used systems is on the order of 55 percent (19). Tests with antisera of the other red cell and serum systems can still provide valuable evidence even though they do not have the medicolegal acceptance of A-B-O, M-N-S, and Rh testing.

The use of the Gm system in conjunction with the blood group systems has been reported as a possible adjunct in paternity cases (20). Human sera retain the Gm factors well at 20 C. An example of application is seen in Figure 12. The blood groups provide an exclusion on the basis of the MN blood groups for the putative father. However, with the additional Gm typing, another exclusion is provided. The findings are interpreted as showing the child possessing the genetic antigen (Gm f+) which could not have been inherited from the mother or putative father. Such a finding indicates the father can be excluded as the child's father. These findings do not constitute *legal evidence* and, as such, can be used only in addition to the other blood grouping studies.

SPECIAL CONSIDERATIONS

The performance of tests and the variety of specimens received in a forensic testing laboratory have been discussed. Let us now turn to the area of personnel. Staffing with qualified, experienced, and competent personnel is a difficult and continuous problem for the director of any forensic testing laboratory.

Who is qualified to perform forensic tests? There are no specific standards or guidelines for teaching or examining an individual interested in pursuing a career in forensic science. This makes the question of qualifications more difficult. The following criteria have been suggested as representative of the *very minimum requirement*:

a. Medical Technologist or equivalent.

b. Reference laboratory experience - 2 years.

c. Forensic laboratory experience - 1 to 2 years.

d. Continuing education to include:

(1) Formal training - genetics, cytogenetics, biochemistry and immunohematology, leading to advanced degrees.

(2) Liaison with other forensic testing laboratories such as the laboratory of Dr. A. S. Wiener.

(3) Attendance at forensic conferences.

(4) Review of the literature in forensic sciences.

While it is true that the legal and practical use of certain forensic group testing procedures has advanced painfully slow, forensic pathology has grown exceedingly well and centers for expert training exist. We feel that the future and utility of forensic serologic tests lies primarily along this newly trodden path of forensic pathology.

SUMMARY

Current technics of blood group serology are presented as useful tools of forensic identification. These procedures cover human and animal sources of blood, bone, hair, saliva, semen, and nails. Various

animal sources of blood, bone, hair, saliva, semen, and nails. Various problems and pitfalls are discussed as they relate to validity of testing and reporting of medicolegal materials.

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Figs. 1-1, 1-2, 1-3. Form for reporting results of blood grouping.

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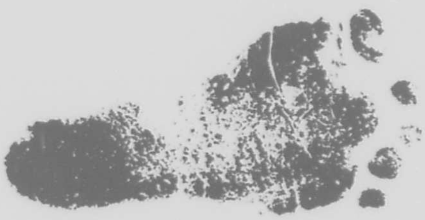


Ellen Marie Smith (daughter)

REPORT ON
BLOOD GROUPING TEST

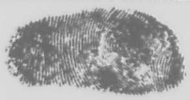
NAME	IDENTIFICATION
Samuel S. Smith 405-65-8341 (Husband)	 Blood sample was certified as received at Fort Knox, Ky
Mary Jane Smith (Wife)	 Blood sample was certified as received at Fort Knox, Ky

Fig. 1-1

Results:

	Blood of	Group and Subgroup	M-N Type	Rh-Hr Type
1.	Samuel S. Smith	O	MNss	R_1R_1
	[redacted]			
2.	Mary Jane Smith	O	MMSS	R_1R_1
3.	Ellen Marie Smith	A_1	MNSs	R_1r
4.				
5.				

Interpretation:

Samuel S. Smith is group O, Rh positive, and type MN
Mary Jane Smith is group O, Rh positive, and type MS
Ellen Marie Smith is group A_1, Rh positive, and type MNSs

The agglutinogen A cannot appear in the blood of a child, unless present in the blood of one or both parents. A second exclusion is provided under IV, the Rh blood types, example 1.

FRANK R. CAMP, JR.
LTC, MSC
Director, Blood Transfusion Division

LAWS OF HEREDITY

I. The Blood Groups (O, A, B, and AB)

1. The agglutinogens A and B cannot appear in the blood of a child unless present in the blood of one or both parents.
2. Group AB parents cannot have group O children, and group O parents cannot have group AB children.

II. The blood types (M, N and MN)

1. The agglutinogens M and N cannot appear in the blood of a child unless

Fig. 1-2

present in the blood of one or both parents.

2. Type M parents cannot have type N children, and type N parents cannot have type M children.

III. The Subgroups of Group A (A_1 and A_2) and group AB (A_1B and A_2B)

1. No child can belong to subgroup A_1 or subgroup A_1B unless one or both parents belongs to one of these subgroups. For example, two parents both of subgroup A_2 cannot have a child of subgroup A_1.
2. Parents of subgroup A_1B cannot have children of subgroup A_2, and parents of subgroup A_2, cannot have children of subgroup A_1B.

N.B. When a man is excluded by these tests, it is highly probable, though not absolutely certain, that he is not the father of the child in question.

IV. The Rh Blood Types [rh, rh', rh", rh'rh" (or rh_y), Rh_0 , Rh_1, (or Rh_0 '), Rh_2 (or Rh_0 ") and Rh_1Rh_2 (or Rh_z)]

The Hr Factors: Rh_1Rh_1=Rh_1hr'-, Rh_1rh=Rh_1hr' +, etc.
Rh_2Rh_2=Rh_2hr"-, Rh_2rh=Rh_2hr" +, etc.
rh'rh'=rh' hr'-, rh'rh=rh'hr' +, etc.
rh"rh"=rh"hr"-, rh"rh=rh"hr" +, etc.

1. Factors Rh_0 rh', rh", hr' and hr" cannot appear in the blood of a child unless present in the blood of one or both parents.

2. Parents who are rh' negative cannot have children who are hr' negative, and hr' negative parents cannot have rh' negative children.

3. Parents who are rh" negative cannot have children who are hr" negative, and hr" negative parents cannot have rh" negative children.

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Fig. 1-3

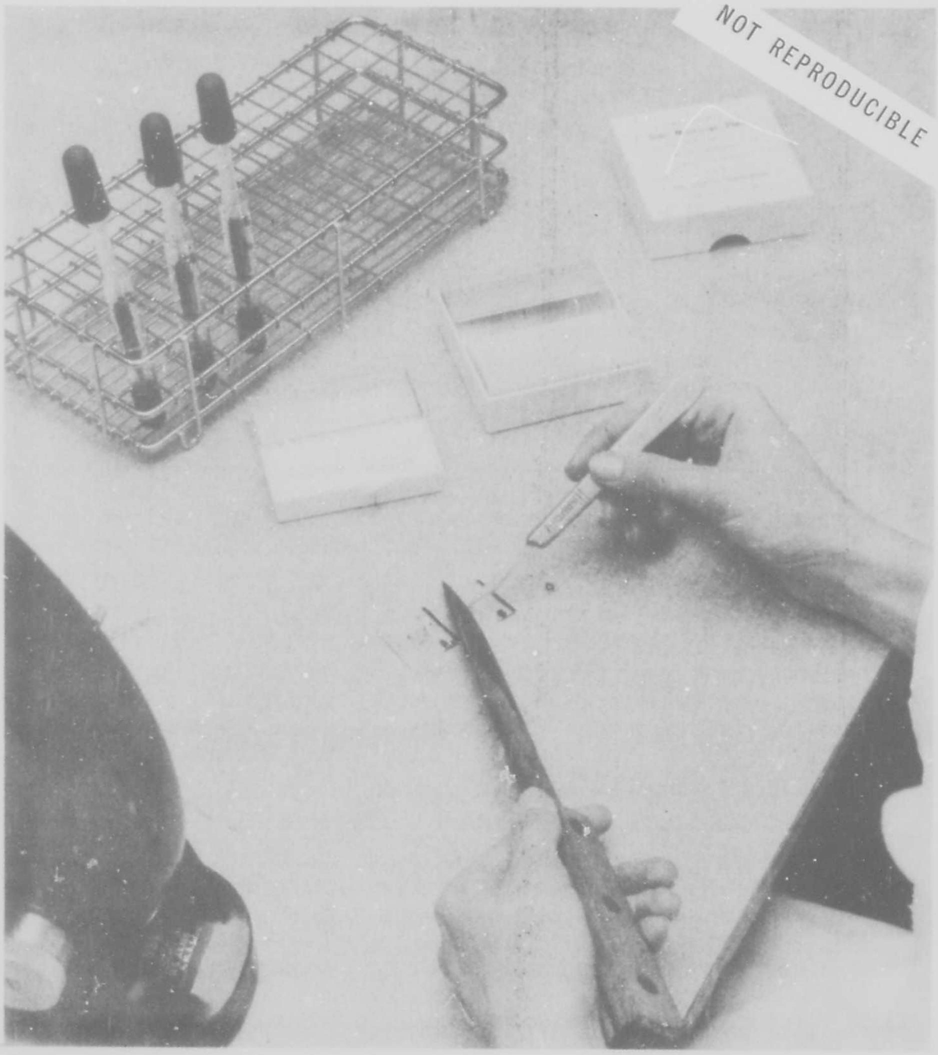

Fig. 2. Placing particles of unknown blood crust on slide.

Fig. 3. Negative reaction with crust and A_1 red cells.

Fig. 4. Positive reaction with crust and B red cells.

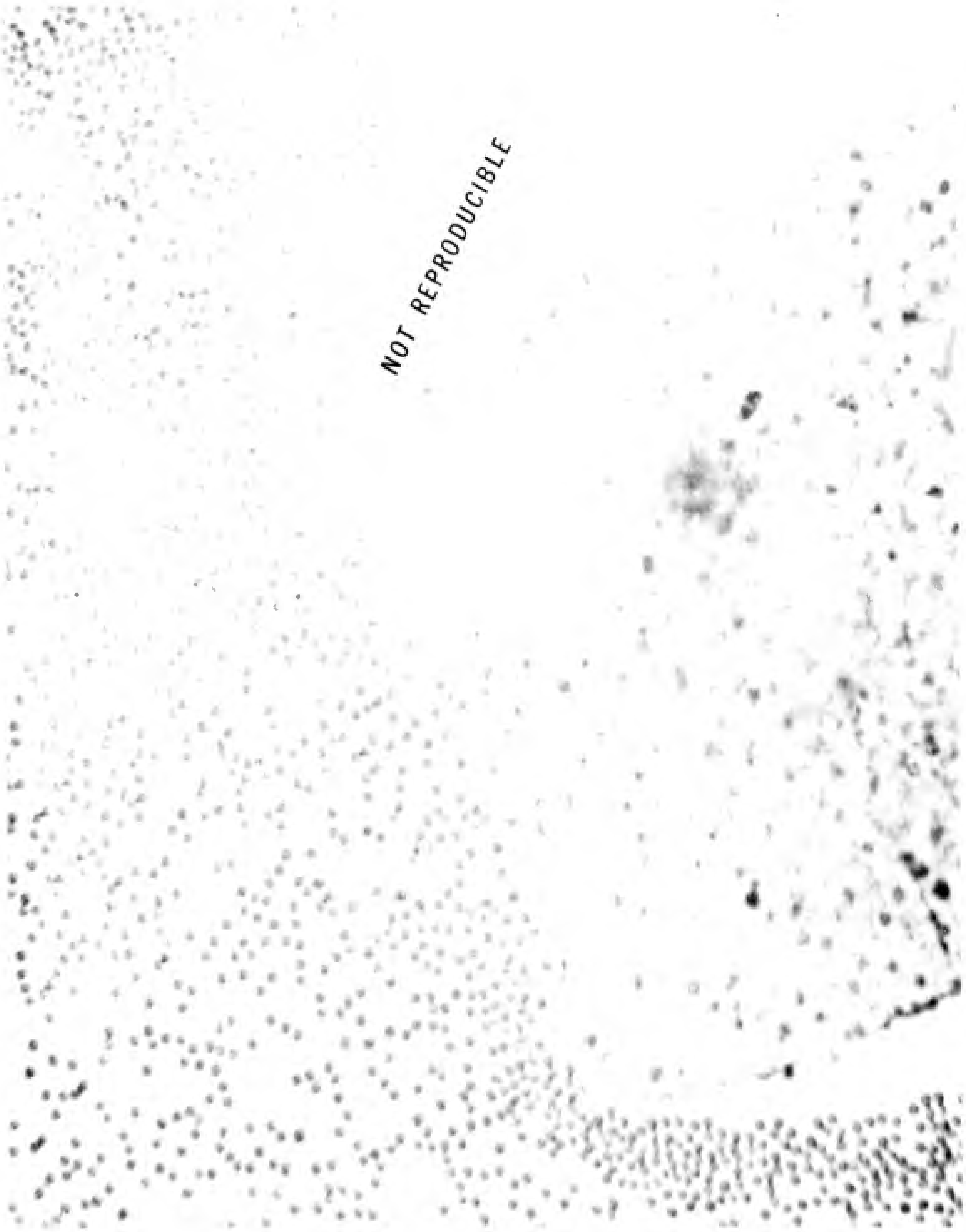

Fig. 5. Negative reaction with crust and 0 red cells.

Fig. 6. Positive reaction with hair sample and red cells seen in lower tube.

Fig. 7. Freeze-drying of bone samples.

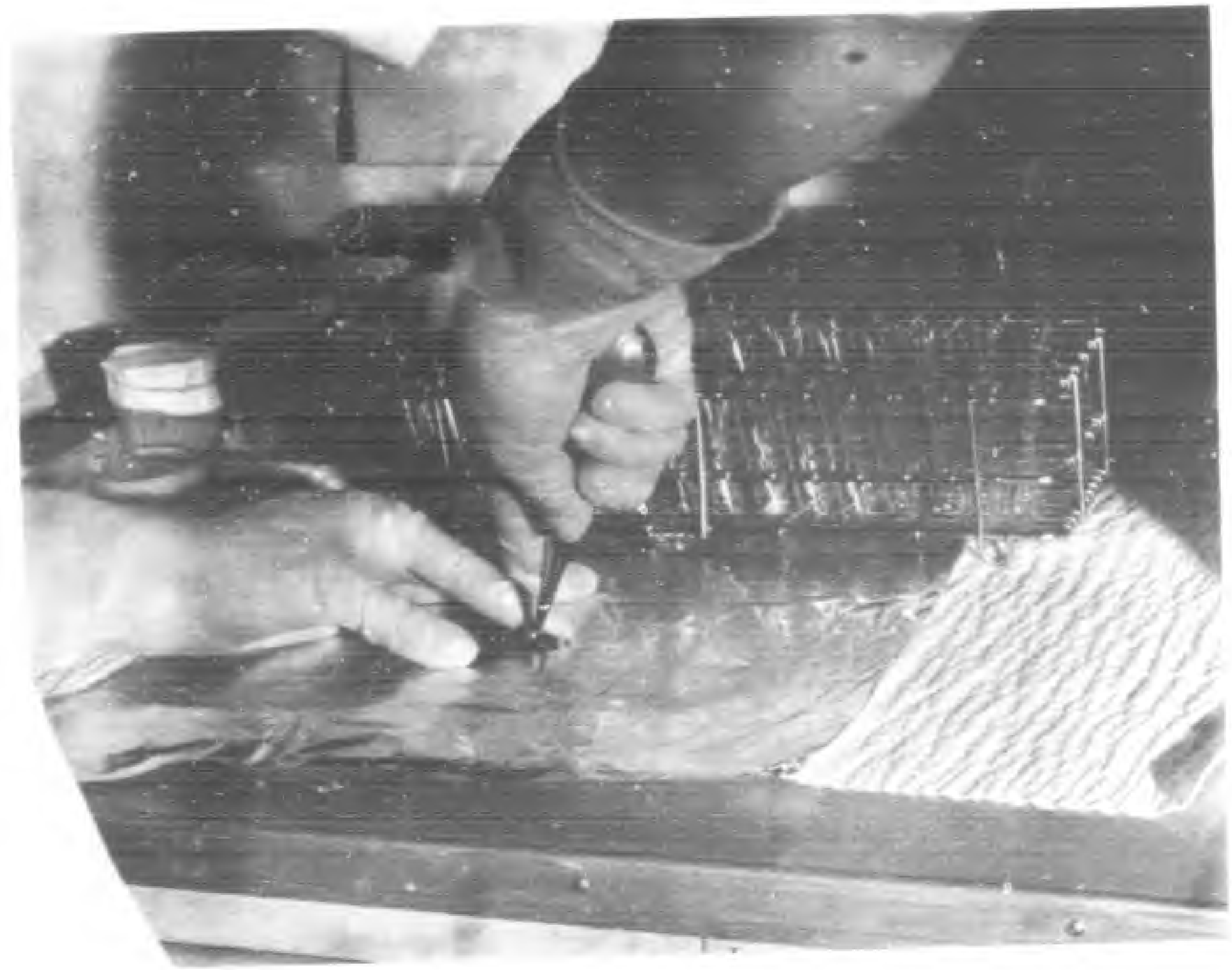

Fig. 8. Obtaining cancellous bone after freeze-drying.

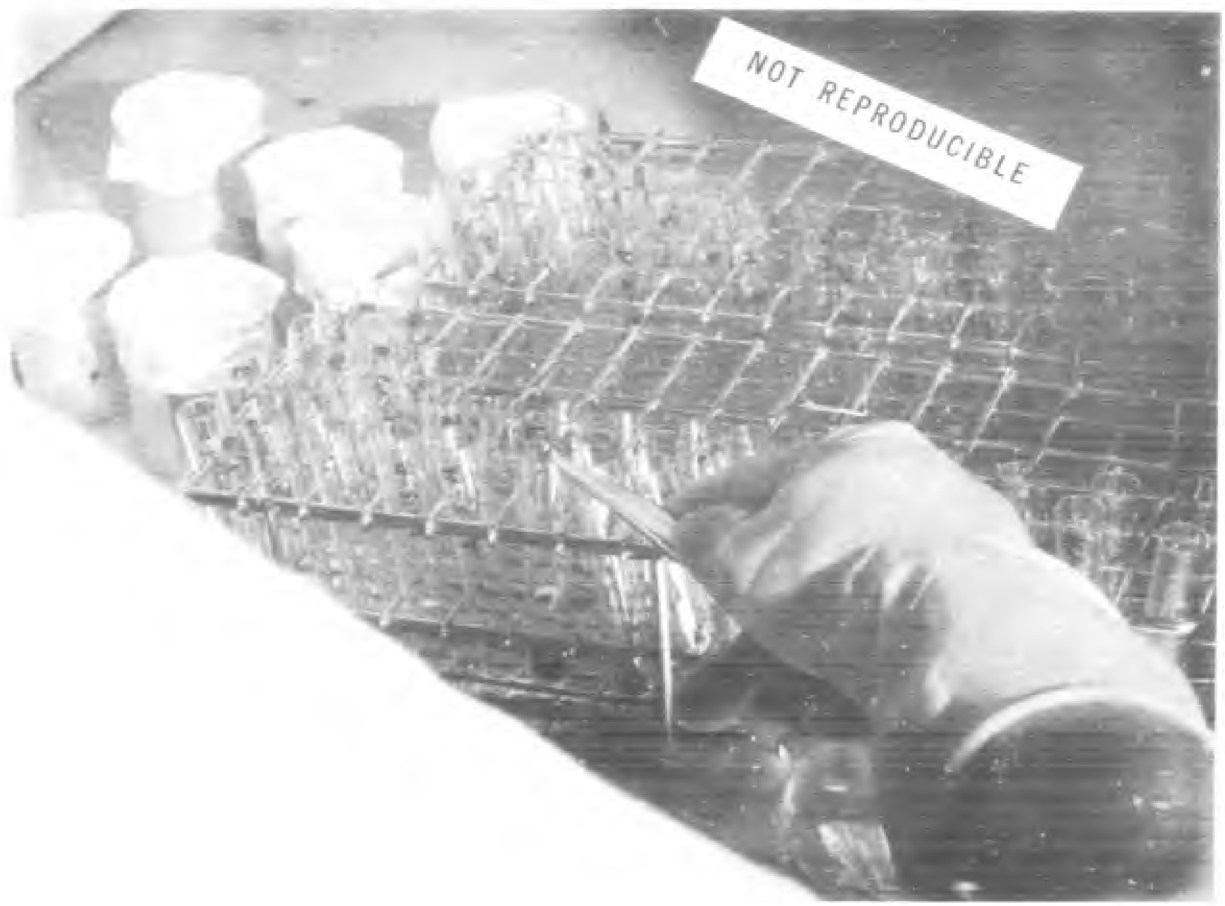

Fig. 9. Placing bone aliquots in test tubes for testing.

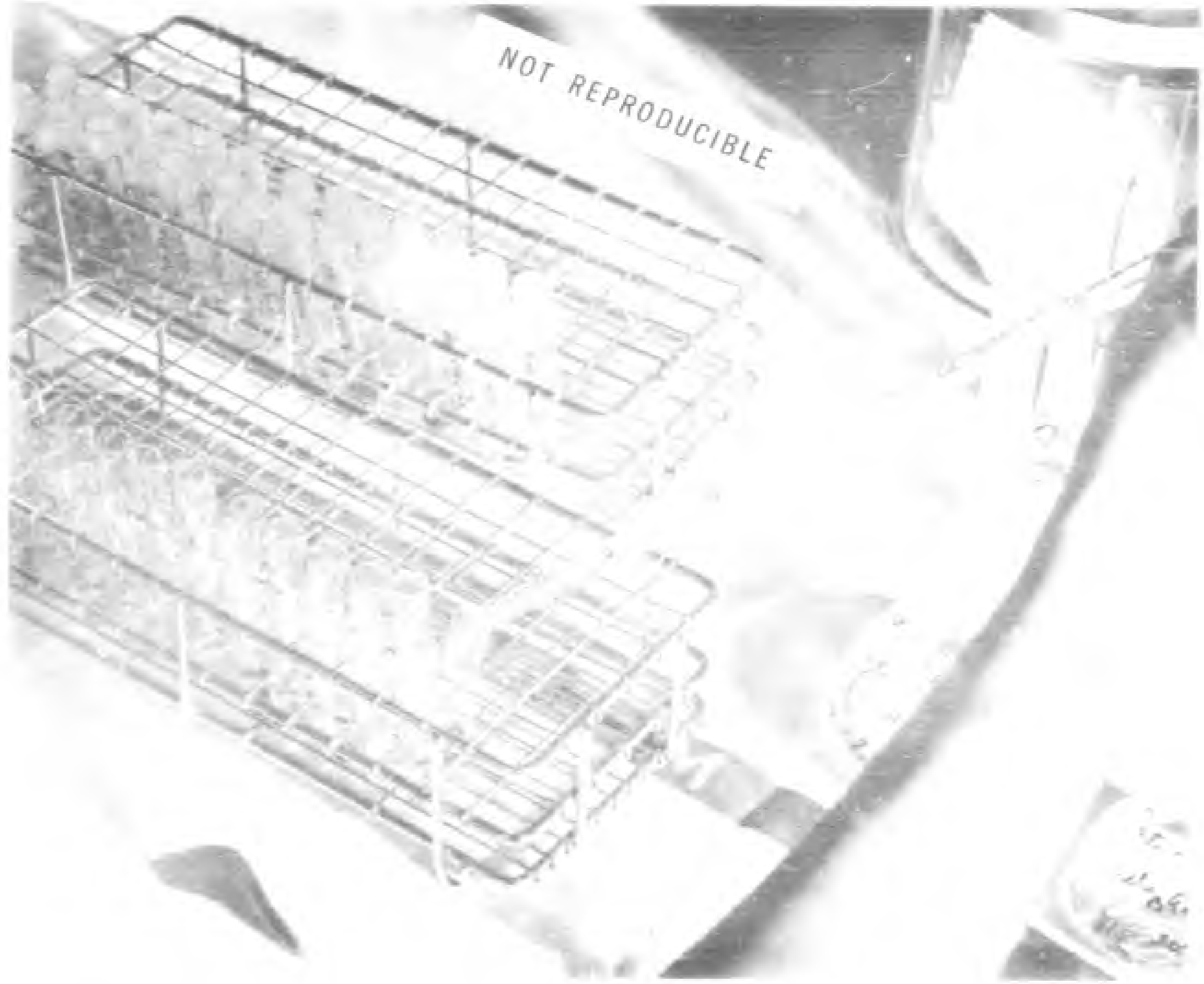

Fig. 10. Adding selected dilution of antisera to bone samples.

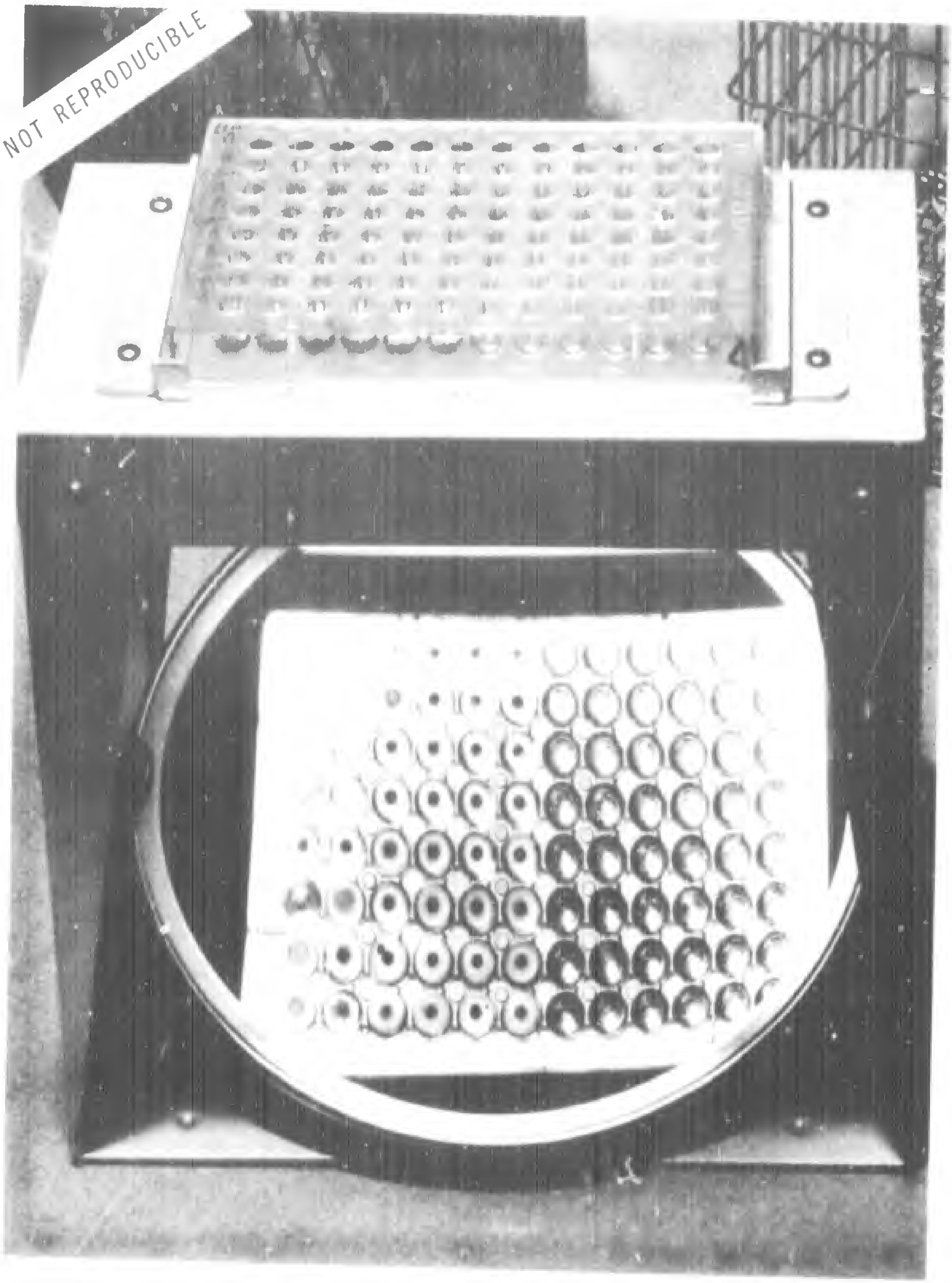

Fig. 11. Pattern of reactions of group A and group B bones.

RESULTS OF PATERNITY TESTING BY TWO METHODS

	Group and Subgroup	M-N Type	Rh-Hr Type
Father	B	MMss	$R_o r$
Mother	A_1	NNss	$R_o r$
Child - I →	O	NNss	rr
Child - II	B	MNss	$R_o r$
Child - III	B	MNss	$R_o r$

Gm TYPING CF SAME FAMILY

Father	Gm (a+, b+, f-, g-)
Mother	Gm (a+, b+, f-, g-)
Child - I →	Gm (a+, b+, f+, g-)
Child - II	Gm (a+, b+, f-, g-)
Child - III	Gm (a+, b+, f-, g-)

Fig. 12. Exclusion based on blood groups and Gm system.