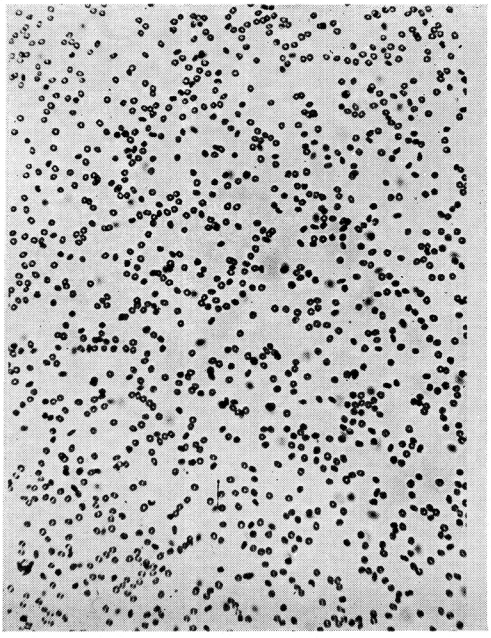
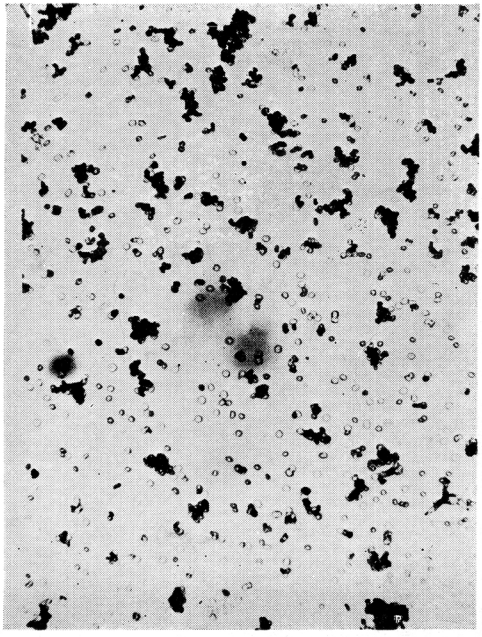
THE TECHNICIAN IN THE POLICE LABORATORY



(1) A sufficiently concentrated suspension of red cells for blood-grouping tests.



(2) Showing clumping of red blood cells a minute or so after treatment with appropriate anti-serum.

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This month's cover photograph illustrates the article on blood-grouping technique, contained in this issue. These photographs, as well as those on the back cover, were taken with "Microfile" film, using the Leica camera and Micro-Ibso attachment, on a Spencer research type microscope. Exposures of approximately five seconds, with visually comfortable illumination, gave satisfactory negatives.

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THE TECHNICIAN

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CONTENTS

BLOOD-GROUPING TECHNIQUE

By John E. Davis (1)

The testing of blood-stained garments, weapons, and other objects found at the scenes of crime is one of the most frequently necessitated procedures encountered in the police laboratory. Yet except for the preliminary and the proof tests for blood, relatively little comment has appeared in current publications regarding this particular type of analysis. In the standard medico-legal references may be found discussions of the precipitin test (for the determination of biologic origin of proteinaceous materials) as well as some mention of blood-grouping procedures in relation to the police laboratory. However, the latter is frequently treated rather lightly, or else, as in specific texts on blood-grouping work, likely to be a little over-complete, and perhaps a little too involved to meet the needs of the technician who is just beginning the study of this particular work. While the technician should be aware of the principles upon which his tests are based, the possible sources of error, new refinements in technique, etc., such information cannot be assimilated in a day, nor well extracted from a highly comprehensive text on the subject, without benefit of a somewhat elementary introduction to the material first.

In the first issue of THE TECHNICIAN, the writer presented a discussion of the preliminary and proof-tests for blood. The information contained therein was based largely on his own personal experiences, and was prepared merely for the purpose of making that information available to others who might find it of value under those circumstances. The present contribution represents a similar situation. It is not intended to appear as the "authoritative" literary product of a specialist in blood-testing technique, but rather should be regarded more as a sort of report on methods which have proved satisfactory in this laboratory during the past two years, and which might be

equally satisfactory elsewhere. To the uninitiated, the testing of blood spots and stains associated with criminal offences may appear a most difficult and laborious procedure -- and so it may be at times; however, there is no reason why these methods should not prove quite satisfactory in the hands of the average police laboratory technician, provided he is willing to undertake the responsibility of testing and re-testing the methods presented, and provided sufficient care is followed in carrying out all steps in the determination. It is hoped that the comments offered here will provide the experienced police laboratory technician with some new ideas which may be of use to him, and yet at the same time be such as to satisfactorily serve others as an initial introduction to the methods described.

It might be well to first mention briefly the significance of blood-grouping reactions in the police laboratory.

To begin with, there are four main blood-groups, and a number of sub-groups or types, into one of which any sample of human blood may be classified. These four blood-groups are commonly known as "A", "B", "AB", and "O". Of the sub-groups the "M" and "N" factors (which are independent of the first four groups) are probably better known than the others. However, our discussions here will not be concerned with these sub-types, nor with the variations which may exist within any one group in the "A" and "B" series.*

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* Not only are there four main groups in this series, but there are differences in "intensity" or strength, of existing factors. For example, there are strong "A" and weak "A" bloods, etc. The factor is the same, but the strength different. In testing dry stains it is not practically possible to differentiate members of these different sub-groups. Accordingly they will not be further considered here.

As stated, the blood of any individual may be classified as belonging to one (and only one) of these four groups. It is either "A", "B", "AB", or "O", and remains the same and unchanging throughout the lifetime. However, it may be well to note that there are two other accepted NAMING systems applied to these SAME factors. These are the "Moss", and "Jansky" systems. Both use Roman Numerals (or Arabic) rather than letters of the alphabet, in designating these factors. Further the number-arrangement is different in the two systems. The alphabetic designation, suggested by Landsteiner, is known as the "INTERNATIONAL" system, and has been much more widely adopted than the other two.

The symbols utilized in these two systems correspond to the "A" and "B" factors (and to each other) in the following manner:

INTERNATIONAL	A	B	AB	O
Jansky	II	III	IV	I
Moss	II	III	I	IV

The convenience and advisability of using the international system in all reference to these blood-groups obviously rests in the fact that there is no possibility of confusion once the factor or blood-group is named. In the other two systems one must designate which naming system is being employed when any reference is made to the AB or O group by numeral, inasmuch as they are not the same in the two systems. It is to be understood, however, that all systems have reference to the same blood-factors, and not to different sub-groups or types of blood.

From the criminological standpoint, blood-grouping factors are significant in permitting the technician to determine the possibility or impossibility of an identity

of origin between two or more blood samples. Thus, if the blood of a certain persons is of group "A", and he is suspected of being the source of blood-stains later demonstrated to be of group "B", then obviously a mistaken suspicion is indicated. By such means it may be possible to divert the attentions of an investigator into more proper channels. On the other hand, had the blood from the suspect and that found elsewhere proved to be of the same group, then it would have shown the possibility of a common origin. Accordingly, one can conclude that two samples could have had the same source, or that they could not possibly have had. The latter, being a definite (positive "negative") conclusion, may have definite value as a means of eliminating certain persons suspected, whereas the former will merely admit the possibility of a common origin in the specimens tested. Thus the blood-group determination may serve as another link in the chain of evidence surrounding a case. Alone, it might mean relatively little, but in conjunction with the other facts similarly ascertained, the blood-group determination may play an important part in the investigation of a criminal offence.

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Although there are these four main blood-groups existent, there is not an equal number of persons belonging to each of the groups. The majority of persons are of group "O" (45%). Ten percent are of group "B", while only three percent are of group "AB". Thus, the percentages may have some small value in indicating the rough probability of an identity between two samples. Obviously if two specimens are both of group "AB", the probability that they had a like source is greater than if both were of group "O". Forty-two percent are of group "A".

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Let us consider for a moment just what is meant by blood-grouping, the determinations involved, and the factors upon which the reactions are based.

Normal human blood, when freshly drawn, consists essentially of a suspension of red corpuscles in a fluid medium known as the serum. For the sake of simplicity, we will assume that it consists of this and nothing more. Ordinarily, these red-cells are freely and separately suspended in the serum, and show no tendency to clump together in groups. If a blood sample is taken, and then allowed to stand for some time, it will clot -- that is, the red cells will all settle to the bottom of the container, in a more or less solid, gellatinous mass. This clotting is not to be confused with "clumping" later referred to, as it is a different thing altogether.

Not only are freshly drawn red blood cells freely and separately suspended in the serum, but if these same cells are placed in a solution of 0.85 percent sodium chloride, they will retain their original shape, size, and form, and continue separate and fee in suspension. Under certain circumstances, however, one may cause such corpuscles to clump together in groups like "bunches of grapes"*, by introducing into the test drop a certain amount of the blood serum of another and different blood-group. Thus, the serum of "B" blood will clump the cells of "A" blood, etc. This clumping is caused by a reaction between two different substances.

One of these, the AGGLUTINOGEN, is contained within the red blood cells. The other, known as the AGGLUTININ, is contained in the serum. That factor which is contained within the red cells determines the blood-group to which an individual belongs. Although there are four blood-groups, there are only two factors in the cells which account for the four groups. The factors are "A" and "B". The absence of both results in a group "O" blood; the presence of both in an "AB" blood. The serum contains a similar set of factors, known as alpha and beta, or "Anti-A" and "Anti-B" respectively. They, too, may both be pres-

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* As contrasted with the "piles of coins" arrangement of cells within a blood clot.

ent, both absent, or separately present, making four possible blood groups on this basis as well.*

The factor present in the serum is always compatible with that present in the red cells of the same blood, and are not capable of clumping cells of that particular group. Thus, group "A" blood has an anti-B factor, but no anti-A factor. Group "B" blood has an anti-A factor, but no anti-B factor. Group "O" blood has both the anti-A and anti-B factors in its serum, while group "AB" blood has neither.**

It is the reaction between these agglutinogens and agglutinins which forms the basis for blood-grouping determinations.

It might be well to consider more specifically for a moment the agglutination reaction between the different types of blood.

(1) Suppose we should mix a drop of "A" blood with a drop of "B" blood. What would happen?

We know that the "A" blood contains in its red cells an "A" factor (agglutinogen). This same blood, in its se-

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* Actually the relationship between the factor present in the red cell, and that present in the serum of the blood is always constant and the same for any one group, so that in designating the blood-group, the factor present in the serum is disregarded, and reference made only to the cell-factor. In other words there are four possible groups on the basis either of the cell factor, or the serum factor, and not sixteen possible blood-groups on the basis of both.

** If it did, obviously it would clump its own cells. Such incompatibility could not exist. Such serum is capable of clumping no cells. Group "O" blood, on the other hand, is capable of clumping any and all red cells except those of group "O".

rum, contains an agglutinin of "Anti-B" nature. In other words, the serum of "A" blood is capable of clumping any and all cells which have a "B" red-cell factor. Accordingly, the serum of the group "A" blood will clump the cells of the "B" blood with which it is mixed.

Nor is this the only reaction which occurs. The serum of the "B" blood contains an "anti-A" factor which is capable of clumping "A" cells. From this it becomes apparent that there is effected a mutual clumping of cells. The serum of "A" blood clumps the cells of the "B" blood; the serum of the "B" blood clumps the cells of the "A" blood.

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(2) Suppose we should mix a drop of group "O" blood with a drop of "AB" blood. What would happen?

The cells of the "O" blood contain no agglutinogens. They therefore cannot be clumped by the serum of any blood. The cells of the "AB" blood, however, have both the "A" and the "B" agglutinogens present, and may be clumped by serum of any other type of blood. As a result, when one mixes the "AB" and "O" blood, the red cells of the "AB" blood are clumped by the serum of the "O" blood. No other reaction occurs, for the cells of the "O" blood are not agglutinable (lacking both "A" and "B" agglutinogens) and the serum of "AB" blood is incapable of clumping cells of any blood, lacking as it does both the anti-A and anti-B agglutinin factors.

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From these explanations, it should be apparent that the following statements hold true:

(1) The cells of "A" blood are clumped by
  (a) The serum of "O" blood (Due to the anti-A factor)
  (b) The serum of "B" blood (Due to the anti-A factor)

(2) The cells of "B" blood are clumped by
   (a) The serum of "O" blood (Due to the anti-B factor)
   (b) The serum of "A" blood (Due to the anti-B factor)

(3) The cells of "AB" blood are clumped by
   (a) The serum of "O" blood (Due to the anti-A and anti-B factors present)
   (b) The serum of "A" blood (Due to the anti-B factor)
   (c) The serum of "B" blood (Due to the anti-A factor)

(4) The cells of "O" blood cannot be clumped.

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In making the blood-group determinations, it is only necessary to use sera of an "Anti-A" type, and of an "Anti-B" type. This involves a minimum of possible confusion, and permits any and all determinations to be made.

In actually performing the blood-grouping tests, there are three different methods which may be used.

The first of these is a simple, and very direct method, in which a suspension (in saline) of the questioned red cells is treated with known anti-A and anti-B sera respectively, and the effects of the treatment observed directly on the cells themselves. It is this system which is commonly employed, in one form or another in clinical testing (as for transfusions etc.) and which finds use in the police laboratory primarily in grouping fresh specimens of blood which are to be compared with dried stains found at the scenes of crime. The procedure obviously requires the availability of the anti-A and anti-B sera, as well as a sufficiency of whole, free red cells in the blood being examined.

A second method, frequently necessitated in the police laboratory, permits a more or less direct typing of dried blood stains. The procedure actually is one which determines the presence of agglutinins (which were in the serum) rather than of the red cell factor, the agglutinogen. Nevertheless, one can (after having determined the agglutinin present) readily deduce the blood-group represented. Determination of the agglutinin content, rather than the agglutinogen content, is necessitated by the absence of whole free red cells in the dried stain. One obviously cannot attempt to clump cells which are not available. It then becomes a problem of attempting to clump cells which are available (in known blood specimens) with the serum factor present in the dried stain.

This method is commonly referred to as the "crust" or "cover-glass" method, and will be discussed in greater detail a little later on.

A third procedure is often necessitated. This is the "indirect" or "absorption" technique. It is also a method applicable to dried stains, but unlike the crust method is a test for the agglutinogen content of the cells which were once present in the blood. This is not done by direct clumping tests on the unknown blood, but rather by an indirect reaction as indicated. The test depends upon the fact that even though the red cells are not present in free and complete form, that the agglutinogen originally present in them is still active, and may react with agglutinins present in the known blood sera, just as if the cells were present. From this it will be seen that the blood-cell clumping (when such cells are present) is not the "end-product" of the reaction or test, but is rather merely incidental to it. The corpuscles, then, when present, clump when the reaction occurs. The reaction itself, however, may occur just as freely even if the cells have hemolyzed or otherwise been destroyed. It is upon this fact that the success of the absorption technique rests.

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I. THE DIRECT METHOD OF GROUPING FRESH STAINS

Blood-grouping tests may be performed in a number of different ways. Probably each has its merits and disadvantages. The reaction may be carried out in a small test tube, or on a microscope slide. Both procedures may be used in combination. If a slide is used, it may be covered, or not, to suit the individual technician.

Essential to the reaction, of course, is a set of standard sera of the anti-A and anti-B groups. These may be obtained by purchase from any of several commercial supply houses, or may be made from known "A" and "B" bloods. For the average laboratory which has not a great amount of blood-testing to do, probably the commercial sources would prove more satisfactory. In addition to the directly taken sera (that from the known blood specimen) one may purchase anti-A and anti-B rabbit sera, just as with the precipitin sera commercially available.

In any event, one should always test the quality of these sera, before accepting them to be "as labeled". The tests run simultaneously with unknowns may prevent mistaken conclusions.

We in this laboratory have, for some time, been using anti-A and anti-B rabbit sera in our tests. This particular sera is put out in dry-powder form by at least one commerical house (Lederle Laboratories, New York) and has proved most satisfactory in our work with it. This sera is particularly suited to use in direct blood-grouping tests on freshly obtained specimens. However, we have also been quite successful in adapting it to use in the indirect absorption method, as will be explained below, and find it convenient in many respects.

As to the liquid sera, both in blood-grouping work, and in the precipitin series, we have found that prepared

by Dr. Wiener* excellent in every respect.

In testing, a drop of saline is placed on the slide, and just enough blood added (with a toothpick) to color the saline a faint but visible pink. Generally speaking, there is probably a tendency for technicians to use too concentrated a blood test-drop. The saline drop itself should be rather small, and should occupy only a small area on the slide.

The blood is well mixed with the saline, and examined under the low-power (100-x) of the microscope. The red cells should be clearly visible, and numerous. They should be freely suspended in the solution, and showing no tendency toward clumping or clotting. There should not be so many cells that the field looks "solid" with them, by any means. It should merely be very well dotted with them, when any one plane is in focus.

After having adjusted the concentration of cells of the proper degree, a drop of the anti-A serum (or a small amount of the powdered type) is added to one of these cell-suspension drops. To a second test-drop is added a like amount of anti-B serum or powder. These may then either be covered with a cover-slip, or left uncovered. It is well to stir the drops every few seconds, (or to agitate the cover-glass by lightly tapping it with a toothpick) in order to keep the cells suspended in the solution. Clumping should occur within from three to five minutes at the most, if the reaction is positive.

If possible, control tests should be run simultane-

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* Alexander S. Wiener, M.D., Laboratories 64 Rutland Road Brooklyn, N.Y. It may be noted that the precipitin sera distributed by Dr. Wiener are of high quality, clear, and with a minimum of sediment. Commerical sera which the writer has seen were in some instances so cloudy that they were valueless. Centrifugation was necessary, and even then failed to clear the samples.

ously on known A and B bloods as a check on the quality of the sera being used.

Interpretation of the test results is quite simple. If the anti-A serum causes the unknown cells to clump, then the cells contain an "A" factor. If the anti-B serum causes them to clump, the cells contain a "B" factor. If both are clumped the blood is group "AB" blood, and if neither clump it is group "O".

It is well to always repeat such tests once or twice to minimize the possibility of an incorrect conclusion. Errors in the grouping of dried stains might be excused -- but not in the grouping of fresh specimens.

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We will not dwell upon possible sources of error otherwise, nor discuss such factors as the keeping quality of sera, effect of temperature, pseudoagglutination, etc. These have been described quite completely in the various texts on the subject. Reference to them may be made at the readers discretion. It is merely the desire here to present the basic steps in performing these tests, and to include a minimum amount of incidental material.

II. THE CRUST METHOD OF BLOOD-GROUPING

The existence of blood-groups, and something of their significance is well known to the average individual. Most persons have had their blood-group determined at some time and are familiar with the apparent simplicity of the whole procedure. Under the circumstances, the uninformed individual is likely to wonder why it is that a police technician is unable to determine the blood-group of a small spot of dried blood on someone's shirt or dress. He may feel that it should have better been sent to a medical technician for analysis (who would actually probably be at a total loss to make the examination). But to the techni-

cian who has found it necessary to perform these examinations, blood-grouping tests on dried stains represents a most tedious and trying problem. Nevertheless, such determinations are possible, and are often something of a pleasure -- particularly when other evidence, later submitted, serves to confirm the conclusions drawn.

Of the procedures which may be followed in such instances, the crust method is the most easily performed, though not the most sensitive and accurate. The success of the test depends upon the detection of the agglutinins originally present in the serum of the stain. Even though the blood has dried, the agglutinins and agglutinogens may still be present. Actually the agglutinins seem to be less stable, and more easily destroyed than the agglutinogens, so that a correct determination may not always be possible by this method. A combination of this, and the indirect method, should always be utilized where practicable, with the greater weight of the conclusion depending upon the results of the latter.

In the crust method, the following procedure is employed:

A small fragment of the dried stain is scraped off the surface upon which it is found, and placed on a microscope slide. Upon this is then placed a single drop of a saline suspension of known "A" cells. A second flake of the unknown dried blood is similarly treated with a saline suspension of known "B" cells. The particles are then covered with cover-slips, and allowed to stand for some little time. The cover-glass may well be agitated from time to time, or the glass immediately over the dried flake pressed down to assure proper distribution of the cells and release of agglutinins from the blood flake.

As the small flake of blood becomes moist with the surrounding saline, it begins to dissolve, permitting the agglutinins to diffuse out into the medium and thus become capable of clumping the known cells previously added. It

is important that one not add too great a quantity of saline (and cells) for the size of the dry flake added. Otherwise the reaction may not occur properly. By using a flake about a sixteenth of an inch square, or thereabouts, the chance of an accurate determination will be fairly good.

Usually, the clumping (if any) will be detectable after about twenty minutes or less, though it may take as long as an hour or more. The first clumping occurs immediately around the edges of the blood flake, where the serum concentration is highest. Later, clumps will appear throughout the field -- particularly as the cover-glass is agitated. One should take care not to mistake mere aggregates of cells for true clumps. The former are easily separated -- the latter adhere tightly together and float around in the solution as a unit.

If the cells in both test drops are clumped, then the unknown blood was of group "O". If neither are clumped, then it was of group "AB" or else those agglutinins which were once present in the serum have deterioriated. If the known "A" cells clump, but not the "B" cells, then the blood has an anti-A factor (group "O" or "B"), and if the known "B" cells clump but not the "A" cells, the blood has an anti-B factor, indicating it to be of group "O" or "A".

If it should happen that the stain in question is not a dried crust on a solid surface, but is a stain on cloth or some other material from which it could be extracted,* the following may be suggested.

If the stain is large enough, extract it as well as possible with distilled water (minimum amount) and concentrate this gradually in one spot on a slide. Let one drop

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* Direct use of a small section of the stained cloth is not recommended, as results are likely to be poor, or negative, where an extraction would yield much more reliable results.

dry, then place a second on the same spot, etc. and continue until an artificial crust has been produced. This should all be done at room temperature and without the use of heat. After the crust has formed and dried, it may be carefully chipped away, and particles of it used for testing purposes, following the methods previously described.

In chipping away the dried crust of blood, one stands a chance of loss of the particles. If sufficient blood is available to begin with, it is often simpler and more convenient to merely make either four or six such crusts and then test them directly with the saline cell-suspensions.

Wiener recommends the simultaneous running of a control, in which the crust is tested against known "O" cells. Inasmuch as no blood should have a factor which would clump "O" cells, the possibility of any abnormal "agglutination" reaction would thus be detected.

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On the outside back cover of this issue there appears an illustration of the effect of clumping by the crust method.

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III. THE INDIRECT OR ABSORPTION, METHOD OF BLOOD-GROUPING

In our discussion of blood-grouping so far, we have described two of the three main procedures which may be followed. The "indirect" method has been frequently alluded to, and we shall now consider it in greater detail. It is this technique which the police laboratory worker must understand, and frequently apply if he is to successfully determine the blood-group of dried blood-stains. As has been said, the crust method may be used either alone, or in conjunction with the absorption technique. However, the accuracy of the crust method cannot be considered suf-

ficiently high to permit such definite conclusions as are desirable in this work. In the indirect, or absorption method, we have a procedure which, under favorable conditions, and with proper care, may permit more accurate conclusions to be drawn, and which therefor must be regarded as the more acceptable technique of the two.

The absorption method is the most difficult of the three procedures, and requires much practice, care, precision, and a rigid adherence to a standard method once that method has been established and shown satisfactory.

In performing any and all of these tests it is absolutely essential that all glassware, slides, test-tubes, cover-slips, pipettes, etc., be absolutely clean, and that the utmost care be taken to keep the laboratory desk and surrounding area as neat as possible. Especially is this necessary in regard to the absorption method, for, being a rather complicated procedure, contamination or other sources of error might well influence the reliability of the final results. The technician who keeps his apparatus clean, and neatly arranged, and who follows through these methods in a systematic manner is not likely to experience too great a difficulty in obtaining satisfactory results.

First let us again briefly review the absorption technique.

Essentially this procedure is one in which a dried blood stain is first divided into two portions, and each of these treated with an anti-serum. One is treated with anti-A, the other with anti-B. The blood must be in excess. Each such treated spot is allowed to stand for a certain length of time, during which period any agglutinogens present may react with agglutinins added. Later, the added sera are removed from the blood (or with it) and utilized as anti-sera in attempts to produce clumping in KNOWN saline suspensions of "A" and "B" cells respectively. If the anti-serum is still effective in clumping cells, we know that it has not lost its agglutination pow-

er. Since the unknown blood was originally in excess of the added sera, it becomes apparent that there was no agglutinogen present of such type as would unite with the agglutinin added via the serum. If the anti-serum is not now effective in clumping known cells, then it is obvious that the clumping power of the serum has been lost--namely because the anti-factors or agglutinins which it did contain have been absorbed by the unknown blood to which it was originally added, and is now "locked" as to speak, and no longer active. We can then deduce the type of agglutinogens originally present in the unknown blood stain, and hence demonstrate the blood-group represented.

The above paragraph contains, in essence, the fundamentals of the absorption method. The technician who expects to attempt this procedure for the first time should understand thoroughly just what is meant in that paragraph before going on to the test itself. Otherwise he is likely to find himself hopelessly entangled in a maze of test-tubes, sera, blood, tooth-picks, etc., the labels on none of which will have any significance to him.

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Before proceeding, it may be well to mention here that the methods which are to be described are essentially standard and accepted. However, they are at the same time, modification, to a degree, of standard methods, and are to be so understood. As stated before, we in this laboratory have found these methods reliable. To the writer, the modifications suggested herein are such as to add to the accuracy of the procedure, without effectively decreasing the convenience of it. As in all such procedures, the personal factor -- the many estimates and judgments which the technician must make as regards quantities, etc., -- plays an important part. Another technician might find the recommended steps unsatisfactory from his own standpoint. In any event they are presented here for the benefit of those who may be interested.

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The absorption process may be satisfactorily carried out either with the liquid anti-sera, or with the dry "powdered" type previously mentioned. In the following discussions we will assume that the powdered sera are being used. Those who use or prefer the liquid type should have no difficulty in replacing the powdered with the liquid sera, by preparing dilutions of approximately the same relative concentrations. In some respects the liquid type would probably be more convenient. The powdered sera of Lederle Laboratories are colored (anti-A with methylene blue, anti-B with eosin) which makes somewhat less difficult the problem of keeping the solutions separate.

Where bloodstains are on cloth, it is generally advised that the sera be absorbed directly into the dry cloth and stain, then squeezed out for use in testing known cells. With a powdered serum, one would obviously have to prepare a solution before such an absorption could be attempted. This may be done by dissolving a small amount of the powdered sera in saline, after which it would be utilized in the same way as an originally liquid-type sera.

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The writer prefers not to absorb the sera or solution directly into the cloth or substrate under any circumstances. More uniform conditions may be obtained, both in testing bloodstains from cloth as well as from other sources, by first extracting the blood with saline, and then working with the extract so obtained. The latter procedure, though more time consuming, has a number of advantages, and makes more consistent and exact the procedure which follows.

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For convenience in explaining the testing procedure, let us imagine that the stained area is on a cloth base, and that it is of such size as will permit our cutting

from it at least one section of stained cloth the size of a quarter, or half-dollar.* From here we will proceed in steps, following the method through just as it would be followed in actual practice.

(1) Cut out one of the stained areas of the size indicated. An area larger than a half-dollar is not needed if the stain is fairly concentrated. If sufficient material is desired for a number of repeat tests, it would be better to extract them as needed than to permit the blood to extract all at once and then stand around the laboratory awaiting the attention and time of the technician.

(2) Place the section of cloth in a test-tube (small), and cover it with saline solution. The amount of saline used will depend on the amount of blood which appears to be present, the size of the stained cloth, etc. One should ordinarily attempt to get as concentrated an extract as possible (up to a medium deep red) and yet have sufficient liquid to perform the test in a proper manner. This may require as much as two cubic centimeters, although less may be used if one is careful. One cubic centimeter of extract is just about the minimum which may be safely used. Chances of success are poor if the extract is not a fairly deep red, although with care, a light red to deep pink solution may be tested.

By placing the cloth in a small test tube (about 8 cm. in length, and having an internal diameter of about 8 mm.), and pressing it with a glass rod while in contact with the saline, a good extract should be obtainable.

(3) Cut from an UNSTAINED region of the garment (adjacent to the stained area) a specimen of similar size to

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* We have successfully performed this grouping test on stains as small as a dime, even though not particularly concentrated. This, however, is just about the minimum size which may be so tested.

that previously removed. This is to be placed in a like quantity of saline and "extracted" in a manner identical to that used for the blood-stained area. This is to be used as a control, and is absolutely essential to the proper performance of the procedure being described. It is always possible that agglutination factors may be detected in the clothing itself, as from perspiration, etc. which would be mistaken for blood factors. By so testing the cloth, this possible source of error will be eliminated.

(4) Carefully remove the extracted cloth from each of the two test-tubes. Squeeze each section out as much as possible, permitting the liquid to remain in the appropriate test-tubes. This may be conveniently done with small clamps (such as cover-glass forceps or a glass rod). The cloth particles may then be discarded.

(5) Centrifuge the two solutions. Particles of lint, dust, and dirt from the cloth will settle to the bottom and minimize interference in the tests. If conveniently possible, decant, or pipette off the supernatent clear liquids and place them in two new test-tubes. The blood extract should amount to one or two cc. of light to medium dark red solution. The other solution should be practically clear and colorless. (A pure saline solution may be used as a control where the blood has been scraped from a solid surface for testing.)

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This completes the extraction process.

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(6) The next step involves the preparation of the sera to be used. One must prepare a solution of the anti-A serum, and one of the anti-B serum of such a strength (titer) as may be used to best advantage in the tests which will follow. The concentration utilized depends largely on the concentration of the blood solution with

which one is working. Generally speaking, the writer uses sera of such strength as requires between five and ten minutes to clump known, fresh "A" and "B" cells of a concentration such as was previously recommended for use in the direct clumping tests.

If one is using a liquid sera, it should be relatively simple to dilute it with saline to such an extent that it will take approximately eight minutes to clump known sensitive cells.

In using the dry, powdered sera, the writer has found that not over one cubic-centimeter of the final solution is needed (actually about half that much is generally all that is used) and that this may be made by using that much saline and approximately two or three "toothpick-fulls"* of the powdered sera. It now becomes necessary to test the strength of the solution so prepared and to then adjust it to the proper concentration. One must take care to see that all of the sera has dissolved before so testing -- otherwise errors may result.

(a) Test the solution of anti-A serum against known sensitive "A" cells. Clumping should not occur before five minutes, nor be delayed beyond ten. The best time will be around eight minutes.**

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* The amount of powder which will rest on a 2.5 mm length of the broad end of a flat toothpick.

** In all clumping tests it is absolutely essential that an identical procedure be followed in respect to the amounts of sera used, the area over which the test drops are allowed to spread on the slide, cell concentration, and the amount of agitation given the cells during the test period. Each of these factors influences the rate of clumping. Since the Time Element plays an important part in the procedure, it is imperative that the technician observe these precautions throughout, and be consistent in his methods.

(b) Test the solution of anti-B serum against known "B" cells. Clumping should occur at around 8 minutes.**

When the concentrations of these two sera have been so adjusted, they are ready for use.

The technician should record in his notebook the time taken for the clumping to occur in each instance. The test should be repeated at least once, as a check.

(7) Having arrived at the proper concentration of serum, the next step will be the dividing of the blood and cloth extracts.

First it is necessary to take equal portions of the blood extract and of the unstained cloth-extract, for testing purposes. It is also necessary that precisely equal portions of each of these be taken for absorption of the anti-A, and the anti-B factor. Accordingly, for the absorption procedure one must have four test-tubes, each with an identical amount of liquid content. Two of these must contain blood extract, and two must contain the unstained-cloth-extract. Remaining are the two original tubes which contain a reserve supply of each.

We have, then, at this point, two test tubes, each containing approximately one cc. each of anti-A and anti-B sera. In addition there are six other test tubes, corresponding to the following:

(a) Reserve supply of blood extract (probably around one cc.)

(b) Reserve supply of unstained cloth extract (also around one cc.)

(c) Portion of blood extract for absorption of the anti-A factor. (probably around 0.4 cc)

** Refer to footnote at bottom of page 23.

(d) Portion of blood extract for absorption of the anti-B factor. (Identical quantity.)

(e) Portion of unstained cloth extract for absorption of anti-A factor. (Identical quantity.)

(f) Portion of unstained cloth extract for absorption of anti-B factor. (Identical quantity.)

(8) The two reserve supplies (tubes 1 and 2) are used solely for the purpose of dilution, in the event an excess of blood does not seem to have been obtained in the tubes 3 and 4. They can be dispensed with in case sufficient blood is not available to permit maintaining a part separately for reserve, but a more precise technic is necessitated thereby.

We must now add to tubes 3 and 5, a small amount of the anti-A serum previously prepared, and to tubes 4 and 6 a small amount of anti-B serum. The amount added depends on the concentration of the blood extract, and on the amount of solutions being tested. The object, of course, is to add such an amount that the serum will not be so diluted as to be no longer effective merely on the basis of dilution, and yet to add an amount small enough to permit the blood to be in excess when present. One then is continually faced with a problem of not adding too much, and yet adding enough. It is here that the reserve blood-extract plays its part.

In making all these additions and dilutions it is essential that exact quantitative methods be followed. The writer uses standard graduated pipettes, delivering 0.2 cc at a time. These have been found quite convenient for the work, although other calibrated pipettes or dropping pipettes might as well be utilized. The important thing is that one be able to make equal additions to, and removals from any of these tubes at all times.

As to the amount of sera to add, the writer generally begins with a single 0.2 cc addition of sera for each 0.4 cc. of blood extract used, when the latter is fairly concentrated. After making the proper additions (of anti-A to tubes 3 and 5; of anti-B to tubes 4 and 6) the tubes are allowed to set for at least 45 minutes. An hour is better, and even two or three hours does not seem to be excessive.

(9) Next, one must test the effect on these sera, of the absorption process which has been carried out. This is done by testing known "A" cells with the absorbed anti-A sera from tubes 3 and 5, and known "B" cells with the absorbed anti-B sera from tubes 4 and 6 respectively. Thus there are necessitated a minimum of four grouping tests -- two against "A" cells, and two against "B" cells. In taking this absorbed sera for grouping tests, it is obviously essential that identical amounts of each be taken. If this precaution is not observed, then subsequent dilutions (which may be necessitated) and all the accuracy of previous work as well, will be affected.

In performing the grouping tests on known cells with this absorbed sera, one must record the time range over which grouping occurs. For this, the writer finds most convenient one of Eastman's photographic "Kodak Timer" clocks having a second and minute hand.

Under the conditions of our procedure, the "unstained cloth extract" absorbed anti-A and anti-B sera should normally cause clumping in "A" and "B" cells in between twelve and fifteen minutes. (Time increase over original eight, due merely to dilution, unless some agglutination factor is present in the cloth, which is not generally the case.) This test is repeated two or three times, and the time recorded in each instance. One should standardize on a "degree of clumping" at which he records his time, or else accept a range such as "initial", "poor", "fair", "good", "excellent", etc, and record the time for such qualities of clumping. A conclusion such as "initial

stages of clumping at 12 minutes. Good clumping at 14", is a representative observation.

After one has observed the times required by the saline-absorbed sera, the same experiment is performed with the blood-extract-absorbed sera, and the times of clumping just as carefully noted.

Then by comparing the times, one may be permitted to draw a conclusion as regards the blood-group represented. One can expect minor variations in time amounting to as much as three in ten, or five in twenty, due to slight inaccuracies in measurement, observation, etc. However, if the blood is absorbing a serum, and the latter is not appreciably in excess, any clumping observed will not be noted for two or three times the length of time required by the controls. One might obtain, in an actual case, results such as the following,

(a) Unabsorbed anti-A serum -- clumping time for "A" cells -- 8 minutes.
(b) Unstained cloth extract absorbed anti-A serum -- clumping time for "A" cells - 13 minutes (good)*.
(c) Blood extract absorbed anti-A serum - clumping time for "A" cells -- 28 minutes (poor).
(d) Unabsorbed anti-B serum -- clumping time for "B" cells -- 8 minutes.
(e) Unstained cloth absorbed anti-B serum -- clumping time for "B" cells -- 12 minutes (good)*.
(f) Blood extract absorbed anti-B serum -- clumping time for "B" cells -- 14 minutes (good).

Indication in this case is obviously that the "unknown" is group "A" blood, and that the anti-A serum was in slight excess, or else the absorption time was insufficient.

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* If these take fifteen or twenty minutes to clump, it might be well to add a little more sera (0.1 or 0.2 cc), to each of the tubes.

Had the clumping time of both the anti-A and anti-B blood-absorbed sera been markedly increased, then we might fairly assume that the unknown blood was of group "AB".

Suppose that the clumping time of none was increased over that of the unstained-cloth absorbed sera. What is indicated? The answer, of course, is that the blood is either of group "O", or else such an excess of serum was added to all that the effect of absorption could not be detected. (or that agglutinogens once present have been destroyed.) The next step must now be a dilution of the absorbed sera with unstained cloth extract, and blood extract respectively until a more definite conclusion may be drawn. Accordingly, to tubes 3 and 4 there must be added another volume of blood-extract -- say 0.4 cc; and simultaneously there must be added to tubes 5 and 6 a like quantity of the saline, or unstained cloth extract. (So that the effect of dilution alone may be detected.) This is again allowed to stand for a half hour or so, and the clumping tests repeated. If the blood was of group "O", then it will probably take around 25 minutes or more in each instance, for the clumping to occur. If it was not of group "O", then there will probably be a noticeable time difference between the blood-absorbed, and cloth-absorbed sera, amounting to about fifteen minutes. After the dilution has been made so great that over twenty-five minutes is required in each case, and yet no appreciable difference detected between tubes, then one may safely conclude that there are no agglutinogens present. A repeat test should be run on a new sample if possible.

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This covers, for the most part, the absorption technique. In each case the technician should perform two or three grouping tests on each absorbed specimen before drawing a conclusion, and if possible should re-work the entire experiment on a different blood-stained section of cloth.

First attempts to perform these analyses will prob-

ably result in inconsistent and varied results. Only experience and practice will lead to the even reasonably consistent results which are so desirable.

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In making blood-group analyses, it is suggested that the absorption method be tried first in those instances where only a dried spot remains. The crust method is likely to give misleading results, and influence the observations and conclusions of the technician in later tests. Further it is advisable that the "most questioned" spots be analyzed first. Thus the stains on the clothing of a suspect should be analyzed before the blood-group of the victim is determined. By such means completely unbiased determinations are assured, and the significance of an accurate determination increased.

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As to the practical applications of this procedure, we have found it quite reliable in those instances where sufficient blood was available. Analyses performed on questioned stains have on a number of instances been shown accurate by later evidence or tests performed. It is unfortunate that so tedious and time-consuming a procedure should not generally have more significant an outcome than does the blood-grouping determination. As stated in the introduction, blood-group determinations do not mean a great deal (except as negative reports) for the most part. Nevertheless, if it can be shown that spots and stains could not have had a like origin, that fact may prove most important at times.

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It may be of interest to note here that this laboratory received, in June of this year, the clothing of a soldier suspected of having committed rape. The victim in the case was a girl of about 19, and had been badly beaten

during the offense. The clothing of the victim was also submitted. The soldier's clothing had been submitted to a dry-cleaner, and had already been placed in the naphtha cleaning solution. At the direction of the investigating Trooper, it was removed for our examination.

The stains on the shirt gave positive blood-tests, and were so submitted to the absorption grouping test. Results of the examination indicated the stains almost certainly to be of group "A". These stains were for the most part badly "washed out" smears, and not very concentrated. Tests repeated four times, twice on new sample.

The stains on the dress were similarly tested and proved to be of group "A". Results were more clear-cut, and definite. Test performed twice.

An automobile robe or blanket, stained with blood was likewise tested. Conclusion formed on basis of two such absorption tests indicated blood to be of group "A".

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In another and more recent case, a hammer was submitted as being the lethal weapon in a murder case. Blood had all dried on the hammer, but there was a rather large quantity available for testing. Blood scraped off, dissolved in saline, and tested by absorption technique first.

Observation indicated the blood to be of group "B". Repeat tests gave same results.

Crust method then attempted. Gave indication that the blood was of group "B", but clumping of "A" cells did not occur for almost an hour. Conclusion was drawn that the blood was of group "B". A few moments later, a teletype message was received in the laboratory to the effect that the victim in the case was of Moss type 3 (International "B"). Blood of suspect was later received and shown to be

of group "O". Spots on his clothes too small to type.

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(1) Mr. Davis is a graduate of the University of California at Berkeley, where in December of 1941 he received an A.B. degree in "Technical Criminology". He has been employed as a civilian laboratory technician by the Missouri State Highway Patrol since Feb. 9, 1942.

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OF INTEREST:

With the November issue of THE TECHNICIAN, there was sent out a questionnaire form, regarding state police laboratories, and accompanying the article on that subject, prepared by Mr. Davis of this laboratory. The form was included merely as a sample of the original one which was used in the survey discussed. It is of interest to note, however, that some of the departments receiving the publication have filled out and returned these forms to us. We had not requested nor expected such a reaction. Nevertheless we are appreciative of the additional information so submitted to us by those departments. If any other agency would care to return the forms as well, we should be pleased to obtain the data called for.

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EDITORIAL APOLOGY

Due to a shortage of assistance in preparing the November TECHNICIAN, an insufficient number of copies were issued. It was necessary that we delete from our mailing list about a dozen departments who would otherwise received copies, and to send to a few departments issues lacking page 4 of the bulletin. We regret that this was necessary, and shall try in the future to publish a greater number, and to do a better job of mimeographing on those issued.

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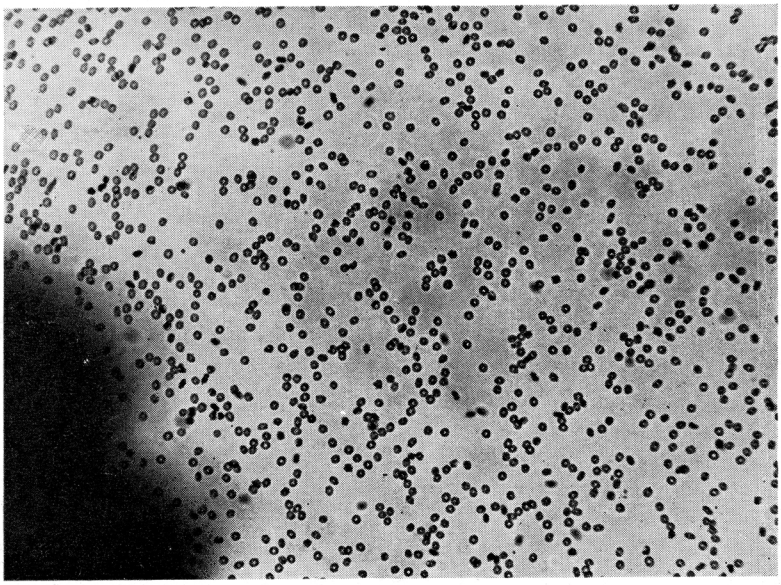
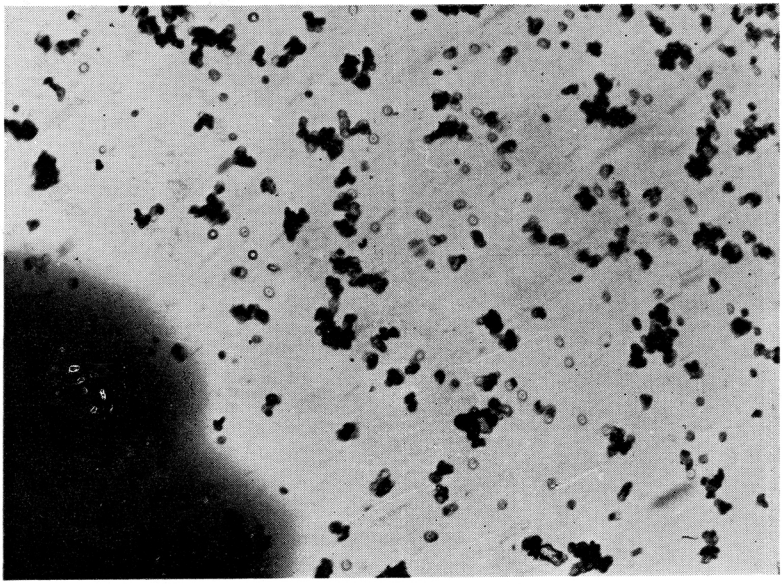


(1) The crust method of blood-grouping. Cells as yet unaffected by serum from the blood-flake.



(2) Same after about ten minutes. Clumping has been caused in the added "A" cells, by agglutinins present in the blood flake being tested.