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SEPARATION OF BLOOD STAINS AND OTHER SOLUBLE MATERIALS BY CAPILLARY ACTION*

Paul L. Kirk, Harry L. Roth, and William R. Clayton

Paul L. Kirk, Ph. D., is Professor of Biochemistry and Criminalistics, University of California, Berkeley, and an Associate Editor for Police Science of this Journal. Professor Kirk has contributed a number of scientific papers to this Journal, and this article is his second of a series on blood testing.

Harry L. Roth and William R. Clayton are both recent graduates of the School of Criminology, University of California. Mr. Roth is continuing his studies at the Medical School, and Mr. Clayton is now on active duty with the U. S. Navy.—
EDITOR.

Blood stains particularly, and some other soluble materials as well, are located in evidence in such a form as to make their removal and testing by conventional methods exceptionally difficult. For example, blood may be mixed with soil, dry leaves, wood fragments, and miscellaneous debris in such a way that ordinary methods of testing are ineffective. Furthermore, blood may be distributed over irregular objects and in very small quantities, and it may have been spread and diffused by washing and other procedures to such an extent that its recovery is very difficult. Automobiles suspected of being involved in hit-run cases are among the more common sources of this type of situation. For example, blood was found on the lower end of a windshield wiper arm in a region so irregular as to prohibit the possibility of soaking it off by the conventional use of wet filter paper. The amounts were too small to be visible and could only be located by color tests. On the same automobile there were areas which also obviously had been washed but which contained invisible amounts of blood which could be detected chemically. The difficulties of removing blood under these circumstances in sufficient quantities to obtain useful results is very considerable. When blood has soaked into dirt or debris in between floor boards or has been shed in the out of doors where it can mix with a large variety of miscellaneous materials, the problems of the criminalist in detecting it are, if not impossible, at least unlikely to yield useful results.

A technique has been developed, and will be described in this article, which eliminates many of these difficulties and allows very precise and sensitive testing for blood in numerous conditions which otherwise could not be carried out readily or perhaps at all. The technique depends on the removal of the blood from the material in which it is found by the capillary action of water in climbing a filter paper column. All soluble materials found in the mixture can be isolated and concentrated by this method, and for this reason the procedure has also been found useful for

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location of nitrate ion in clothing. It is very probable that it likewise may be found applicable to several similar types of problem.

The method is inexpensive, relatively rapid, and consumes a minimum of the operator's time.

EXPERIMENTAL

The essential technique involved, regardless of the type of material from which blood is removed, is the application of a filter paper strip which is kept wet with the water used to transfer the blood and which allows it to creep up by capillary action and evaporate from paper at a point above the location of the blood-containing material. The soluble materials are carried to the highest point reached by the water and concentrate there in a narrow band. The application of the basic technique to various types of situations will be described in greater detail in the succeeding paragraphs.

Miscellaneous Debris. When blood is mixed with soil, dried leaves, fragments of branches, sweepings, or material dug from cracks in the floor, the material in question is placed in an appropriate sized glass dish. It is covered or at least well moistened with an amount of water which will allow a definite layer of water on the bottom of the dish. A strip of filter paper which may be a foot or more in length is suspended so that the bottom end of it is dipping in this puddle of water. The top is held by means of a clip or rack. A system which is satisfactory for this type of technique is shown in Figure 1. No further attention on the part of the operator is necessary until the material has evaporated to dryness or until an appreciable quantity of the water has risen on the paper strip giving in many cases a visible line of bloodstain at the top of the rise, that is, the highest position which the water reaches on the paper strip.

At this point, the strip is removed, and may be cut in lengthwise pieces through the line containing the blood which will in general be quite narrow and each piece may be subjected to whatever test may be desired. For example, the presence of blood may be checked by use of the benzidine, leucomalachite green, phenolphthalin or luminol tests as described in a previous publication,¹ or the blood may be utilized for grouping or for the precipitin test. As will be discussed later, it was found that these tests are also possible with the blood separated in this manner, as was the application of the Teichmann test.

This technique has a general applicability to small objects, as well as to debris, for example, a metal object which is so irregular as to make

1. Grodsky, M., Wright, K., and Kirk, P. L., J. Crim. Law, Criminol., and Pol. Sci., 42 (1); 95-104 (1951).

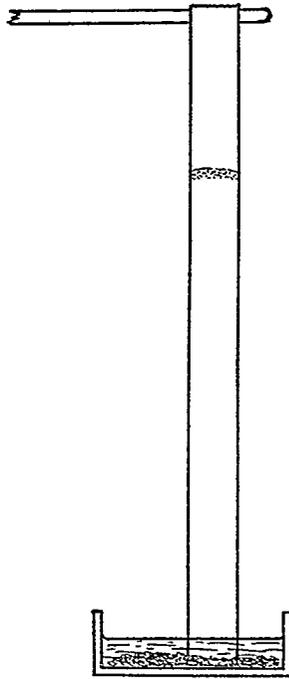


Figure 1

Simple arrangement for collecting blood from debris by filterpaper.

soaking it difficult may be placed in the dish, covered with water, and treated as though it were made up of finer materials. These objects would include nuts removed from bolts, or the ends of wires, windshield wiper arms, knives, and a large variety of possible items of interest.

Removal of Blood from Clothing. When clothing contains large enough amounts of blood to leave definitely visible stains, the operations of testing these stains which are used conventionally are ordinarily quite adequate. It is only when the clothing has been washed or when the amount of blood carried on it is so small as to make the collection of the blood difficult that the paper strip technique shows great advantage over conventional methods. When this is the case, the area which shows chemilluminescence, as revealed by spraying with luminol reagent,¹ may be marked for testing by means of the paper strip method. To do this it is convenient to make a ring around the area believed to carry blood, by means of wax or paraffin so as to avoid general spreading of the liquid through the cloth. A dish, containing a piece of sponge such as is commonly used for moistening postage stamps is very satisfactory as a source of water for the soaking operation. This dish containing water

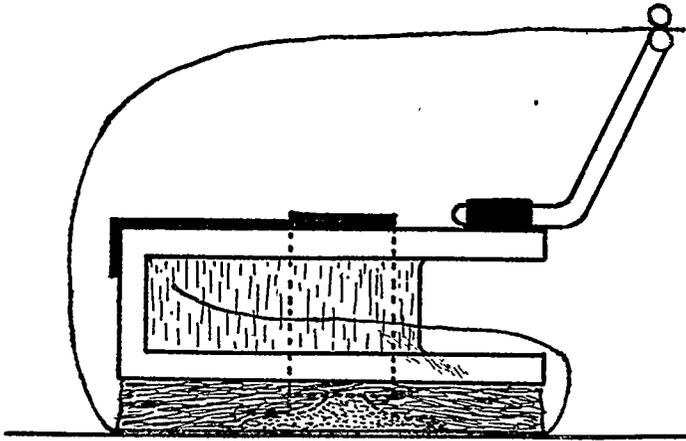


Figure 2

Arrangement for Collecting Blood from Vertical Surface. Metal frame and suction cups indicated.

is placed under the cloth to be tested at the point which has been ringed with wax. The end of a long filter paper strip (one foot or longer) is placed on the cloth and covered with a piece of rubber strip such as may be obtained from an old inner tube. On top of the rubber strip is placed a weight sufficient to hold the cloth, paper, and sponge in close contact. The free end of the strip is raised in the air, suspended as before from a support. Water supplied by the sponge can only soak through the cloth to the paper, transferring the blood to the latter and up the paper strip as the water creeps up the latter. At the end of the soaking period, all or most of the blood will be found to reside in a narrow strip across the paper at the maximum distance reached by the ascending water column. It may be advantageous when the amounts of blood are particularly small, to use a tapered strip so that the upper meniscus in which the blood is found will be considerably narrower than the region which is being soaked. These are modifications which the operator will readily recognize. The method of removal of blood from clothing by this means has been shown to be successful when the stain was invisible or barely discernible due to dilution or the minute quantity of the blood involved.

Application to Solid Surfaces. One of the most important sources of blood stained surfaces is the hit-run automobile. Blood may, however, be found on tables, walls, and any other type of surface which is in the neighborhood during criminal activity. Again, if the amount of blood is sufficient to be readily discernible and removed by scraping or some other common technique, it is not worth while to apply the paper-strip method. When the blood has been removed nearly completely by wash-

ing or other operations, the remaining quantity may, however, be removed and concentrated as before with the paper strip method. In order to apply the method to more or less vertical surfaces it is necessary to arrange a water supply and a holder which will press the paper firmly to the surface which is to be soaked. A device shown in Figure 2 is satisfactory for this purpose. It consists of a deep but narrow plastic dish which contains a supply of water and on the outside of which is attached a sponge rubber backing prevented from direct contact with the paper by a layer of rubber or plastic sheeting on the surface. The dish carrying the rubber pads may be attached to a vertical or sloping surface by means of a light metal framework carrying two suction cups. This framework may also carry a vertical standard which will suspend the upper end of the paper strip.

To use this apparatus some water is placed in the plastic container, a long strip of filter paper is dipped at one end into the water, is passed out over the length of rubber sheet, brought around the bottom of the container, and is suspended from the vertical standard. The paper surface next to the rubber strip is pressed firmly against the surface which has shown a luminol or other test for blood. The entire assembly is held in place by the suction cups. Water will pass by capillary action up the paper from the reservoir, soak down over the stain, and will follow the paper strip up to a point at which all of it is evaporated. The action is identical with that described previously, the blood appearing at the end of the liquid column in a thin line. It is frequently possible to observe on the paper a stain of blood which has been invisible on the original surface.

When the surface carrying, or believed to carry, blood is horizontal, or virtually horizontal, such as the top of an automobile hood or a table, the apparatus described is not suitable. It must be modified as shown in Figure 3. In this case a dish of the same general dimensions as the one described previously is used but with the opening on the broad flat side rather than the end. This dish is fitted with rubber pads as described but mounted on the bottom of the dish. The paper, which leads from the water reservoir, around the rubber pad, and to a vertical support is pressed down directly on the horizontal surface. If convenient, a light weight may be placed upon the dish to hold it in place. If the surface is slanting at an angle which does not allow the use of a weight, suction cups and a metal frame may be employed. The action is exactly as described for the vertical surface apparatus except for the slight difference in the plastic dish and in the mounting arrangements.

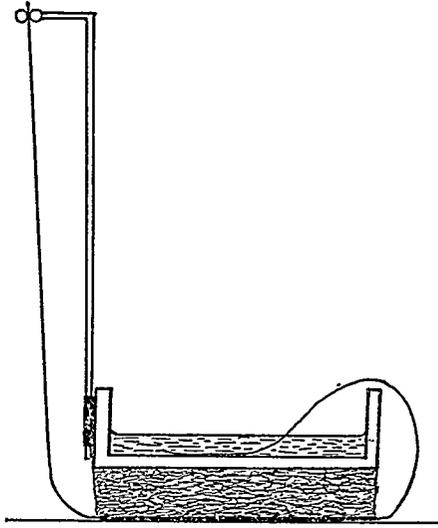


Figure 3

Arrangement for collecting blood from horizontal surfaces.

Modifications of this general procedure may be required to suit special cases which will arise. This should ordinarily not be difficult if the basic principles are understood and the fundamental equipment is available. Regardless of the particular adaptation of the generalized technique, it should result always in a high degree of purification of blood from all the insoluble debris commonly associated with bloodstains. The blood should be recovered in a highly concentrated form as compared with the original stain and on material which is peculiarly susceptible to any kind of testing procedure that is desired. In the authors' laboratory the color and luminescence tests are applied to small strips cut through the cross-wise area containing the blood on the paper. If the Teichmann or Takayama crystal tests are employed, a little bit of the strip is soaked off in water on a slide and evaporated for the test as though it were common blood extract. The blood is readily removed from the filter paper by soaking it in a drop or two of water to obtain it in a small volume in relatively high concentration. These tests are ordinarily successful with blood separated by means of the filter paper strip.

Two crystal tests for identification of hemoglobin were tested on the concentrated area of blood on the paper. By soaking off the blood in a minimum quantity of water and drying it, both the Teichmann and Takayama tests were found to be positive if color was shown on the paper. As so often happens with the Teichmann test particularly, the results were not completely uniform, occasional failures being en-

countered. However, the test was in no case applicable to the starting material, and the difficulties that exist are known to be inherent in the test itself which is not always successful even with ample quantities of blood. Spectroscopic tests for blood were not made with the paper strip procedure. These also depend on the presence of hemoglobin which could be demonstrated by all the methods used, so that the presumption is that sufficient quantities of blood would give the test. The quantities necessary are such as to limit this test regardless of the technique used.

It was of special interest to ascertain if the paper strip method would allow precipitin testing and blood grouping as well as chemical blood testing. The precipitin test depends on the presence in the blood of various proteins which serve as antigens when mixed with the corresponding anti-sera. These proteins are largely in the serum fraction, and probably do not include hemoglobin. Blood grouping also depends on the presence of substances other than hemoglobin, viz. specific materials, presumably complex polysaccharides either alone or in combination with proteins. If these various materials move on the strip in a manner similar to that of hemoglobin, the paper strip method would be of general applicability in blood study.

Tests were made by soaking off the concentrated band at the top of the strip and testing it with the required materials. Human blood so treated was found to react positively with anti-human precipitin serum in a number of tests, while animal bloods gave negative reactions. The quantity of blood necessary was such that a faint red coloration on the paper was necessary, as is true also when the paper strip method is not used. Blood grouping tests were also made with similarly treated blood samples, using the indirect or adsorption method which is a test for the A and B agglutinogens which are carried in the blood cells. Known A and B bloods were employed. It was found that all the known bloods tested were properly grouped when the amount of blood was sufficient to impart a pink color to the paper in the concentrated zone.

It is apparent then, that all of the common blood tests, both by color and luminescence, and by crystal tests may be performed by the paper strip method and that species testing and blood grouping are also possible. The method should therefore be of general application and make possible many tests which are impossible with the spots or areas of blood-stains as they are often found, viz. washed, or in high dilution or mixed with so much debris as to make testing by the conventional methods impractical or impossible.