James J. Harrington,[1] *M.S.; Rolf Martin,*[2] *Ph.D.; and Lawrence Kobilinsky,*[3] *Ph.D.*

Detection of Hemagglutinins in Dried Saliva Stains and Their Potential Use in Blood Typing

REFERENCE: Harrington, J. J., Martin, R., and Kobilinsky, L., **"Detection of Hemagglutinins in Dried Saliva Stains and Their Potential Use in Blood Typing,"** *Journal of Forensic Sciences*, JFSCA, Vol. 33, No. 3, May 1988, pp. 628-637.

ABSTRACT: Since 1928, hemagglutinins have been known to exist in saliva; however, they have not been utilized as evidence in criminal investigations because in the past, techniques for measuring them have not been sufficiently sensitive. In this paper we describe improved techniques for detecting salivary hemagglutinins and report initial results obtained with these methods. The stability of salivary hemagglutinins at several different temperatures was examined in liquid samples and in dried stains on filter paper, cigarette butts, and envelope flaps. Our observations indicate that salivary hemagglutinins may be sufficiently stable, over periods of one to several days at ambient room temperatures, to be of value to forensic science investigators. The results of the hemagglutinin assay are not affected by the age or sex of the sample donor. Because salivary hemagglutinins can be used to determine ABO blood type, analyses of this kind can serve as an important confirmatory test which the forensic serologist can use in conjunction with salivary agglutinogen determinations.

KEYWORDS: forensic science, hemagglutinins, saliva, serology, blood typing

Because a majority of the research involving salivary hemagglutinins has been conducted by investigators whose primary interests are dentistry and periodontology, fresh liquid saliva samples, similar to that found in a patient's mouth, have been used by most workers. Obviously, this type of sample is not always available for analysis as evidence in criminal cases unless the propositus is present to supply saliva. Generally, saliva samples collected at crime scenes are in the form of a dried stain. The forensic science value of salivary stains is limited by a number of factors, such as the secretor status of the donor, the age and quantity of the stain, the effects of temperature and humidity on the stain, and the degree of abiotic contamination. Bacterial or fungal contamination or both also can be a limiting factor in the interpretation of blood typing results.

Unfortunately, there is no published research concerning detection of salivary hemagglutinins in dried stains, and no detection method is currently available for forensic science use.

Received for publication 16 March 1987; revised manuscript received 22 July 1987; accepted for publication 17 Aug. 1987.

[1]John Jay College of Criminal Justice, City University of New York, New York City, Chemist and North Regional Laboratory, Division of the New Jersey State Police, Little Falls, NJ.

[2]Assistant professor of chemistry, Chemistry Department, West Virginia State College, Institute, WV.

[3]Associate professor of biology and immunology, John Jay College of Criminal Justice, City University of New York, New York City, NY and Graduate School and University Center, Ph.D. Program in Biochemistry, New York City, NY.

As a result, evidence concerning salivary blood group antigens cannot be confirmed by an independent testing method.

The current study was undertaken to determine if recently improved detection methods are sufficiently sensitive to measure salivary hemagglutinins and to determine if such hemagglutinins are sufficiently stable, under normal circumstances, to be of evidentiary value.

We could find no reports in the literature on the detection of hemagglutinins in dried saliva samples, presumably because existing techniques of detection and analysis are not sufficiently sensitive for researchers to conduct these studies. To begin our investigation, we developed a new assay method that includes five complementary strategies to increase assay sensitivity:

(1) concentrating salivary hemagglutinins by extraction with toluene;
(2) adding polyvinyl pyrrolidone, dextran, albumin, *and* anti-immunoglobulin A (IgA) antibody to the hemagglutination mixture;
(3) pretreating the red blood cells in the hemagglutination mixture with a proteolytic enzyme, bromelin;
(4) using low concentrations of erythrocytes during hemagglutination; and
(5) completing the hemagglutination reaction at low (0°C) temperature.

These modifications are described in detail in the Methods section. Together they may provide a basis for developing a much needed, complementary test for the currently used absorption-inhibition test that only detects salivary blood group antigens.

Materials and Methods

Collection Methods

One hundred volunteers, blood typed in the ABO system, donated whole saliva. After chewing paraffin to increase saliva production, the subjects salivated into clear plastic cups, and then we transferred their saliva to glass test tubes. Each saliva sample to be used for stain production and subsequent hemagglutinin assay was number coded. The samples were either used fresh or were frozen at −20°C for 24 h or until tested. In preliminary studies it was determined that the age of the donor has no significant effect on the detection of hemagglutinins. Saliva donors ranged in age from 16 to 60. In addition, our early studies indicated that the sex of the donor has no effect on hemagglutinin assay results. These results confirm those of Wilson and Green [*1*].

Hemagglutination Assays for Liquid Samples

Stock Solutions—Phosphate buffered saline (PBS), pH 7.4, is prepared by addition of 1.15 g of sodium phosphate, dibasic (Na_2HPO_4), 0.20 g of potassium phosphate, monobasic (KH_2PO_4), and 8 g of sodium chloride (NaCl) to 1 L of distilled water. Bromelin solution is prepared by mixing 0.1 g of bromelin with 10 mL of PBS, treating the mixture briefly with a homogenizer, and then centrifuging for 5 min at 1000 × *g*. The supernatant is stored at −20°C. Albumin, dextran, and polyvinyl pyrrolidone-PBS (ADP-PBS) is prepared by dissolving 0.5 g of powdered albumin, 0.5 g of dextran, and 0.5 g of polyvinyl pyrrolidone in 100 mL of PBS.

Sample Preparation—Before analysis, frozen saliva samples are allowed to thaw for 10 min at room temperature. One volume of toluene is then added to two volumes of saliva and mixed for 30 s using a vortex mixer at medium speed. The mixture is then centrifuged at 450 × *g* for 5 min. Three layers become visible: the top layer contains a cloudy mucus, the middle layer is a less cloudy solution, and the bottom layer contains amorphous debris. The

middle layer is carefully removed with a Pasteur pipette for agglutination tests, and the other layers are discarded.

Preparation of Test Cells—Erythrocytes to be used as test cells are washed three times with PBS. After the first and second washings, the cells are centrifuged at 450 × *g* for 2 min; after the third washing they are spun for 15 min at 450 × *g*. One part of the red cell sediment is then mixed with two parts of bromelin solution and incubated at 37°C for 1 h in a water bath. The cells are then washed three times with PBS in a ratio of 1:4 and spun after each washing at 450 × *g* for 2 min. From the final sediment, a 0.5% cell suspension is prepared in ADP-PBS. Less concentrated solutions of cells (0.2%) are used when extracts from saliva stains are examined.

Hemagglutination Assays—Of bromelin treated cells, 50 μL are added to 150 μL of toluene-treated saliva and the mixture is allowed to stand for 2 h at 0°C. The agglutination reaction is then read visually using a hand-held, ×15 magnifying lens. Red cell buttons that have smooth edges and run slowly down the vertical surface of the inverted test tube are scored as negative. Agglutination clusters that have rough edges and do not run, but remain attached to the bottom or fall down as one or a small number of clumps, indicate a positive hemagglutination reaction. Positive results are expressed on a scale from one to four, with four indicating maximum hemagglutination. Type O cells, which remain unagglutinated regardless of the type of saliva under investigation, serve as a control to detect nonspecific agglutination.

Preparation of Saliva Stains

Saliva stains are produced on cigarettes by soaking cigarette butts in fresh, whole saliva for 2 min. These samples are coded as described above so that the analyst has no knowledge of the donor's blood group. The cigarette filter and filter paper are separated from the body of each cigarette and the paper is cut into 1-mm squares. Each square is then eluted in 400 μL of PBS for 23 h at 4°C and 1 h at 50°C in a disposable glass test tube. Similarly, 800 μL of the same solution was used to elute the filter itself under the same conditions. Although stains prepared in this manner are not representative of typical stains found in forensic science casework, this method was adopted so that calculations of protein extraction efficiency could be made.

In a preliminary experiment, saliva stains were prepared by placing 200, 400, 600, and 800 μL of fresh, whole saliva onto filter paper. These stains were allowed to dry for 2 h at 25°C. The stains were then located using ultraviolet light, cut into 1 mm squares, and eluted in PBS.

Measurement of Protein and IgA Antibodies in Stain Extracts

To determine the extent to which IgA protein is transferred from stains to the PBS eluant, stain extracts are examined by immunoelectrophoresis using goat anti-human IgA antiserum (heavy chain specific) obtained from Cappel Laboratories.[4] The buffer used during electrophoresis is 0.02*M* PBS at pH 7.3 with 0.05% sodium azide, as described by Grabar et al. [2]. The total concentration of protein in the saliva extract is compared with toluene extracted and untreated saliva using a standard Bio-Rad assay.[5] Dilutions of an IgA protein standard (also from Cappel Laboratories) are prepared at concentrations ranging from 0.2 to 1.4 mg/mL. The protein concentration in liquid saliva samples and stain extracts is determined, after completion of the assay, by graphing absorbance at 595 nm and comparing the results with those obtained with standard solutions. For both types of saliva samples, dilu-

[4]West Chester, PA, catalogue Number 8601-0021.
[5]Bio-Rad Laboratories, Richmond, CA, catalogue Number 500-0001.

tion before assay is necessary to read the protein content accurately from the standard curve. The dilution is subsequently factored into the calculations for protein determination.

Hemagglutination Assays with Stain Extracts

Hemagglutinins in each saliva stain extract are measured as follows: One volume of toluene is added to two volumes of stain extract. The solutions are mixed and subsequently centrifuged as described above. The upper and bottom layers are discarded, and 20 μL bromelin treated test cells are added to 60 μL fluid obtained from the middle layer. The resulting solution is gently mixed and allowed to stand for 1 h at 0°C. Two microlitres of anti-IgA antiserum are then added, and the mixture is once again allowed to stand for 1 h at 0°C before being examined for hemagglutination. If agglutination has not occurred, the mixture is again centrifuged and the supernatant is removed. Sixty microlitres of additional stain extract is added and mixed, and the incubation and reading are repeated. This procedure is repeated until agglutination occurs or until the stain extract is completely consumed. The results of each assay are then compared to the known blood group of the saliva donor.

Hemagglutinin Stability Studies

Liquid saliva samples and dried stains were aged at −20, 25, and 56°C for periods of 0, 2, 8, 24, and 72 h, and 1, 2, 6, 8, 10, 12, and 14 weeks. Hemagglutination assays were then conducted as described above. Liquid samples were aged in sealed, disposable glass test tubes. Stains were prepared by drying 800 μL of fresh saliva on filter paper.[6]

Results

Stain Extraction Efficiency

The objective of our first experiments was to determine the efficiency with which salivary proteins, IgA antibodies, and hemagglutinins can be extracted from dried stains. A variety of eluant solutions were tested, including PBS, 0.1*M* NaCl (isotonic saline), 5% ammonium hydroxide, and distilled water. The Bio-Rad dye binding protein assay, described in the Materials and Methods section, was used to determine the total protein eluted in each case.

Extraction with each of these solutions yielded similar eluant protein concentrations, with little apparent loss of protein. PBS was chosen as the eluant for subsequent experiments because it is relatively inexpensive and easy to prepare, and because it buffers samples against pH changes that might affect protein stability.

The protein concentration in PBS stain extracts was approximately equal to (96%) that of whole saliva, which measured 1.67 mg/mL. The protein concentration of toluene extracted saliva was somewhat lower (78%) than that of whole saliva. Immunoelectrophoresis was used to determine the relative concentration of eluted IgA antibodies. The very faint precipitation bands which were observed indicated that IgA proteins were present only at very low levels. At this point it was apparent that an extremely sensitive assay method would be needed to detect the extracted hemagglutinins, which constitute only a very small fraction of the total IgA protein.

Assay Sensitivity

Filter paper stains were prepared from 200, 400, 600, and 800 μL of fresh saliva and dried at room temperature. Figure 1 summarizes the results of hemagglutination assays per-

[6]Nine-centimetre diameter, manufactured by Reeve Angel, Clifton, NJ.

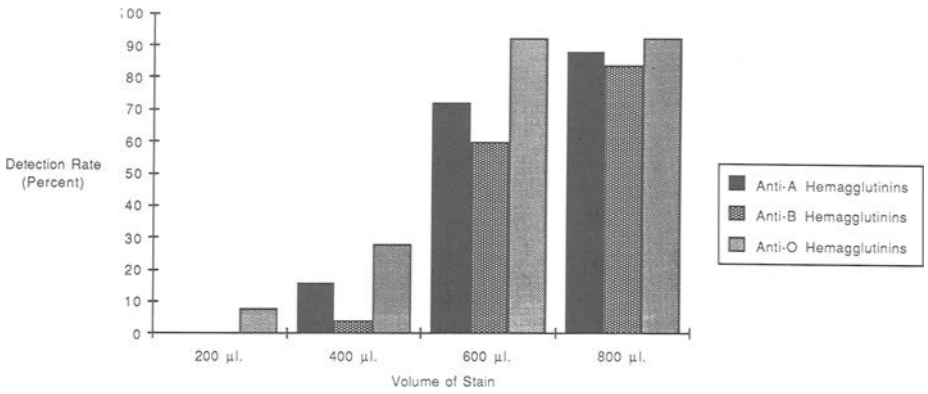

FIG. 1—*Effect of stain volume on hemagglutinin detection rate. Legend for this figure applies to all subsequent figures. Detection rate is based upon 25 different samples which were tested.*

formed after 2 h. The percent detection rate is calculated based upon the 25 samples tested. The results shown in Fig. 1 indicate that stains of 800 µL saliva are generally required to ensure a high rate of hemagglutinin detection with the assay methods presently in use.

The hemagglutinin assay of the saliva stains is performed using a number coding system so that the analyst has no knowledge of the donor's blood group or agglutinogen secretor status before making a determination based on the detection of hemagglutinins. Note that one false positive was observed in the AB class. In addition, anti-A but not anti-B hemagglutinins were observed in samples donated by one volunteer with type O blood. This same individual had tested positive for both hemagglutinins two months earlier during fresh saliva studies. It was later learned that this volunteer was being treated with antibiotics for a bacterial infection. Subsequent examination of another fresh saliva sample from this individual indicated that both hemagglutinins were again present, suggesting that the bacteria, antibiotics, or both, may have influenced the secretion or detection of anti-B hemagglutinins. No attempt has been made to correlate ABO typing results based on hemagglutinin detection with the presence of ABH antigens within the stain. However, there is an excellent correlation between the detection of A and B hemagglutinins with the donor's blood type.

Hemagglutinin Stability

Fresh saliva samples and dried stains were then incubated at various temperatures to determine hemagglutinin stability after different time periods. Figures 2 through 6 summarize our observations concerning these samples. Hemagglutinins appear to be stable for up to two months when stored at −20°C; however, they are stable only for 12 h to one day when kept as stains at room temperature. These results indicate the importance of refrigerating, or preferably, freezing collected samples as soon as possible to protect the hemagglutinins. More important, they also indicate that hemagglutinin stability in dry stains is sufficient for collected stains to retain forensic value for periods of one to several days or longer, if temperatures are low or assay sensitivity is high.

Analysis of Stains on Cigarette Butts and Envelope Flaps

The detection procedure developed was used to evaluate whether ABO blood type could be determined from stains on cigarettes and envelope flaps. Our results indicate that the determination of specific hemagglutinins from cigarette butts is generally not possible using presently available methods, however ABO bloodtype could be determined in almost 50% of the

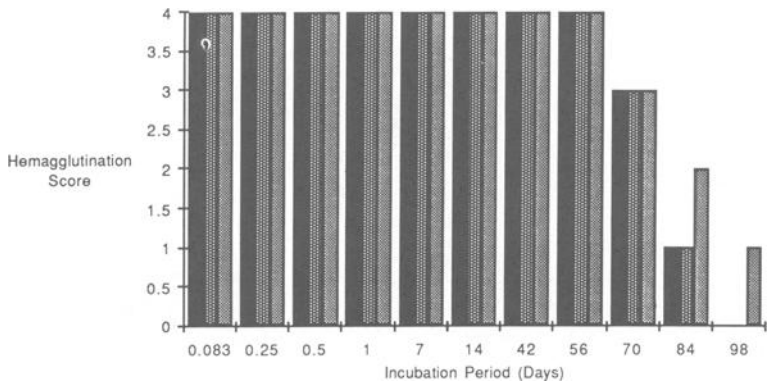

FIG. 2—*Hemagglutinin stability in 25 different liquid saliva samples incubated at −20°C.*

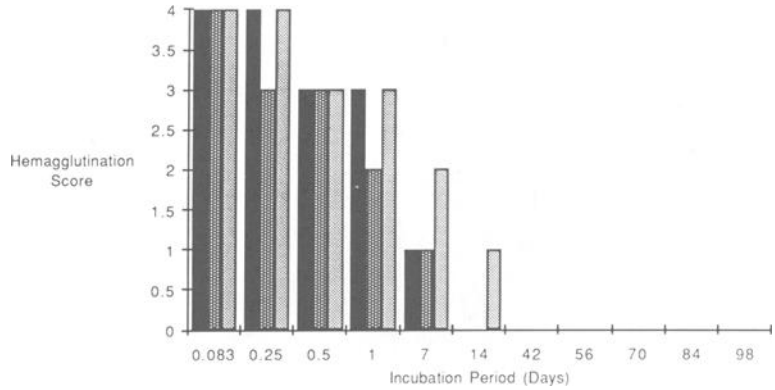

FIG. 3—*Hemagglutinin stability in 25 different liquid saliva samples incubated at 25°C.*

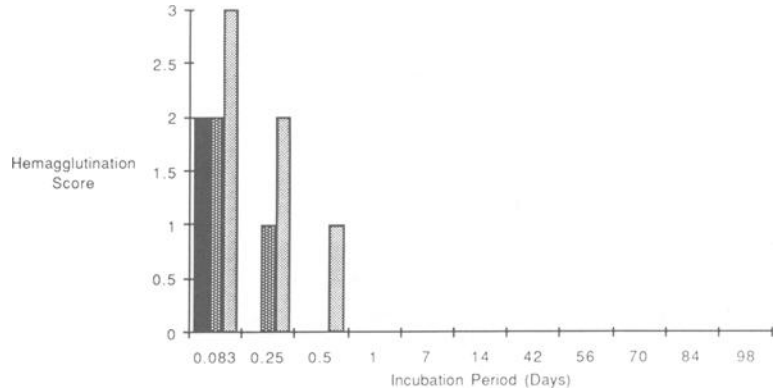

FIG. 4—*Hemagglutinin stability in 25 different liquid saliva samples incubated at 56°C.*

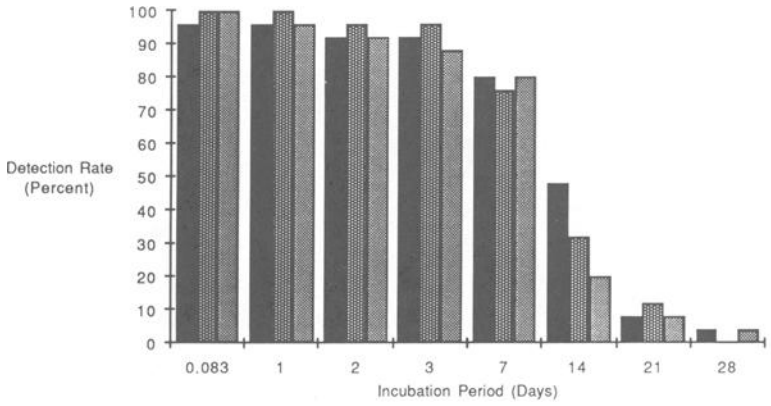

FIG. 5—*Hemagglutinin stability in dried saliva samples incubated at −20°C. Detection rate is based upon 25 different samples which were tested.*

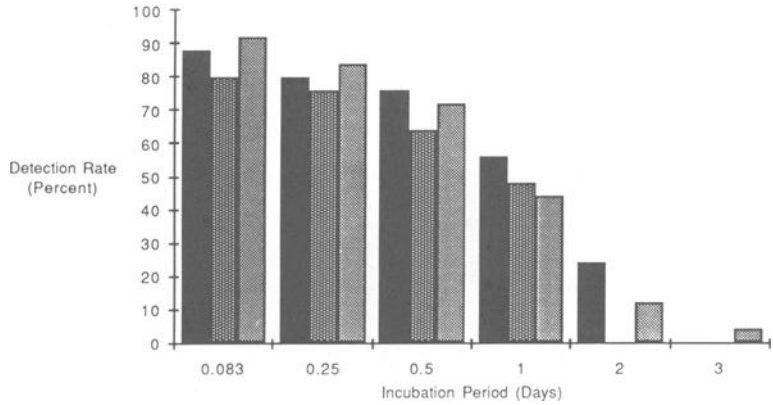

FIG. 6—*Hemagglutinin stability in dried saliva samples incubated at 25°C. Detection rate is based upon 25 different samples which were tested.*

envelope flaps tested. It thus seems likely that relatively minor improvements in assay sensitivity may be sufficient for stain analysis in actual casework. This approach can provide valuable information when used as a confirmatory procedure in combination with established methods for agglutinogen determination.

Phadebas Procedure/Hemagglutinin Assay Compatibility

Various procedures have been developed to determine if a stain is in fact saliva. Some of these might interfere with ABO hemagglutinin bloodtyping. For example, Willot and Griffiths [*3*] developed a procedure in which Phadebas tablets are dissolved in water, sprayed on filter paper, and then placed on suspected stains. To determine if this procedure interferes with our hemagglutination assay, 25 800-μL saliva stains were prepared and tested with the Phadebas procedure and subsequently tested with the hemagglutinin assay. In 22 of 25 cases, ABO blood types were correctly determined, indicating that Phadebas tablets do not interfere with detection. In the 3 unsuccessful cases, the results were inconclusive. It is possi-

ble that these stains contained hemagglutinins at levels below the sensitivity of the assay employed.

Discussion

There is a remarkable difference between the stability of hemagglutinins in bloodstains and hemagglutinins in saliva stains. Blood hemagglutinins are readily detectable and can be used for blood typing after aging at room temperature for as long as two weeks, in most cases, and occasionally for several months, while salivary hemagglutinins are useful for approximately one day, even when relatively sensitive assay techniques are used. An understanding of the reasons for this difference is important because it may enable the development of methods for extending the useful time period for salivary stain analysis.

In one of our studies, 90 fresh saliva samples for "O" blood group donors were analyzed for the presence of A and B hemagglutinins. In 2 of those cases, anti-A hemagglutinin was not detected, and in 1 case anti-B hemagglutinin was not found. This would indicate that in these cases either salivary secretion of an immunoglobulin is taking place minimally or not at all, or that one hemagglutinin is less stable than the other. Of the 2 possibilities, the first seems more likely since we have never seen any case of preferential instability of IgA hemagglutinins. Our results also indicate that antibiotics may alter salivary hemagglutinin secretion to the extent that the hemagglutinin detection assay may result in errors. Thus it must be emphasized that this procedure should be used as a confirmatory rather than a primary test.

Hemagglutinins may be more stable in bloodstains than in stains of saliva for a variety of reasons. For example, blood has higher concentrations of various salts and a much greater buffering capacity than saliva. Many natural antioxidants and free radical scavengers, such as the vitamins C, E, and A, bioflavenoids, and various thiols, may protect blood hemagglutinins from oxidative damage. And the presence of high concentrations of albumin and other serum proteins may also serve to stabilize native hemagglutinin conformation. The possibility that one or more stabilizing factors are present in blood is supported by the Karsner and Koeckert's observation that hemagglutinins are more stable in whole blood stains than in stains prepared from serum [*4*].

Of course, stabilizing factors in blood, or destabilizing factors in saliva, may not significantly affect hemagglutinin stability. Blood hemagglutinins are predominantly IgM and IgG antibodies, while salivary hemagglutinins are exclusively IgA [*5*]. Differences in stability between them may simply be an inevitable consequence of the primary structures of IgG, IgM, and IgA antibodies.

Normal adults have between 60 and 330 mg of IgA/100 mL of blood, but have less than 5.0 mg IgA/100 mL of saliva [*5*]. Because saliva contains significantly lower quantities of IgA, it is also possible that the apparent instability of salivary hemagglutinins may be due partly to difficulties in detection of small amounts of these hemagglutinins.

Regardless of the reasons for the apparent instability of salivary hemagglutinins, the steady increase in hemagglutinin assay sensitivity during the past two decades suggests that the importance of salivary hemagglutinins to the forensic serologist will increase markedly in the future. Improvements in assay sensitivity since 1960 have resulted from addition of gum acacia [*6*], albumin [*7*], PVP [*8*], dextran [*9*], and homologous antibodies [*10*] to the assay mixture; use of low concentrations of test cells in well slides [*11*]; pretreatment of test cells with enzymes like papain [*12*] and bromelin [*13*]; extraction of saliva with ether or toluene [*14,15*]; and performance of the assays at low temperature [*16*].

In our laboratory we have recently found that the combined use of PVP, albumin, *and* dextran raises sensitivity more than any of these alone [*17*]. We have also observed that inclusion of goat anti-human IgA antibodies in the assay mixture leads to an additional improvement in sensitivity.

Future increases in assay sensitivity may result from extraction of stains with buffers composed of cations and anions which can stabilize protein conformation, from extraction at low temperature in the absence of oxygen, and from use of radioimmunoassay and enzyme-linked immunoassay techniques.

Use of these assay strategies can make a critical difference in the forensic science value of saliva stains. Very sensitive techniques for determination of salivary blood group antigens are currently in general use when saliva samples are obtained in criminal cases. However, the results of these antigen determinations are not definitive in all cases. This test, like most others, has an associated error rate [*18,19*]. Presently, results cannot be confirmed by an independent method of testing. Further development of hemagglutinin assay techniques, as described, would provide the desired confirmatory test.

Acknowledgments

This research was conducted with the help of funding obtained from the Professional Staff Congress, City University of New York, Grant PSC-BHE 664120 and from the U.S. Department of Education, MISIP Grant G-008641165. We would like to thank Professor Peter R. DeForest, John Jay College of Criminal Justice, for his advice and assistance throughout the conduct of this research.

The research was performed in partial fulfillment of the requirements for the M.S. degree in Forensic Science, John Jay College of Criminal Justice, by James J. Harrington.

References

[*1*] Wilson, R. M. and Green, G. E., "Genetic Aspects of Salivary Secretion of Isoagglutinins" in *Proceedings of the Society of Experimental Biology and Medicine*, Vol. 115, 1964, pp. 982–985.

[*2*] Grabar, P. and Williams, C. A., "Methode Permettant l'Etude Conjugee des Proprietes Electrophoretiques et Immunochimiques d'un Melange de Proteines. Application au Serum Sanguin," *Biochimica Biophysica Acta*, Vol. 10, 1953, pp. 193–194.

[*3*] Willot, G. M. and Griffiths, M., "A New Method for Locating Saliva Stains—Spotty Paper for Spotting Spit," *Forensic Science International*, Vol. 15, 1980, pp. 79–83.

[*4*] Karsner, H. T. and Koeckert, H. L., "The Influence of Dessication on Human Normal Isohemagglutinins," *Journal of the American Medical Association*, Vol. 73, 1919, pp. 1207–1210; cited in R. E. Gaensslen, *Sourcebook in Forensic Serology, Immunology, and Biochemistry*, National Institute of Justice, U.S. Department of Justice, Washington, DC, 1983, p. 300.

[*5*] Tomasi, T. B., Jr. and Zigelbaum, S., "The Selective Occurrence of $Gamma_{1A}$ Globulins in Certain Body Fluids," *Journal of Clinical Investigation*, Vol. 42, 1963, pp. 1552–1560.

[*6*] Wiener, A. S., "Isoagglutinins in Dried Bloodstains. A Sensitive Technique for their Demonstration," *Journal of Forensic Medicine*, Vol. 10, 1963, pp. 130–132.

[*7*] Funk, H. J. and Tostiak, W., "A Practical Method for Detecting ABO Agglutinins and Agglutinogens in Dried Bloodstains," *Journal of Forensic Sciences*, Vol. 10, 1965, pp. 455–465.

[*8*] Matsuzawa, S., Yoshida, N., and Hata, Y., "Secretion of Anti-A and Anti-B Agglutinins into Human Saliva," *Japanese Journal of Legal Medicine*, Vol. 26, 1976, pp. 19–23.

[*9*] Van Oss, C. J., Mohn, J. F., and Cunningham, R. K., "Influence of Various Physicochemical Factors on Hemagglutination," *Vox Sanquinis*, Vol. 34, 1978, pp. 351–361.

[*10*] Kissling, E. and Neumann, W., "Beitrag zum Nachweis von H-Substanzen an Trockenblutspuren im Absorptionsverfahren und zur Erfassung schwacher Agglutinine im Agglutininwirkungsversuch nach Lattes," *Archiv für Kriminologie*, Vol. 150, 1972, pp. 141–145; cited in R. E. Gaensslen, *Sourcebook in Forensic Serology, Immunology, and Biochemistry*, National Institute of Justice, U.S. Department of Justice, Washington, DC, 1983, p. 29.

[*11*] Outteridge, R. A., "Recent Advances in the Grouping of Dried Blood and Secretion Stains" in *Methods of Forensic Science*, A. S. Curry, Ed., Vol. 4, New York Interscience, 1965, pp. 299–332.

[*12*] Ducos, J., "Utilisation d'Hematies Papainisees pour la Detection des Anticorps Anti-A et Anti-B dans les Taches de Sang Sec," *Annales de Medecine Legale*, Vol. 40, 1960, pp. 519–521.

[*13*] Hummel, K. and Schoch, J., "Untersuchungen uber die Ausscheidung von Anti A und Anti B Hamagglutinen im Menschlichen Mundsperchel," *Zeitschrift Immunitaetsforschung, Allergie und Klinische Immunologie*, Vol. 133, 1967, pp. 80–100.

[*14*] Boettcher, B., "ABO Blood Group Agglutinins in Saliva," *Acta Haematologica,* Vol. 38, 1967, pp. 351–360.

[*15*] Saneshige, Y. and Woodfield, D. G., "Detection of ABO Isoagglutinins in Saliva using Toluene," *Journal of the Forensic Science Society,* Vol. 24, No. 5, 1984, pp. 489–493.

[*16*] Hardman, P. K. and Hardman, J. T., "Salivary ABO Antibodies and Periodontal Disease," *Journal of Periodontology,* Vol. 54, 1983, pp. 351–353.

[*17*] Kobilinsky, L. and Harrington, J. J., "Detection and Use of Salivary Hemagglutinins for Forensic Blood Grouping," *Journal of Forensic Sciences,* Vol. 33, No. 2, March 1988, pp. 396–403.

[*18*] Scott, M. L. and Corry, J. E. L., "Effect of Blood Group Active Micro-organisms on the ABO Grouping of Human Whole Saliva," *Forensic Science International,* Vol. 16, 1980, pp. 87–100.

[*19*] Pereira, M. and Martin, P. D., "Problems Involved in the Grouping of Saliva, Semen and Other Body Fluids," *Journal of the Forensic Science Society,* Vol. 16, 1976, pp. 151–154.

Address requests for reprints or additional information to
Professor Lawrence Kobilinsky
Department of Forensic Science
John Jay College of Criminal Justice
City University of New York
445 W. 59th St.
New York, NY, 10019