J. L. Mudd,[1] *M.S.*

A Capillary Tube Method for the Lewis Typing of Red Blood Cells

REFERENCE: Mudd, J. L., **"A Capillary Tube Method for the Lewis Typing of Red Blood Cells,"** *Journal of Forensic Sciences*, JFSCA, Vol. 28, No. 1, Jan. 1983, pp. 231-234.

ABSTRACT: A simple and inexpensive capillary tube procedure, which can be applied in the forensic science laboratory, is described for the detection of the Lewis^a (Le^a) and Lewis^b (Le^b) antigens on red blood cells. This procedure will permit approximately 4000 tests to be performed from a single 2-mL bottle of Lewis antiserum.

KEYWORDS: pathology and biology, antigen systems, genetic typing, blood, hemagglutination, Lewis blood group system, blood grouping and cross matching

The detection of the Lewis^a (Le^a) and Lewis^b (Le^b) antigens on red blood cells as a means of classifying an individual as a nonsecretor, Le(a+b−), or secretor, Le(a−b+), is finding increased use in forensic science laboratories. However, one obstacle that some laboratories have encountered in implementing the Lewis system on a routine basis is the high cost of the Le^a and Le^b antisera. In addition, the standard tube test used for Lewis typing requires approximately 100-μL of neat Lewis antiserum per test and therefore limits the number of tests to approximately 20, which can be obtained from a 2-mL bottle of Lewis antiserum.

In 1944, Chown introduced a capillary tube technique for the Rh typing of red blood cells [*1*]. Subsequently, various modifications of the capillary tube method have been used in blood banking for the detection of the Lewis [*2,3*], Kell [*4*], Bg^a (HL-A7) [*5*], and Bg^b (W-17) [*5*] antigens on red blood cells. Later, using hemagglutination inhibition in capillary tubes, Milner et al [*6*], successfully detected the hepatitis B antigen on red blood cells.

Using a method introduced by Crawford in 1977 [*2*], this paper describes a simple and inexpensive capillary tube procedure that can be applied in the forensic science laboratory for detection of the Lewis blood group antigens on red blood cells. Preliminary reports of this procedure were presented by the author in 1980 [*7,8*].

Materials

Chown-type capillary tubes (0.4 mm inner diameter by 90 mm length) were obtained from Diagnostic Technology, Great Neck, NY.

One part Lewis antisera (Ortho Diagnostics) was diluted with four parts of 100-m*M* phosphate buffered saline (PBS), pH 7.0, for use in the capillary tube test.

Blood specimens of known Lewis type were obtained from volunteer donors by finger puncture. Human blood specimens of unknown Lewis type were obtained from a Washing-

Date received 6 April 1982; revised manuscript received 3 June 1982; accepted for publication 4 June 1982.

[1]Research chemist, FBI Academy, Quantico, VA.

ton, DC area hospital and from cases submitted to the FBI Laboratory. The red blood cells (RBC) to be tested were washed three times with PBS and a 50% v/v suspension was prepared by mixing 50-μL packed RBC with 50-μL Alsever's solution. Immediately before testing, 25 μL of a 4% (w/v) ficin solution (Sigma, No. F8629) was added to the 50% RBC suspension and mixed. The 4% ficin solution, prepared in Alsever's solution and filtered through Whatman No. 1 filter paper, was stored at −35°C until used. Ficin-treated RBC are stable for 2 h at 4°C.

A hematocrit tube sealant (Fisher, No. 2-676-20) was used to seal the ends of the capillary tubes.

Method

By capillary action, the diluted Lewis antiserum was drawn into the capillary tube to a distance of approximately 2.5 cm and the tip of the capillary tube was wiped dry. Failure to completely wipe the capillary tip may contaminate the ficin-treated cells with antiserum. While keeping the capillary tube in a vertical position to prevent air bubbles from being trapped in the tube, the ficin-treated red cells are drawn into the capillary tube to a distance of approximately 1 cm and the tip is again wiped dry. The end of the capillary tube used to draw up the antiserum and RBC was sealed by pushing the tip into the hematocrit tube sealant. The tube was then inverted and placed at a 60° angle to allow the red cells to flow down through the antiserum. Agglutination results are read after 10 min.

To ensure the accuracy of the results, known blood specimens from Le(a+b−) and Le(a−b+) individuals were run as controls alongside the questioned blood specimens. In addition, with each questioned blood specimen, a blank control having Alsever's solution substituted for the Lewis antiserum was run to ensure that the questioned blood specimens had not been contaminated with either of the Lewis antisera.

The qualitative tube test used to verify the results of the capillary tube procedure was that described by Ortho Diagnostics [*9*].

Results

Typical agglutination results obtained for the phenotypes Le(a+b−), Le(a−b+), and Le(a−b−), using the capillary tube method, are shown in Fig. 1. Positive results, Tubes 1 and 4, are indicated by a clumped, broken column of red cells along the length of the capillary tubes. Negative results, Tubes 2, 3, 5, and 6, are indicated by an unbroken needlelike column of red cells along the length of the capillary tubes.

A study, comparing the test tube and capillary tube methods, was conducted on 300 individual liquid blood specimens obtained from a Washington, DC area hospital. Of the 300 specimens tested, 174 were grouped as Le(a−b+), 62 as Le(a+b−), 60 as Le(a−b−), and 4 as Le(a+b+). Because information regarding the racial origin of the blood specimens was unavailable, no attempt was made to calculate the population frequency of these specimens.

Results obtained from this study showed no inconsistencies in the various Lewis phenotypes observed with either the test tube or capillary tube method.

Of the 174 specimens grouped as Le(a−b+) the test tube and capillary tube methods, ten specimens produced very weak agglutination for the Le^a antigen when grouped by the more sensitive capillary tube method. The weak Le^a antigenic activity observed in these specimens is not surprising since the Le^a antigen has been reported to be the precursor to the Le^b antigen [*10*], although the exact pathway is still uncertain [*11*], and there usually remains in the serum of Le(a−b+) individuals very low concentrations of the unconverted Le^a antigen [*12*]. This unconverted Le^a antigen, however, was not readily detected on red cells by the test tube method. The weak agglutination caused by unconverted Le^a antigen, observed in the capillary tube method, is easily distinguished from the typical strong agglutination normally

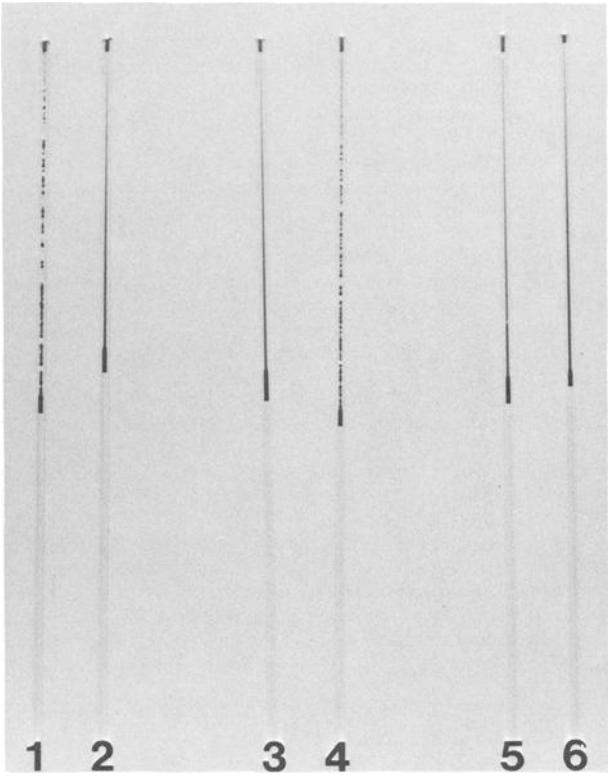

FIG. 1—*Typical results obtained for each of the known Lewis phenotypes: Tubes 1 and 2, known Le(a+b−) cells with anti-Le^a and anti-Le^b, respectively; Tubes 3 and 4, known Le(a−b+) cells with anti-Le^a and anti-Le^b, respectively; and Tubes 5 and 6, known Le(a−b−) cells with anti-Le^a and anti-Le^b, respectively.*

observed with a positive result and therefore does not interfere with the correct identification of the Lewis phenotype.

Twenty-five liquid blood specimens, which ranged in age from two weeks to six months, were obtained from cases currently under examination in the FBI Laboratory and were tested for the presence of the Lewis antigens. The Lewis phenotype obtained for each specimen was found to be the same using both the capillary and the test tube methods.

Conclusion

The capillary tube method described in this paper has been shown to be a simple and reliable technique for determining the Lewis phenotypes of liquid blood specimens and can be used in the forensic science laboratory. Furthermore, this procedure will permit approximately 4000 tests (2.5 μL of antisera per test) to be performed from a single 2-mL bottle of Lewis antisera (diluted 1:5), thereby reducing the cost per test from approximately $1.50 for the test tube method to less than one cent per test for the capillary tube method.

In addition, preliminary work, performed by this laboratory and by others [*13,14*], indicates that the capillary tube method described in this paper can also be applied to the ABO and Rh typing of liquid blood and in the hemagglutination inhibition test for Lewis and ABO blood group substances in dried semen and saliva stains.

References

[1] Chown, B., "A Rapid, Simple and Economical Method for Rh Agglutination," *American Journal of Clinical Pathology*, Vol. 14, 1944, pp. 114-117.

[2] Crawford, M. N., "Rapid Testing of Ficinized Cells in Capillary Tubes," *Transfusion*, Vol. 18, No. 5, Sept.-Oct. 1978, p. 598.

[3] Crawford, M. N., "Capillary Tube Testing," in *Special Serological Technics Useful in Problem Solving*, R. B. Dawson, Ed., American Association of Blood Banks, Washington, DC, 1977, Chap. 1, pp. 1-5.

[4] Lewis, M., Kaita, H., and Chown, B., "Kell Typing in the Capillary Tube," *Journal of Laboratory and Clinical Medicine*, Vol. 52, No. 1, July 1958, pp. 163-168.

[5] Crawford, M. N., Gottman, F. E., and Rogers, L. C., "Capillary Tube Testing and Enhancement with 30% Albumin," *Vox Sanguinis*, Vol. 30, 1976, pp. 144-148.

[6] Milner, L. V., Ellis, J. L., Viljoen, M. H., Marimuthu, T., and Godefroy, J., "Hemagglutination Inhibition in Capillary Tubes—a Simple and Reliable Test for HBsAg," *Transfusion*, Vol. 17, No. 3, May-June 1977, pp. 233-237.

[7] Mudd, J. L., "The Use of the Ficin Capillary Method for Lewis Typing of Red Blood Cells," presented at the Fall Meeting of the Southern Association of Forensic Scientists, Tallahassee, FL, 5-6 Sept. 1980.

[8] Mudd, J. L., "Use of the Ficin Capillary Tube Method for Lewis Typing Red Cells," presented at the Fall Meeting of the Mid-Atlantic Association of Forensic Scientists, Arlington, VA, 10-11 Oct. 1980.

[9] "Qualitative Test for Recognition of the Le^a Antigen on Human Red Blood Cells," Ortho Diagnostics, Inc., Raritan, NJ, 1978.

[10] Prohaska, R., Schenkel-Brunner, H., and Tuppy, H., "Enzymatic Synthesis of Blood-Group Lewis-Specific Glycolipids," *European Journal of Biochemistry*, Vol. 84, 1978, pp. 161-166.

[11] Watkins, W. M., "Biochemistry and Genetics of the ABO, Lewis, and P Blood Group Systems," in *Advances in Human Genetics*, Vol. 10, H. Harris and K. Hirschhorn, Eds., Plenum Press, New York, 1980, pp. 1-136.

[12] Issitt, P. K. and Issitt, C. H., "The Lewis System," in *Applied Blood Group Serology*, Spectra Biologicals, Oxnard, CA, 1976, Chap. 7, pp. 91-101.

[13] Long, J. C., "Lewis Capillary Method in Absorption—Inhibition of Saliva," First Combined Seminar of the California and Northwest Association of Forensic Scientists, Stateline, NV, 4-6 Nov. 1981.

[14] Hardman, J. T., Pierce, S. R., Crawford, M. N., and Beck, M. L., "A Modified Capillary Test Using 4% Ficin," *Transfusion*, Vol. 21, No. 3, May-June 1981, pp. 330-331.

Address requests for reprints or additional information to
James L. Mudd
FBI Academy
Room 214A, FSRTC
Quantico, VA 22135