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P. L. Zajac,[1] *B.S. and B. W. Grunbaum,*[1] *Ph.D., M.Crim.*

Determination of Group Specific Component Phenotypes in Dried Bloodstains by Immunofixation on Cellulose Acetate

A rapid method is described for the determination of the genetically controlled group specific component (Gc) system. The method uses electrophoresis on cellulose acetate membranes followed by immunofixation with a specific anti-Gc antiserum. The method has been successfully applied to samples of plasma, whole blood, and dried bloodstains. Multiple specimens, up to 16 samples, can be analyzed simultaneously in a total analysis time of less than 60 min. In contrast to conventional immunoelectrophoresis on agar gel, or immunofixation on agarose or starch gel [*1*], this method of immunofixation on cellulose acetate is rapid, reliable, and economical.

Materials and Methods

The method for the determination of the Gc phenotypes in dried bloodstains was standardized by using the Beckman Microzone Electrophoresis System, automatic one- to eight-sample applicator and accessories [*2*], Sartorius cellulose acetate membranes, and anti-human Gc antiserum (from Atlantic Antibodies). The method is essentially that described by Grunbaum and Zajac [*3*], with minor modifications.

Samples of dried bloodstains, either scrapings or stains on fabric, were placed in 250-μl microcentrifuge tubes and a minimum amount of water (5 to 10 μl) was added to make the solution as concentrated as possible. The tube was vortexed for 10 to 15 s and allowed to soak for 5 to 10 min. A drop or two of chloroform was then added, and the solution was vortexed for 10 to 15 s and then centrifuged for 1 min. The top layer of blood sample was then ready for application to the cellulose acetate membrane.

The samples were electrophoresed for 15 min at 500 V (or 20 min at 400 V). After electrophoresis, the membrane was immersed in the anti-Gc antiserum (diluted 1:6 with normal saline) for 5 to 7 min. The membrane was then rinsed in fresh saline for 10 min with agitation; the saline was changed once after 5 min. The original procedure was modified by the use of anti-Gc antiserum of sufficiently high titer to enable the elimination of the mineral oil immersion. The membrane was then stained with Ponceau S and finally washed with 5% acetic acid.

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[1] Criminalist, and research biochemist and consultant, respectively, Criminalistics Laboratory, Alameda County Sheriff's Department, Pleasanton, Calif.

Results and Discussion

This method was first standardized by using known fresh serum standards and whole blood. The method was then applied to prepared stains of whole blood and, eventually, stains in evidence casework. Thus far, dried bloodstains up to two months old have been successfully typed for Gc phenotypes (Fig. 1). Although one application of serum of the known Gc types is best for good resolution, two or more applications of the bloodstain sample may be needed, depending on the degree of persistence of the Gc protein.

It has been found that the addition of chloroform to the bloodstain solution greatly improves the clarity of the results by removing the stroma which normally causes streaking (Fig. 2).

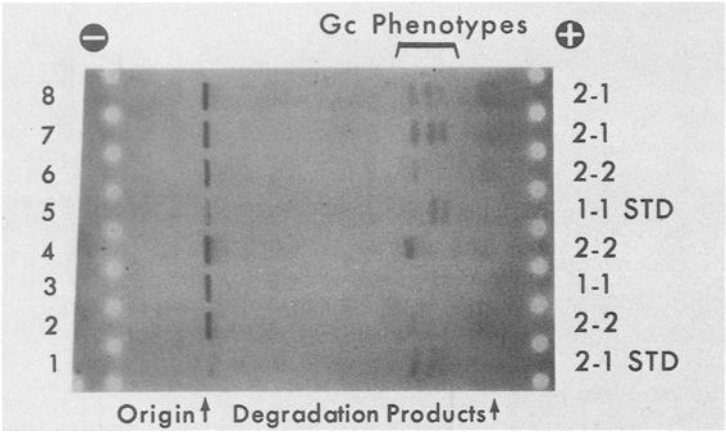

FIG. 1—*Cellulose acetate electrophoretogram showing fresh and dried blood samples of the three common Gc phenotypes. Position 1 is a fresh serum of Type 2-1, and Position 5 is fresh whole blood of Type 1-1. Position 8 is a one-week-old bloodstain of Type 2-1. Positions 2, 3, 4, 6, and 7 are stains two months old of Types 2-2, 1-1, 2-2, 2-2, and 2-1, respectively.*

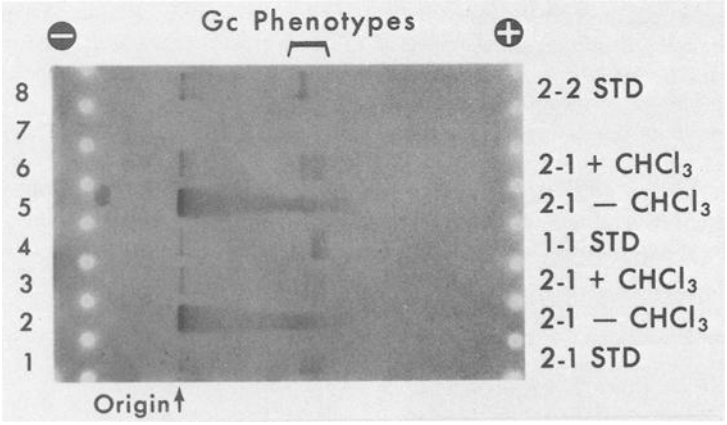

FIG. 2—*This electrophoretogram shows the effect of chloroform on the preparation of dried bloodstain samples. Positions 2 and 5 are ten-day-old stains of Type 2-1 without chloroform. Positions 3 and 6 are the same bloodstain samples prepared with chloroform and show marked improvement of clarity of results.*

Summary

Group specific phenotypes in dried bloodstains can be rapidly and reliably determined by immunofixation on cellulose acetate membranes. Up to 16 samples (Fig. 3) can be analyzed simultaneously in less than 60 min, and the cellulose acetate electrophoretogram is retained as a permanent record.

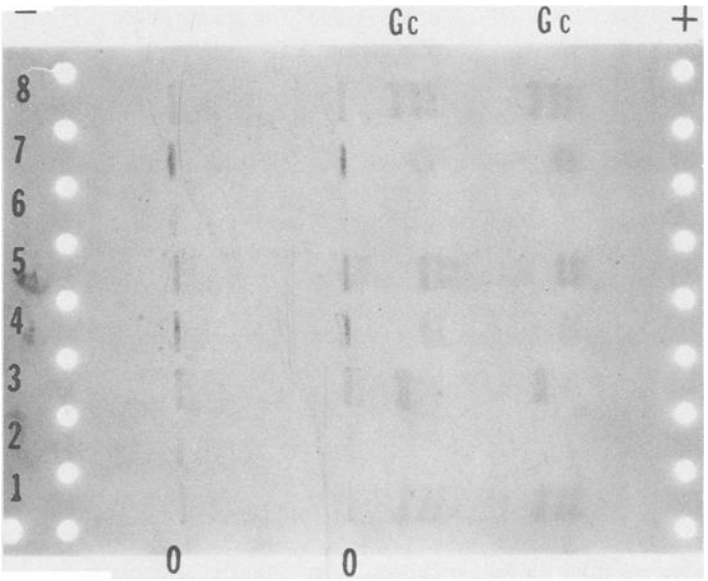

FIG. 3—*Electrophoretogram showing 16 samples run simultaneously on the same membrane. Positions 1, 5, and 8 of each row are fresh serum samples. The remaining samples in both rows are dried bloodstains.*

Acknowledgment

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References

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Address requests for reprints or additional information to
Patricia L. Zajac
Criminalistics Laboratory
Alameda County Sheriff's Department
P.O. Box 87
Pleasanton, Calif. 94566