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Rapid Phenotyping of the Group Specific Component by Immunofixation on Cellulose Acetate

A new method for phenotyping the group specific component (Gc) yields clear and unambiguous results within 60 to 80 min on multiple samples. This procedure employs electrophoresis using a cellulose acetate supporting medium, followed by immunofixation.

Phenotyping of Gc is traditionally done by immunoelectrophoresis on agar gel using the method of Hirschfeld [*1*]. This is a lengthy procedure, requiring approximately 48 h. If the antiserum is placed incorrectly in relation to the electrophoretic axis the precipitation arcs may take an inordinate length of time to form. Also, the method may give ambiguous or erroneous results. Nonspecific antisera tend to confuse the recognition of the precipitin arcs formed from the Gc variant. High quality monospecific antisera may be used with good results, but such large quantities are required that the determination becomes very expensive. Because immunoelectrophoresis is a lengthy, cumbersome, and expensive procedure, application of the technique is limited.

Immunoelectrophoresis can also be carried out using cellulose acetate supporting media [*2,3*]. In this laboratory we have successfully differentiated the Gc variants 1-1, 2-2, and 2-1 on cellulose acetate. However, immunoelectrophoresis with cellulose acetate still requires 24 to 48 h to complete and the results may be ambiguous, as with the gels. In contrast, immunofixation on cellulose acetate is rapid, accurate, reliable, and economical.

Materials and Methods

Equipment and Reagents

1. The method was standardized using the Beckman Microzone Electrophoresis system (Beckman Instruments Inc., Fullerton, Calif.), an automatic one to eight-sample applicator and accessories described by Grunbaum [*3*], and cellulose acetate membranes (Sartorius Filters, Inc., South San Francisco, Calif.).
2. A tris(hydroxymethyl)aminomethane (Tris)-glycine buffer (21.8 g glycine and 4.5 Tris to 1 litre) at pH 8.4 was found to be best for the electrophoresis cell buffer and membrane buffer.
3. Anti-human Gc antiserum (Atlantic Antibodies, Westbrook, Maine) was diluted 1 part with 6 parts normal saline.
4. The staining solution consisted of 0.2% (w/v) Ponceau S made up in 6% sulfosalicylic acid.

Received for publication 13 Dec. 1976; accepted for publication 30 Dec. 1976.

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5. Additional chemicals included mineral oil, petroleum ether (30 to 60°C boiling range), 5% acetic acid, normal saline, and water.

Method

The electrophoresis cell was filled with cell buffer. A cellulose acetate membrane was wetted with the cell buffer, blotted between clean filter papers, and placed on the bridge in the cell. Samples of serum or whole hemolyzed blood were applied to the cathodic side of the membrane. The eight-sample applicator applies all eight samples simultaneously, delivering approximately 0.2 μl of each sample. The samples were then subjected to electrophoresis for exactly 20 min at 500 V (2 to 4 mA) at room temperature.

The ends of the membrane were then cut off with a razor blade just outside the index hole and the suspending holes, leaving them intact. The membrane was lifted from the bridge assembly and immersed in the 1:6 dilution of antiserum for about 5 min. The membrane was then removed from the antiserum and totally submerged under mineral oil for 30 min. The membrane was next rinsed three times in petroleum ether to remove the mineral oil. The membrane was then suspended on the bridge assembly and placed in a bath containing normal saline. The saline solution was agitated with a magnetic stirrer to facilitate the washing process. The saline was replaced after 5 min and the washing continued for another 5 min. Excess antiserum and all remaining soluble proteins were removed by this washing. The membrane was then rinsed with water and stained for 5 min with Ponceau S. Excess stain was removed with 5% acetic acid until the background was white. The membrane was finally rinsed with water, dried between blotters, placed in a transparent plastic envelope, and appropriately labeled. Unlike the starch gel, the cellulose acetate electrophoretogram of the Gc variants remains as a permanent record.

Results and Discussion

Figures 1 and 2 clearly show the Gc phenotypes on cellulose acetate by immunofixation. Figure 1 shows the reproducibility of the components and the three common phenotypes. Figure 2 shows results of a random eight samples in which all three phenotypes are clearly distinguishable.

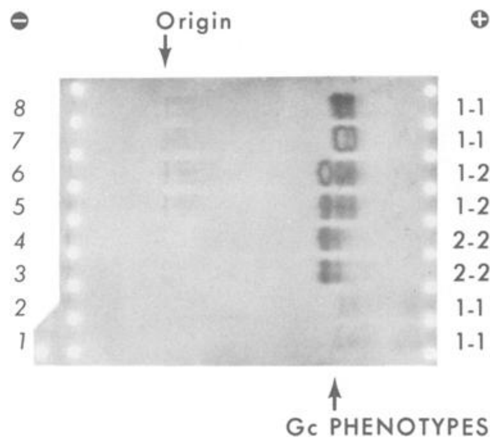

FIG. 1—*Duplicate patterns of the three common Gc phenotypes as seen by immunofixation on cellulose acetate membranes. In Positions 1 and 2 is the Gc phenotype 1-1 pattern of an old bloodstain.*

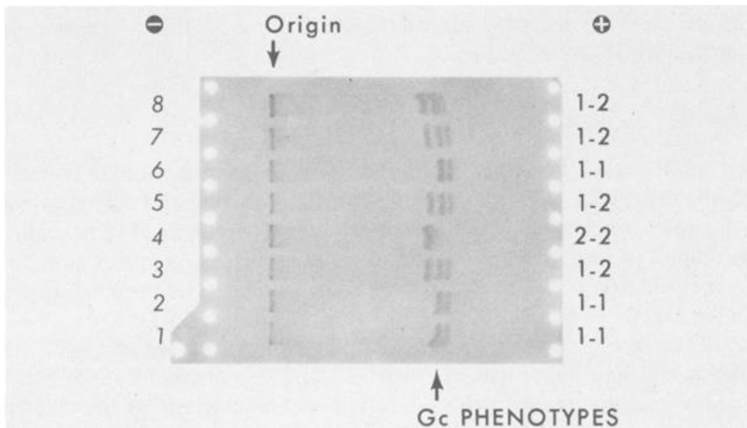

FIG. 2—*This cellulose acetate electrophoretogram shows the three common Gc phenotypes from a random population sampling.*

A single application delivers approximately 0.2 μl of sample to the membrane. Although one, two, and three applications were tested, one application gave the best results for both serum and whole blood.

One millilitre of the Gc antiserum when diluted sixfold with normal saline is sufficient for 80 samples on ten cellulose acetate membranes. In this laboratory, where many samples are analyzed daily, it is standard procedure to determine 80 specimens for Gc simultaneously. The degree of dilution of a particular antiserum depends of course on the titer. The cost of the Gc antiserum used in our present study is about $10 per millilitre. Since 80 samples are determined with 1 ml of antiserum, the cost per sample is 12.5 cents.

This procedure of immunofixation on cellulose acetate membranes yields good results with fresh samples of serum, whole hemolyzed blood, and dried plasma. A dried plasma specimen showing a Gc phenotype 1-1 in duplicate is seen in Fig. 1.

This method holds promise for use in forensic analyses of fresh or dried blood collected as evidence. The Gc variants have a good population frequency distribution; consequently, their identification is an important factor in civil and criminal investigations.

The Gc variants have also been determined in this laboratory by electrophoresis on gradient acrylamide gel, which is a high resolution medium. However, the minor Gc fractions appear too close on the electrophoretogram to the albumin fraction, which constitutes about 60% of the total protein in the serum. When the electrophoretogram is stained for the total protein, the Gc fractions may be obscured by albumin. Other non-Gc minor fractions may also interfere. This procedure is lengthy, costly, and not always reproducible. Electrophoresis followed by immunofixation is the preferred method for Gc phenotyping.

Immunofixation on cellulose acetate has been tried successfully for the polymorphic proteins on transferrin and ceruloplasmin. It should also be possible to determine other polymorphic proteins and enzymes by immunofixation.

Summary

Determination of the genetically controlled variants of the polymorphic Gc system was achieved by electrophoresis on cellulose acetate membranes followed by immunofixation with a specific anti-Gc antiserum. The method is applicable to plasma, whole hemolyzed blood, and dried blood. Multiple specimens can be analyzed simultaneously within 60 to

80 min. The cellulose acetate electrophoretogram of the Gc variants remains as a permanent record.

Acknowledgment

The authors wish to thank the administration of the Alameda County Sheriff's Department for their encouragement and support for the work conducted in the bloodstain analysis program.

Addendum

Benny Del Re of the White Mountain Research Station Laboratory has successfully phenotyped for Gc variants without immersing the cellulose acetate membrane in mineral oil. The oil apparently is not needed if the Gc antiserum is of sufficiently high titer.

References

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