Michael A. Podlecki,[1] *B.S. and Mark D. Stolorow,*[2] *M.S.*

Detectability of Group-Specific Component (Gc) in Aged Bloodstains

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ABSTRACT: An improved method of group-specific component (Gc) typing was conducted electrophoretically on agarose gel. Individual bloodstains randomly collected from different individual donors over a five-year period at intervals of approximately one month were checked for Gc activity. Group-specific component was typed accurately in dried bloodstains stored at room temperature up to 43 months in age. From 100 different donors, bloodstains ranging in age from 38 to 43 months were tested by the methods described and 73% of the samples were interpretable for Gc.

KEYWORDS: criminalistics, group-specific component, genetic typing, primary immunofixation, secondary immunofixation, agarose, double extraction

Group-specific component (Gc) is a polymorphic alpha-2 serum protein. It consists of three major phenotypes: 1, 2-1, and 2. Gc is an important genetic marker in forensic serology. It has a good polymorphic distribution in the population. Gc is also persistent and detectable in bloodstains.

Because the age of a bloodstain is one of the most important factors affecting its detectability, several studies concerning genetic marker persistence of bloodstains have been conducted. Denault et al [*1*] presented the first comprehensive study in the United States of the detectability of selected genetic markers as a function of time and temperature of storage. Gc was not included in this study. Berg and Ladiges [*2*] showed Gc subtypes could be determined in bloodstains up to seven months old and were more easily detectable by electrofocusing then by electrophoresis. Bashinski and Kalish [*3*] showed the viability of genetic markers in bloodstains stored frozen for up to five years. They also studied the markers in frozen lysates and dried bloodstains stored at room temperature. Gc was not included in this study. Zajac and Grunbaum [*4*] demonstrated successful typing of dried bloodstains up to two months old in Gc phenotypes on cellulose acetate membrane.

Because of the absence in the literature of any age studies in Gc, this project was undertaken to show the persistence of Gc in bloodstains produced and stored at room temperature under laboratory conditions.

Sample Preparation and Extraction Procedure

Bloodstains, each from a different donor, were produced from fresh whole blood at room temperature on clean cotton cloth under laboratory conditions. Bloodstains were prepared monthly and ranged in age from one week to more than five years at the time of this study.

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[1]Forensic scientist III, Illinois Department of Law Enforcement, Bureau of Scientific Services, Maywood, IL.

[2]Serology coordinator, Training and Applications Laboratory, Illinois Department of Law Enforcement, Bureau of Scientific Services, Joliet, IL.

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Extraction of the bloodstains was carried out using a "double extraction" procedure. The procedure involves cutting two pieces of bloodstained fabric, each measuring 3 mm square. The first piece of fabric was extracted overnight in 30 μL of distilled water at 4°C and the extract separated by centrifugation according to the method of Baxter and Rees [5] (Fig. 1). The second piece of bloodstained fabric was immersed in the previous extract and the same extraction procedure was repeated.

The double-concentrated extract was vortexed with chloroform to precipitate unwanted hemoglobin degradation products and the aqueous extract was separated from the chloroform by centrifugation [6]. This step prevents the degradation products of hemoglobin from obscuring the Gc electrophoretogram. One modification of this procedure is to reduce the vortexing time of the extract in chloroform from 1 or 2 min to 5 or 10 s.

The supernatant was collected with a double-drawn pipet, with care being exercised to avoid disturbing the precipitated hemoglobin degradation products at the interface. The aqueous extract was applied directly into the sample origin of the electrophoresis gel.

Electrophoretic Procedure

In this study the analysis of group-specific component (Gc) was performed by immunofixation agarose gel electrophoresis using FMC Gel Bond NF® to support the agarose gel [7,8]. Several simple modifications of the original method of Gc electrophoresis according to Wraxall [9] were introduced, namely, the electrophoresis voltage was set at 100 V for the first 30 min of the run. The remaining 2½-h portion of the run was set at 300 V. The initial reduction of voltage is designed to slow down the entry of the Gc protein into the gel. It was observed that this modification reduced the typical cone-shaped appearance of the Gc bands.

For the immunofixation phase of the Gc visualization procedure, a primary and a secondary immunofixation were conducted [10]. Goat anti-human Gc antiserum (Atlantic Antibodies) was diluted one part antiserum to three parts saline for the primary immunofixation step. The secondary immunofixation technique used rabbit anti-goat antiserum (Cappel Lab-

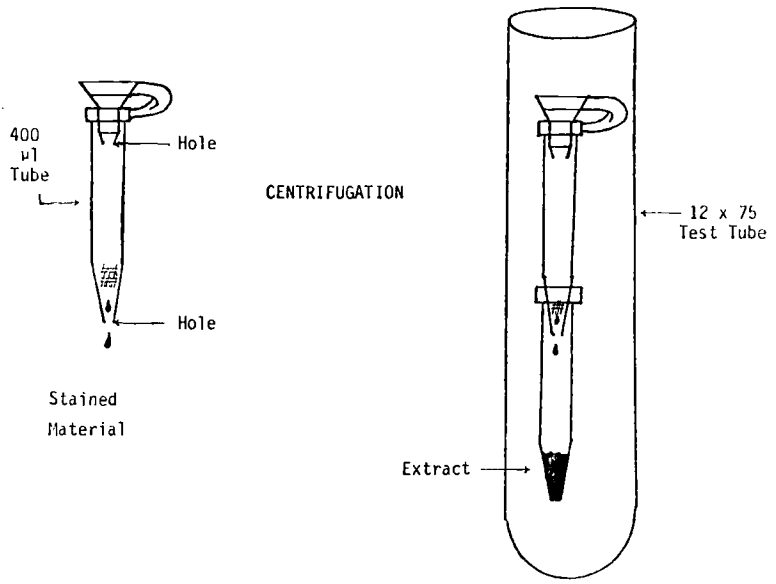

FIG. 1—*Modified double extraction technique for concentrated dried bloodstains.*

oratories) diluted one part antiserum to twelve parts saline. No information concerning the titre of the commercial antiserum was available from either manufacturer.

Cellulose acetate membranes soaked in diluted Gc antiserum were placed upon the agarose following electrophoresis and the gel allowed to incubate in a moisture chamber for 2 h at room temperature. The overlay was then removed and the gel was washed in 1*M* saline overnight. The gel was washed in distilled water for 30 min to remove the saline.

The secondary immunofixation incorporated the same technique above, in addition utilizing rabbit anti-goat antiserum to fix the first overlay of goat anti-human Gc.

The gel was then pressed, dried, and stained. Following immunofixation, the gel was covered with filter paper moistened with distilled water. Several layers of dry paper towels were placed over the gel to absorb water. Lead weights placed on a glass plate were laid over the paper towels for pressing. The pressing dehydrated the gel, thereby reducing the gel thickness on the gel bond. The gel was pressed for 30 min. The gel was then incubated at 65°C until dry. The gel was stained in a solution of 0.1% Commassie Blue, 50 mL of methanol, 10 mL of acetic

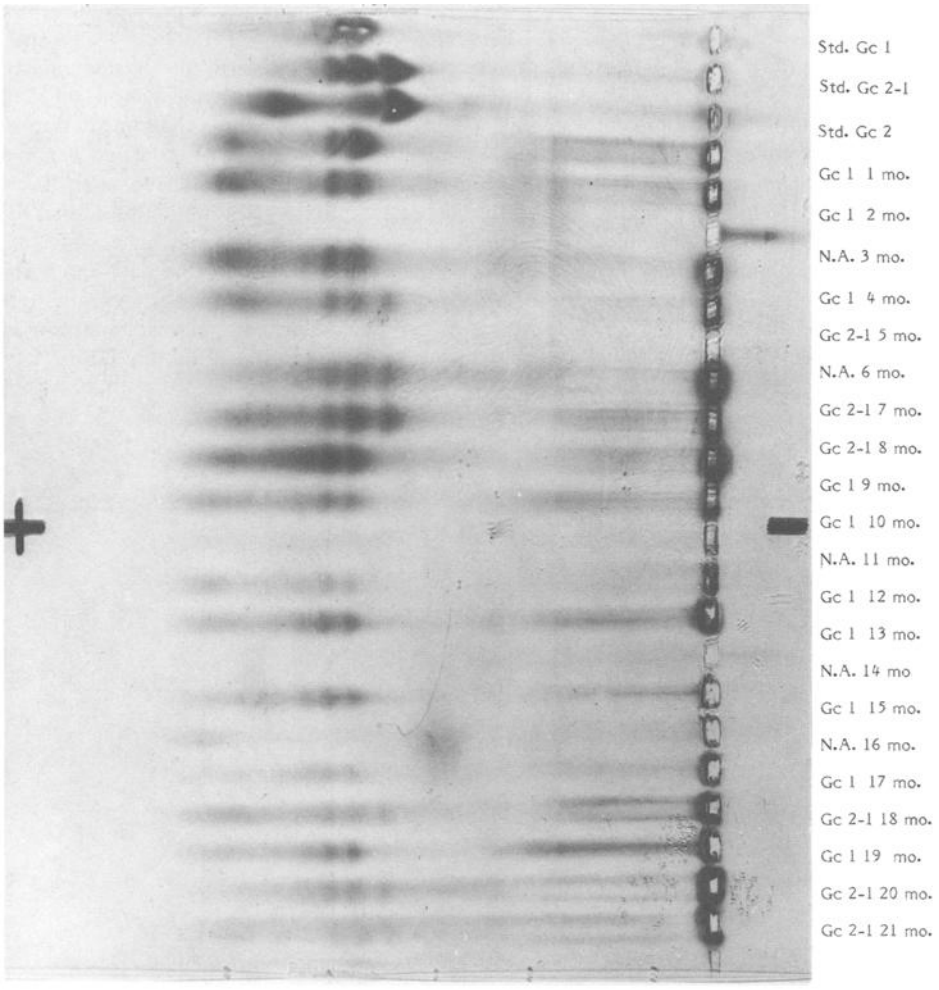

FIG. 2—*Primary immunofixation electrophoresis of Gc in bloodstains 1 to 21 months old.*

acid, and 50 mL of distilled water for 5 min. The gel was destained in a solution of 50 mL of methanol, 10 mL of acetic acid, and 50 mL of distilled water for 15 min. The gel was washed with water and blotted dry before interpretation.

Results and Discussion

Bloodstains ranging in age from less than 1 month to 43 months old were accurately phenotyped in Gc by agarose immunofixation electrophoresis. Electrophoresis using primary immunofixation gave reliable results in phenotyping Gc. Figures 2 and 3 show a sampling of the reproducibility of the components of the three common Gc phenotypes from 1 month to 43 months in age using primary immunofixation.

It was noted during the study that as bloodstains age, the Gc patterns for the three common phenotypes faded in intensity but displayed no evidence whatsoever of selective deterioration of any of the various Gc bands relative to one another. The relative intensities of the anodal Gc

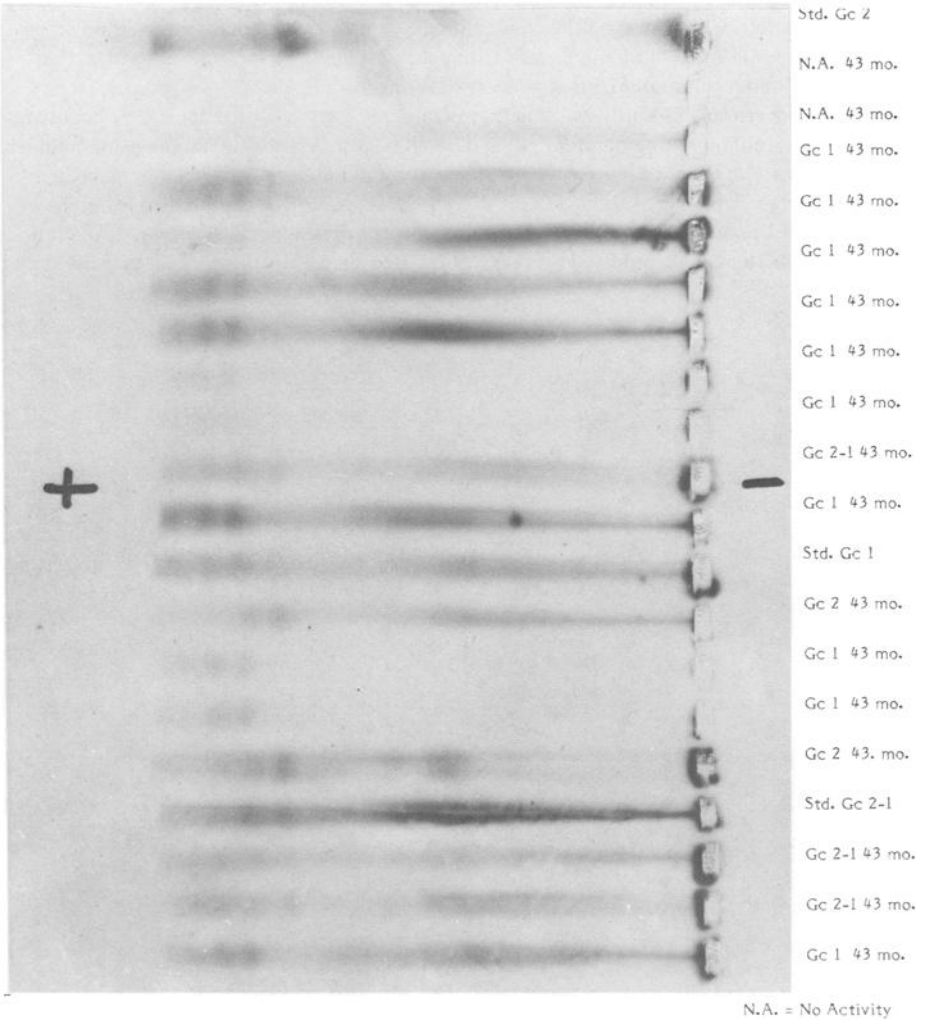

FIG. 3—*Primary immunofixation electrophoresis of Gc phenotypes in bloodstains 43 months old.*

decomposition bands was quite variable from one sample to the other, regardless of the age of the stain.

Note that the implementation of a modified double extraction technique has a profound effect in increasing the concentration of serum proteins from bloodstains. The chloroform extraction procedure eliminates any dark streaking caused by denatured hemoglobin. The reduction of time in vortexing the extract in chloroform is critical because the longer the extract is vortexed in the presence of chloroform, the more Gc is precipitated with the degraded hemoglobin. The substitution of isotonic saline for distilled water in the dilution of the Gc antiserum stabilizes the antibody in solution by providing proper physiological ionic strength.

Because the primary immunofixation electrophoresis procedure detected Gc in stains up to 43 months old, the authors decided to run 100 randomly selected bloodstains from 38 to 43 months in age using primary immunofixation on all samples and a secondary immunofixation technique whenever necessary to enhance any of the 100 samples showing no activity from the primary immunofixation step. Figure 4 represents a sampling of the enhancement by secondary immunofixation of the Gc phenotypes in stains 43 months in age.

As Fig. 5 shows, 65 of the 100 bloodstain samples (38 to 43 months old) were detectable by primary immunofixation alone. Of the remaining 35 samples which demonstrated no activity by primary immunofixation alone, 7 were enhanced sufficiently for proper interpretation by applying a secondary immunofixation process to those 35 samples.

Another concern in this study was whether or not any particular common Gc phenotypes appeared less stable upon aging or whether they all persisted in relatively the same manner during the course of three to four years of storage at room temperature. In looking at the frequencies given in Table 1, we noticed that the Gc phenotypes of 100 randomly selected bloodstains (38 to 43 months old), all from different donors, produced a frequency distribution which was consistent with published reports of Gc frequencies in the general population of

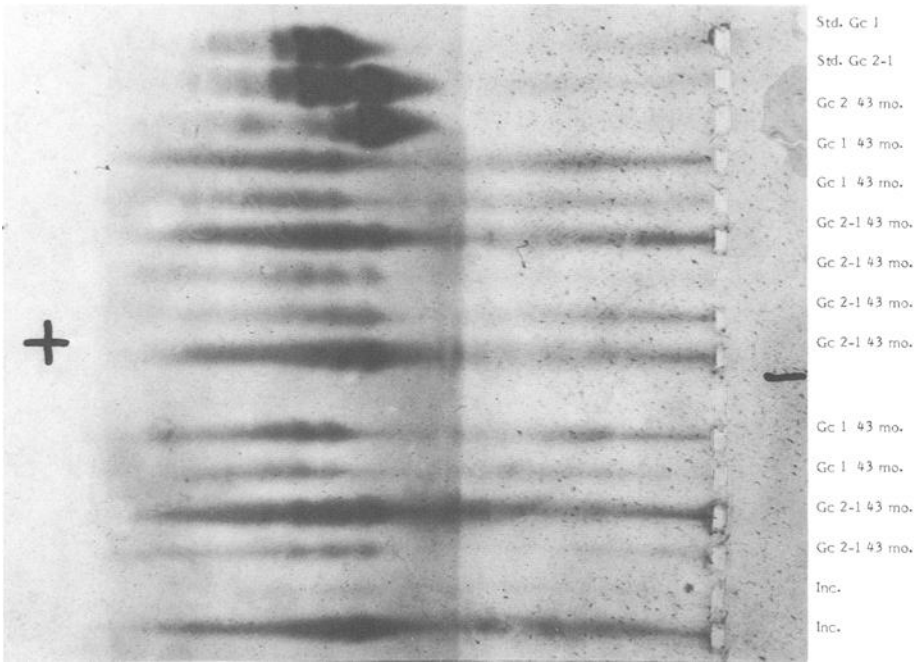

FIG. 4—*Secondary immunofixation of Gc in bloodstains 43 months old.*

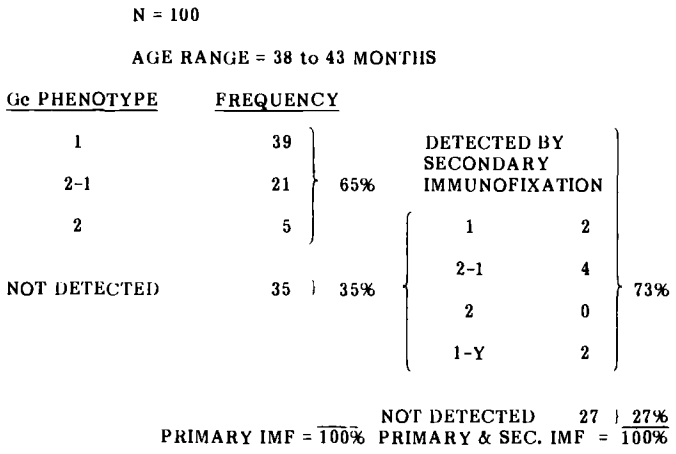

FIG. 5—*Increase in detectability of Gc phenotypes using secondary immunofixation.*

TABLE 1—*Gc phenotypes of 100 randomly selected bloodstains (38 to 43 months old). (The racial composition of the randomly selected samples was indeterminate.)*[a]

System	Phenotype	Observed in this Study	Whites [11]	Blacks [11]
Gc	1	0.5616	0.4811	0.7262
	2-1	0.3425	0.4235	0.2222
	2	0.0685	0.0895	0.0079
	1-Y	0.0274	0.0020	0.0357

[a] $n = 100$.

whites and blacks [11]. From this, one can conclude that there is no evidence of apparent differences in the relative persistence of the various common Gc phenotypes.

Summary

Group-specific component (Gc), a polymorphic protein, was typed successfully using immunofixation electrophoresis on dried bloodstains stored at room temperature up to 43 months. Modifications incorporated into this procedure included a double extraction and centrifugation procedure, reduction of initial electrophoresis voltage, substitution of isotonic saline for distilled water in the dilutions of the antisera, and the introduction of a secondary immunofixation step in the protein visualization procedure.

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Address requests for reprints or additional information to
Michael Podlecki
Illinois Department of Law Enforcement
Bureau of Scientific Services
1401 S. Maybrook Dr.
Maywood, IL 60153