TECHNICAL NOTE

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Increasing the Sensitivity of Detection of the Electrophoretic Marker Group Specific Component in Agarose Gels by Double-Staining with Coomassie Brilliant Blue R250 and Silver

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ABSTRACT: A method is described that increases the sensitivity of detection of group-specific component (Gc) in bloodstains following agarose gel electrophoresis. Gels are stained first with Coomassie Brilliant Blue R250 (CB) and subsequently stained with silver. Double-staining with CB first enhances the sensitivity of the silver stain from 10- to 80-fold over staining with CB alone. The method is reproducible and can easily be incorporated into the laboratory as a routine procedure.

KEYWORDS: forensic science, group specific component, genetic typing, electrophoresis, bloodstain, Coomassie Brilliant Blue R250, silver stain, agarose gels

Immunofixation conventional agarose gel electrophoresis followed by staining with Coomassie Brilliant Blue R250 (CB) is one of the methods used for phenotyping bloodstains for group specific component (Gc) [*1*]. A limitation of this method is that the concentration or extractable amount of Gc or both in some bloodstains may be below the sensitivity of detection of CB. The inability to detect proteins present in low concentrations has limited the application of phenotyping bloodstains for Gc in agarose gels. By employing a histologically derived silver stain (SS) for proteins in agarose gels, Willoughby and Lambert [*2*] observed a tenfold increase in sensitivity of protein detection compared with CB. Budowle et al [*3*] described silver staining Gc which can achieve similar sensitivity. A drawback of this silver staining technique is that it consumes large amounts of silver nitrate and tungstosilicic acid. Therefore, it was desirable to develop a staining method that would be more economical and yet retain the sensitivity for detecting Gc in agarose gels as described by Budowle et al [*3*]. Such a system might involve initial

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staining with CB and, if necessary, counterstaining those samples with no observable Gc patterns with the more sensitive SS. This paper describes a technique for double-staining Gc in agarose gels with CB followed by a SS to observe the less detectable Gc patterns.

Materials and Methods

Blood samples were obtained by finger prick from donors at the FBI Academy. Bloodstains were prepared on washed cotton and air-dried before use.

Cuttings (5 by 5 mm) from the bloodstains were teased apart and extracted in 50 μL of distilled water for 30 min. Ten microlitres of each extract were applied to the gels for 24 min using an application foil (LKB). Immunofixation agarose gel electrophoresis for Gc was performed as previously described by Budowle and Gambel [*1*].

Four microlitres of various dilutions of bovine serum albumin (BSA) (100 ng/mL to 1 mg/mL) were applied to the gel in the same manner as the bloodstain extracts. Since it is difficult to determine staining sensitivity of various dilutions of immunofixed proteins, various concentrations of BSA were used as a guideline for stain sensitivity. Agarose gels were cast 1 mm thick on 20- by 15-cm gel bond (Marine Colloids) or glass plates. Electrophoresis was performed according to the method of Teisberg [*4*]. The gels in which BSA was applied were fixed with 45% ethanol and 10% acetic acid for 10 min, pressed, and dried.

All gels (containing Gc and BSA) were stained in a solution containing 0.25% CB, 45% ethanol, and 10% acetic acid and destained in a solution containing 45% ethanol and 10% acetic acid. The gel background was completely destained. The gels were then washed in distilled water for 10 min, dried, and subjected to silver staining according to Willoughby and Lambert [*2*] (Table 1).

Results and Discussion

Figure 1 shows CB stained immunofixed Gc proteins from blood stains in an agarose gel. The Gc in Samples 1 through 6 could not be detected by this method. Subsequent staining with silver resolved this problem in Samples 2 to 6 (Fig. 2). This double-staining technique has been reproducible on over 100 separate gels. To obtain these results it is very important to destain CB completely from the gel background. Otherwise, silver will deposit across the gel surface, thus, making it difficult if not impossible to determine the phenotypes of the Gc variants.

The silver stain technique of Willoughby and Lambert [*2*] has two limitations: cost and a limited increase in sensitivity compared with CB staining. Budowle et al [*3*] previously reported that it took a total of 300 mL of silver solution, 200 mL as a primer and 100 mL for development of the Gc protein patterns, and over 10 min for direct silver staining of Gc for one agarose gel. However, when a gel was first stained with CB, no more than 100 mL of silver solution were necessary, and the silver staining time was reduced to only 2 min. The CB appears to act as a primer intensifying the SS effects. The negatively charged sulfenyl groups on the CB molecule may have some role in this regard [*5*]. An 80-fold increase in sensitivity was observed for double-staining compared with CB staining using various dilutions of BSA (Table 2).

It would not be cost-effective and convenient to use double-staining on all samples. A more

TABLE 1—*Silver stain solution as described by Willoughby and Lambert* [2].

Solution A =	5% anhydrous sodium carbonate.
Solution B =	0.2% ammonium nitrate,
	0.2% silver nitrate,
	1.0% tungstosilicic acid, and
	1.4% formalin.
Mix 32 mL of A with 68 mL of B and pour directly on agarose gel.	

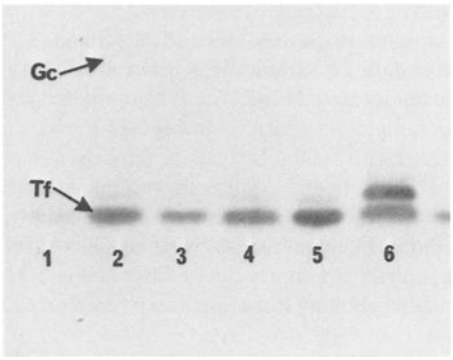

FIG. 1—*A Group III agarose gel* (1) *displaying CB stained proteins. The Gc proteins could not be detected using CB as the stain, only transferrin (Tf) could be visualized. The anode is at the top.*

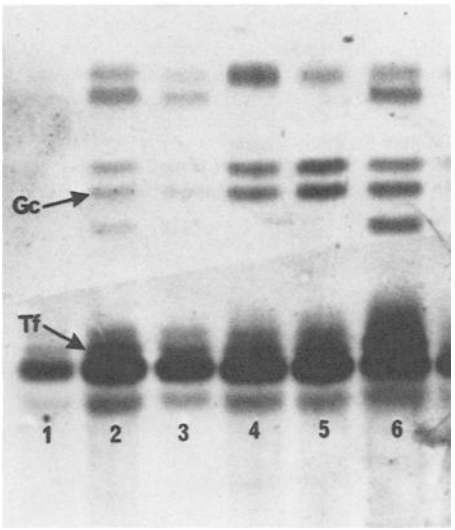

FIG. 2—*The same gel as in Fig. 1 that has been subsequently stained with silver. The Gc patterns in Samples 2 through 6 could be observed. The phenotypes for Samples 2 through 6 are 2-1, 2-1, 1-1, 1-1, and 2-1, respectively. The anode is at the top.*

TABLE 2—*Sensitivity of BSA detection of staining procedures.*

Stain[a]	Sensitivity of Detection of BSA, ng[b]	Increase Fold in Sensitivity over CB
CB	400	X1
SS	40	X10
CB + SS	5	X80

[a] CB = Coomassie Brilliant Blue R250 and SS = silver stain described by Willoughby and Lambert [2].

[b] BSA isolated by conventional agarose gel electrophoresis according to the method of Teisberg [4].

reasonable approach would be to cast agarose gels on gel bond and, after CB staining, cut out with scissors only those samples which need to be silver stained. For example, if 10 gels (170 samples) were CB stained and the Gc variants detected in all but 15 samples, then only these 15 samples would require to be double-stained. These 15 samples could all be stained at 1 time without staining the other samples, in which Gc has already been detected. This would require only 100 mL of SS compared with 1000 mL of SS for ten gels.

The greater sensitivity of silver staining, especially with the intensifying effect of CB, considerably facilitates qualitative analysis of Gc variants in fresh bloodstains. The double-staining technique is rapid, reproducible, and more sensitive than CB or silver staining alone and is relatively inexpensive when individual samples can be silver stained. Therefore, it would be very easy to incorporate this double-staining technique as a routine procedure for Gc phenotyping.

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