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Gc in bloodstains

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Summary. Gc-subtyping was carried out on blood stains that had been made on cotton and glass and stored under a variety of conditions ranging from −20° to +56°C. The limits of detection ranged from 2 weeks at 56°C up to 92 weeks at +4°C and greater than 116 weeks at −20°C. Additional bands that have been reported in other studies could not be detected during this study, and this difference is thought to be due to storage of the samples in the liquid state.

Key words: Gc-subtyping – Bloodstains, Gc subtyping

Zusammenfassung. Gc-Subtypisierung erfolgte an Blutspuren, welche auf Baumwolle oder Glas angelegt worden waren und unter zahlreichen unterschiedlichen Bedingungen im Bereich zwischen −20°C und +56°C gelagert wurden. Die Nachweisgrenzen variierten zwischen 2 Wochen bei 56°C und bis zu 92 Wochen bei 4°C und mehr als 116 Wochen bei −20°C. Zusätzliche Banden, die in anderen Untersuchungen beschrieben wurden, konnten in dieser Studie nicht nachgewiesen werden. Dieser Unterschied ist wohl auf Lagerung (der alterierten Proben) im flüssigen Zustand zurückzuführen.

Schlüsselwörter: Gc-Subtypisierung – Blutspuren, Gc-Subtypisierung

In a previous paper (Rand et al. 1987) we described an extremely sensitive method for the detection of the Gc subtypes, together with preliminary results. In this paper the results of a more detailed study on Gc typing in bloodstains over a 2-year period are reported.

Materials and methods

Bloodstains of the six commonest Gc subtypes (1F, 1F–1S, 1S, 2–1F, 2–1S, and 2), each equivalent to about 40 µl blood were made on cotton and on glass and dried for 1 day at room temperature. These were then arranged in seven separate series and stored at (1) −20°C, (2)

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+4°C, (3) room temperature, (4) humid chamber at room temperature, (5) 37°C, (6) humid chamber at 37°C, (7) 56°C.

For extraction small pieces of cotton or the blood flakes were soaked for 1 h in 6 M urea to break the Gc-actin-complex bonds. The volume used was approximately 10 times the original blood volume. Two millilitres of the supernatant was then immediately applied to the gel 1 cm from the cathode.

Isoelectric focusing was performed in ultra-thin polyacrylamide gels (0.15 mm), C = 3%, T = 5%, pH 4.5–5.4. Focusing data: 3000 V, 5 mA, 10 W as maxima. Focusing was carried out over a total of 110 min including 5 min prefocusing. Semi-dry electroblotting was carried out as previously described (Rand et al. 1987) onto nitrocellulose membranes.

Visualization was achieved using anti-Gc from rabbit (Dakopatts) as primary antibody, peroxidase-linked-anti-rabbit antibody (Dakopatts) as secondary antibody, and 3-amino-9-ethylcarbazole as substrate. Alternatively a biotinilated secondary antibody was combined with peroxidase-conjugated avidin-biotin complex using the same substrate for colour development.

Results and discussion

The storage conditions had a large influence on the time limits of detectability of the Gc subtypes (Table 1) and can be summarized as follows:

- 2 weeks after dry storage at 56°C (group 7)
- 8 weeks after humid storage at 20°C; by this time stains were covered with mould (group 4)
- 22 weeks after dry storage at 37°C (group 5)
- 62 weeks after dry storage at 20°C (group 3)
- 92 weeks after dry storage at 4°C (group 2)
- at least 116 weeks after dry storage at −20°C; this series is still giving good results (group 1).

Examples of the results obtained from stains stored under these conditions can be seen in Fig. 1.

Table 1. Gc subtyping on bloodstains stored under various conditions

Weeks	−20°C	+4°C	+20°C	+37°C	+56°C	Humid chamber
2	+	+	+	+	+	+
4	+	+	+	+	−	+
8	+	+	+	+	−	+
16	+	+	+	+		−
22	+	+	+	+		−
30	+	+	+	−		
38	+	+	+	−		
46	+	+	+			
54	+	+	+			
62	+	+	+			
74	+	+	−			
86	+	+	−			
92	+	+	−			

+, Positive identification of Gc-subtypes; −, inconclusive or no result

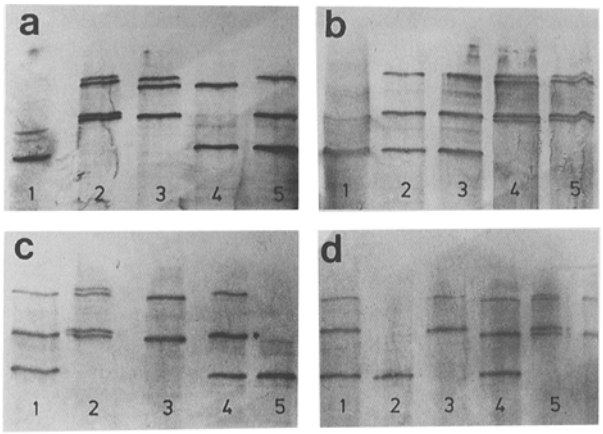

Fig. 1a–d. Gc-subtyping from **a** fresh bloodstains (*1*,2; *2*,1S–1F; *3*,1S–2A7; *4*,2–2A7; *5*,2–1S), **b** bloodstains stored at 20°C for 6 months (*1*,2; *2*,2–15; *3*,2–1S; *4*, 1S–1F; *5*,1S–1F); **c** bloodstains stored at −20°C for 10 months (*1*,2–1S; *2*,1S–1F; *3*,1S; *4*,2–1F; *5*,2); **d** bloodstains stored at 4°C for 7 months (*1*,2–1F; *2*,2; *3*,1F; *4*,2–1S; *5*,1S–1F)

Whilst storage conditions and air humidity had the greatest influence on the detectability, there was obviously no influence from other factors, such as the carrier material. This is in some contrast to earlier observations on other protein and enzyme systems, which are more labile and therefore susceptible to oxidation (Prokop and Bundschuh 1963; Brinkmann et al. 1972). The application of the avidin-biotin technique led to a distinct increase of detectability and was therefore used exclusively for older stains. As has been pointed out (Pflug 1988), isoelectric focusing on ultra-thin polyacrylamide gels leads to an excellent distinction between the 1S and 1F bands and the whole procedure to a 100- to 1000-fold increase in sensitivity.

Pflug (1986) recommended separation in immobilized gel gradients and reported even higher sensitivity. Isoelectric focusing is, however, easier to carry out and is probably more reproducible. This was therefore the preferred method for this study, although it is slightly less sensitive. The dependency on storage conditions has not previously been reported in such detail and therefore only some points of comparison could be made. Baxter and White (1984) reported a time limit of 13 weeks (room temperature); Kido et al. (1984), 25 weeks (4°C); and Westwood and Werrett (1986) at least 28 weeks (room temperature) using conventional methods. Pflug (1986) used the immuno-enzyme-linked detection method and reported a time limit of 6 years in stains stored at room temperature. He concluded that the demonstrability is less dependent on protein degradation than on the successful dissociation of the actin/Gc complex. These observations demonstrate the influence of the methods used.

Another observation was that extra bands, which have been reported by Budowle (1987) and also by Rand et al. (1989), were not found in this study. In our previous investigations these were mainly observed in post-mortem blood samples, in so-called blood alcohol samples and in different body secretions, which were subjected to liquid storage conditions prior to the investigation. The extra bands are probably mainly due to incomplete breakage of the actin/Gc complex, but do not play such an important role in dried bloodstains as in liquid samples.

Our investigations were performed on experimental stains produced on clean substrate materials and therefore do not accurately represent true "casework" stains. However, they can act as a frame-work for the analysis when extensive

casework experiences have been collected. As previously pointed out, the technical procedures of extracting and redissolving the stains must not only take into account the different physicochemical properties of the individual proteins, but also the requirements of the minimum sample size. If a sample size of only 2–5 $\mu l \times 10^{-3}$ for serum or the equivalent of a dried bloodstain is necessary for detection then it is necessary to optimize micro-extraction procedures.

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