Gc in Human Saliva Stains

J. Lötterle, J. Bolt

Institute of Forensic Medicine, Friedrich-Alexander University,
8520 Erlangen FRG

Introduction

The best method for sub-typing the vitamin-D-binding protein Gc in stains is the isoelectric focusing on immobilised pH gradients with subsequent immunoblotting. Many authors have shown the Gc-types in dried blood with this method (THOMAS et al. 1989, ARNDT 1989, KEIL et al. 1988, KIDO et al. 1984, PFLUG 1986, RAND et al. 1987, 1990, WESTWOOD and WERRETT 1990). MILLS and CHASE (1989) described a method for Gc-typing in urin after concentration. In many laboratories the Gc-typing of seminal stains is also successfully carried out. Demonstration of Gc-types in saliva stains has not yet been reported in the accessible literature.
The identification of saliva stains is presently restricted to the bloodgroup systems ABO/Se/Lewis (IKEMOTO et al. 1990). Therefore, a better differentiation of saliva stains using more bloodgroup systems would be desirable.

Materials and Methods

Saliva stains from donors of the most common Gc-types were collected on filter paper, airdried and kept at 23 °C (for 1, 3 and 7 days), at 4 °C (for 1, 3 and 7 days) and at -24 °C (for 1, 3, 7, 12, 28 and 40 days). For determination of the Gc-phenotype small pieces of the filter paper were allowed to soak overnight in 15 µl 6 M urea. Isoelectric focusing was done on commercial 0.5 mm polyacrylamide gels pH 4,5- 5,4. More detailed information on the preparation, focusing and the blotting technique are given in table 1. Besides the experiments with dried saliva on filter paper, some experiments were performed with smoked cigarette ends.

Tab. 1: Data for preparation, focusing, blotting and staining

Elution: Incubate dried saliva stains on filter paper (2 cm^2 cutted in several small pieces) with 15 µl 6M urea with 0,5% BSA at 4 °C overnight. Spin it [e.g. in a serum filter (Filter Sampler Porex Medical)] and place 5-6 µl on the gel 1 cm from the cathode using an applicator strip.
Gel: Immobiline dry plate (Pharmacia) pH 4,5-5,4, reswelling solution 25% glycerol for 1 h.
Focusing: Electrode solution aqua bidest. 5000 V, 4 mA, 10 W, 4 h.
Blotting: Cellulose acetate sheet (Sartorius, Order Nr. 11200-70-400-6) equilibrated in aqua bidest and soaked with goat anti human Gc globulin (Atlantic Antibodies) 1:50. Passive blotting for 1 h. Washing with Triton X 100 buffer (0,01 M Tris/HCl pH 7,4, 0,9% NaCl, 1% Triton X 100) 3 times for 10 min. Second antibody: rabbit anti goat globulin, peroxidase conjugated (Atlantic Antibodies) 1:200, incubation time 1 h. Washing with Triton X 100 buffer 2 times for 10 min and with washing buffer (0,01 M Tris/HCl pH 7,4, 0,9% NaCl) 2 times for 5 min.
Staining: Prepare staining agar (1% agar purum in destilled water, 0,25 M glycerol/NaOH buffer pH 10,4, 0,1 M $MgCl_2$, 0,1 M $ZnCl_2$, 0,1 M BCIP solution, dissolved in dimethylformamid) on Gel-Bond film (Pharmacia) and incubate the cellulose acetate sheet overnight at 37 °C.

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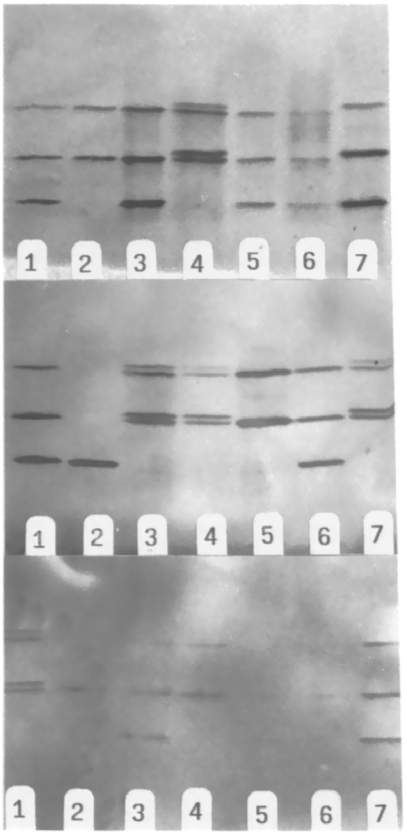

Fig.1a-1c

Results of Gc sub-typing of 8-day old blood stains (1a, from left to right: 2-1S, 1S, 2-1S, 1F-1S, 2-1S, 2-1S, 2-1F), of fresh serum (1b, from left to right: 2-1S, 2, 1F-1S, 1F-1S, 1S, 2-1S, 1F-1S) and of 1-day old saliva stains (1c, from left to right: 1F-1S, 1S, 2-1S, 1S, 2-1S, 1S, 2-1S); anode at the top

Results

Some typical results of saliva stains, stored for one day at room temperature in comparison to results of 8-day old blood stains and serum samples are shown in figure 1a-1c. The Gc-content of the saliva stains is obviously lower than that of serum and some people secrete in their saliva so little Gc that a demonstration with the method used here is not possible. The Gc-phenotype of the saliva stains that could be determined were always the Gc-type of the donor's serum.

Figure 2 shows the demonstration of the Gc proteins' dependence on time and storing temperature. 30 different saliva stains were stored at -24 °C over a period of 40 days and examined, the remaining results refer to 10 different saliva stains respectively. It is recognised, that samples stored at room temperature for more than 1 week for the greater part could not be typed, while the keeping quality of storage at -24 °C is relatively good (66 % of the stains could be typed after 40 days). With the method used it was not possible to determine the Gc-type on smoked cigarette ends.

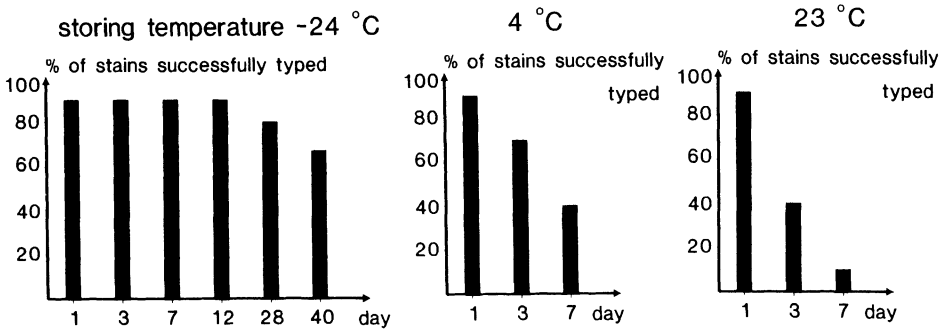

Fig. 2: Dependence of Gc proteins' demonstration in saliva stains on time and storing temperature

Discussion

The results show, that the demonstration of Gc in fresh saliva stains or saliva stains stored for days or weeks at -24 °C on filterpaper seems to be quite promising.
For the determination of the Gc-type on smoked cigarette ends, the method was not sensitive enough. Investigations on gumlack of stamps and/or envelopes have not yet been done.

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