TECHNICAL NOTE

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Gm/Km Typing of Bloodstains in U-Bottom Microtiter Plates

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ABSTRACT: A technique was developed for Gm/Km typing of bloodstains and sera in U-bottom microtiter plates. Gm/Km typing of sweat and urine samples was also attempted with limited success.

KEYWORDS: forensic science, genetic typing, serology, Gm/Km typing, sweat, urine

The excellent discrimination potential for Gm/Km allotyping reported by Kipps [*1*] led this laboratory to investigate the incorporation of this system into our typing methods. Initial attempts to use the test-tube method [2] were found to be time-consuming and cumbersome. Davie [*3*] recommended a V-bottom microtiter plate method and visual reading. This is commonly used for allotyping; however, since U-bottom microtiter plates have been used in this laboratory for several years for ABO typing of body fluids and stains [*4*], the possibility of adapting the method for Gm/Km typing was investigated. The technique was developed using sera from casework and laboratory donors as well as corresponding stains prepared from these blood samples. Internal and external blind trials included 110 stains on different types of material. Gm/Km typing on sweat stains and urine samples was also attempted.

Materials and Methods

The materials required were obtained from the following sources: U-bottom microtiter plates from Greiner, Nuertingen, W. Germany; antisera for the factors Glm(1),(2),(3), G3m(10), Km(1), and their corresponding anti-D coating sera from Dr. Molter, GmbH, Heidelberg, W. Germany, Sangocells-O, Rh positive (CcDEe) from the Behring Institute, Mannheim, W. Germany; and positive and negative control sera for the relevant allotypes from Biotest Diagnostics, Dreieich, W. Germany. Laboratory donors provided paired serum and bloodstain samples. The bloodstains, on sterile cotton cloth, were air-dried at room temperature and then extracted in 0.1% bovine serum albumin in saline (BSA).

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For the blind trials, one hundred and ten bloodstains were made on a variety of materials including cotton, cotton gauze, cotton "wool," viscose (fabric and gauze), cotton and rayon mixture, wool and acrylic mixture, wool tweed, and paper. Blood scrapings from nonabsorbent surfaces were also tested. Fifty stains were prepared in this laboratory, fifty stains were provided by Landeskriminalamt, Baden-Wuerttemberg, W. Germany, and ten stains by Serological Research Institute, Emeryville, California.

Sweat samples were collected from the foreheads of six laboratory donors after vigorous exercise, on sterile cotton cloth, air-dried at room temperature, and tested within a week for Glm(1),(2),(3), G3m(10), and Km(1). Fifteen urine samples and three urine stains were tested only for Glm(1),(2), and Km(1).

Sensitization of Cells

Red blood cells were washed three times in saline. Of the packed cells, 125 μL were diluted with 300 μL of phosphate buffered saline (81.0 mL of 0.1*M* sodium phosphate, dibasic [Na_2HPO_4] + 19.0 mL of 0.1*M* potassium phosphate, monobasic [KH_2PO_4], pH 7.4 [5]. Fifty microlitres of the diluted red blood cells and fifty microlitres of each appropriate anti-D Gm serum and anti-D Km(1) were incubated in test tubes at 37°C for 60 min. After incubation, the cells were washed three times in saline to remove excess antiserum. To check sensitization, one drop of a 5% cell suspension prepared in 0.1% BSA was mixed with one drop of anti-human globulin (Ortho Diagnostics) on a spot plate. Strong agglutination indicated the cells had been sensitized. The sensitized cells were used as a 0.1% suspension in 0.1% BSA.

Antisera Titration

Serial dilutions of anti-Gm and anti-Km sera were made in 0.1% BSA. Of the appropriate 0.1% sensitized cell suspension, 20 μL were added to 20 μL of diluted Gm and Km antisera in the wells of U-bottom microtiter plates, incubated for 1 h at 4°C, and then centrifuged for 3 min at 220*g*. The plates were gently tapped and read at a magnification of ×100 using an inverted microscope. The highest dilution of antisera giving complete agglutination was used as the working dilution for subsequent tests.

Sample Preparation

Serum samples from donors and casework were diluted 1 in 15 with saline or 0.1% BSA. Bloodstains, approximately 0.3 cm^2, were extracted in 120 μL of 0.1% BSA for at least 4 h; this provided sufficient extract to test the factors Glm(1),(2),(3), G3m(10), and Km(1). To obtain good results with weak or old bloodstains or both, extraction overnight in 0.1% BSA at 37°C was necessary.

Of the diluted antisera, 20 μL were added to 20 μL of test serum or bloodstain extract, the contents mixed well, and incubated at 4°C for a minimum of 60 min for sera and overnight for bloodstain extracts. After this absorbtion period, 20 μL of sensitized cells were added to each well, left 1 h at 4°C, and then centrifuged for 3 min at 220*g*. The plates were tapped gently and read using an inverted microscope. Final agglutination was read microscopically and scored as follows: 4 = large clumps, no free cells; 3 = large clumps, some free cells; 2 = some clumps, some free cells; 1 = small clumps, many free cells; and − = free cells only. A positive result was reported only if there was complete inhibition.

Controls

Each set of tests included commercial control sera positive and negative for each antigen tested; sera and stain extracts from known donors at the same dilutions as the samples

to be tested; unstained cloth controls for casework samples; a control that tested for the presence of antiglobulin antibodies in each serum sample (20 μL of diluted unknown serum sample, 20 μL of 0.1% BSA, and 20 μL of 0.1% sensitized cell suspension); an antisera control (20 μL of each suitably diluted anti-Gm and anti-Km serum, 20 μL of 0.1% BSA, and 20 μL of 0.1% sensitized cell suspension); and a BSA control (40 μL of 0.1% BSA and 20 μL of 0.1% sensitized cell suspension).

Results and Discussion

Consistent results were obtained from laboratory donor and casework bloodstains made from whole blood. The 110 bloodstains tested during the blind trial were at least 12 months old, the oldest being 5 years, and were stored either at room temperature or had been frozen and thawed several times. As shown in Table 1, 1 of these stains gave a false negative reaction for Gm(1,10) and 4 stains gave false negative reactions for Gm(10); these were made on viscose gauze and had been frozen and thawed several times. Partial inhibition of Gm(10) was obtained on some of the stains made on cotton "wool," cotton gauze, and blood scrapings; the other substrates had no effect on the results. No false negative reactions were obtained for Gm(2), Gm(3), and Km(1) on any of the stains, and no false positive reactions were observed.

Jorch and Oepen [6] tested sweat samples of six individuals in Km(1) using 10 cm^2 of secretion dried on filter paper. No Km(1) antigen was detected, probably because of the

TABLE 1—*Results of blind trial samples.*

Phenotypes	No. of Stains	Time in Years	Substrates	False Reactions
Gm(1)	6	1–2	cotton cloth	none
Gm(1,2)	3	1–2	cotton cloth	none
	11		viscose gauze[a]	none
Gm(1,2,3,10)	4	1–2	cotton cloth	none
	5		viscose gauze[a]	one false neg. for Gm(1,10) four false neg. for Gm(10)
	4		cotton "wool"	two weak results for Gm(10)
	2		blood scrapings[a]	one weak result for Gm(10)
Gm(1,10)	3	1–2	cotton cloth	none
Gm(1,3,10)	10	1–2	cotton cloth	none
	2		paper	none
	2		cotton/rayon	none
	6		cotton gauze	one weak result for Gm(10)
	14		viscose gauze[a]	none
	2	5	wool tweed	none
Gm(3,10)	13	1–2	cotton cloth	none
	2		cotton "wool"	one weak result for Gm(10)
	2		cotton/rayon	none
	1		viscose fabric	none
	13		viscose gauze	none
	2		blood scrapings[a]	none
	1	5	wool/acrylic	none
Gm(1,2,10)	2	1–2	viscose gauze[a]	none
Km(1)	110	as above	as above	none

[a]These stains had been frozen and thawed several times; all others were stored at room temperature.

TABLE 2—*Results of Gm and Km antigens detected in sweat.*

Donor	Sera/Bloodstains	Sweat
1	Gm(+1,−2,−3,+10) Km(−1)	Gm(−1,−2,−3,−10) Km(−1)
2	Gm(+1,−2,+3,+10) Km(−1)	Gm(+1,−2,−3,−10) Km(−1)
3,4	Gm(+1,+2,−3,−10) Km(−1)	Gm(+1,+2,−3,−10) Km(−1)
5,6	Gm(−1,−2,+3,+10) Km(−1)	Gm(−1,−2,−3,−10) Km(−1)

low levels of Immunoglobulin G (IgG) in sweat. They concluded that the unspecific reactions observed were too weak for sweat to cause interference in the grouping of blood or semen stains.

In contrast, results from the six samples tested in this study (Table 2) showed Glm(1) and Glm(1,2) individuals occasionally have detectable levels in their sweat. Gm(3) and Gm(10) antigens were not detectable in any of the donors tested; a Km(1) donor was not available.

No reactions were observed for Glm(1),(2), and Km(1) on urine stains. Liquid urine tested for these factors gave unreliable reactions which may be due to the insensitivity of the detection system. Work to establish reliable techniques for urine grouping is continuing since Gm/Km grouping may be of help in verifying the origin of urine samples obtained in the U.S. Army drug screening program.

At present in this laboratory, bloodstains and sera from casework are tested only for Glm(1),(2),(3), G3m(10), and Km(1). Negative results must be interpreted with caution; for example, only the presence of Gm(10) is reported, since the blind trial showed that this marker can give false negative reactions. To improve our ability to report negative results, incorporation of a method [7] to verify the amount of available IgG, and the addition of antisera for antigens located on IgG3, and anti-Km(3) are currently being evaluated.

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