CASE REPORT

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Interpretation of Gm Testing Results: Two Case Histories

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ABSTRACT: Two cases in which bloodstains and seminal stain evidence were important were subjected to the identification of Gm antigenic determinants. In the first case, the identification of the seminal stain showed the absence of Gm 1 and 12 determinants and the presence of Gm 4. The interpretation of these results would on the surface suggest that the seminal evidence came from one of two suspects. However, since the absence of Gm 12, which occurs on the immunoglobulin G (IgG) 3 subclass, was not confirmed by the demonstration of another IgG 3 subclass Gm antigenic determinant this result was inconclusive. In the second case, bloodstain and seminal fluid evidence showed that seminal fluid identified on the victim's nightgown had Gm antigenic determinants consistent with those of the suspect and also that blood on the suspect's underpants had Gm antigenic determinants consistent with the deceased's blood. The results of these two cases are interpreted with regard to the Gm results obtained.

KEYWORDS: pathology and biology, genetic typing, antigen systems

In forensic medicine, the determination of blood genetic markers for the identification of bloodstains plays an important role [*1-3*]. The genetic marker system Gm, first described in 1956 by Grubb and Laurell [*4*], consists of polymorphic antigenic determinants carried on the gamma heavy chains of gamma globulin molecules [*5*].

The applicability of Gm typing in dried blood and physiological fluids has been established [*2,6,7*]. This system is valuable for its extreme sensitivity [*2*], stability [*2,8*], discrimination potential, and applicability to determining the racial origin of a stain [*2,9*].

This report is concerned with the application of Gm testing in two separate cases and specific problems encountered in the interpretation of the results.

Methods

Titration of Gm Antisera

Appropriate antisera (Biotest or Behring) were serially diluted (1:2, 1:4, 1:18, 1:16, 1:32, and so on) to 1:512. One drop of each dilution was incubated with one drop of a 2% suspension of sensitized red cells (see below) for 30 min at room temperature. The suspen-

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sion was centrifuged at 1000 *g* for 30 s and the agglutination was read macroscopically. Anti-Gm 1, 2, 4≡3, 10, and 12 sera were used.

Sensitization of Red Cells

One drop of packed (saline-washed four times), Group 0, Rh+ (R_1R_1), red blood cells was incubated with three drops of incomplete anti-D serum containing the appropriate Gm factor. The cells and antisera were incubated for 1 h at 37°C, centrifuged, washed three times in saline, and resuspended in saline to yield a cell suspension of approximately 2%.

Identification of Gm Antigens in Serum

Serum to be tested for the presence of Gm antigens was diluted 1:10 with saline. One drop of the diluted serum was mixed with one drop of appropriately diluted antiserum for 30 min at room temperature. One drop of sensitized red cells was then added and incubated for 30 min at 4°C. The suspension was centrifuged for 30 s at 1000 *g* and the agglutination read macroscopically. The presence of agglutination indicates the absence of the appropriate Gm antigen.

Identification of Gm Antigens in Bloodstains

Bloodstains were cut from cotton sheets and placed into disposable glass tubes (10 by 75 mm). It was found that the Gm antigens tested could be identified from threads [*2*].

One drop of appropriate diluted antisera was added to the threads and allowed to incubate at room temperature for 2 h. After the removal of the thread, one drop of sensitized cells was added to the tube and permitted to incubate at 4°C for 2 h. The suspension was centrifuged at 1000 *g* for 30 s and the agglutination was read macroscopically.

Seminal and Vaginal Stains

Since the concentration of IgG can be low in seminal fluid or vaginal secretions, a larger stain than that used for bloodstains must be used. To date Gm (1), (2), (4), and (12) have been determined in both. A (1-cm^2) section of stain was cut, minced into small pieces, placed into disposable glass test tubes (10 by 75 mm), and extracted with a minimal amount of isotonic saline for 2 h at room temperature. The saline was removed from the stain and concentrated to one drop (0.05 mL) with an Amicon mini-concentrator. This drop was analyzed for the presence of Gm 1 antigen as described for bloodstains above.

MN Typing

The identification of M and N antigens was accomplished by elution of absorbed antibody according to previously published methods [*10*].

Identification of Gm Antigens in Seminal Fluid

Seminal fluid was obtained from volunteers and permitted to liquefy. It was stored frozen (−20°C) until ready for an analysis. One drop of liquefied seminal fluid was diluted with one drop of isotonic saline and frozen and thawed four times and centrifuged before use. Seminal fluid was also dried on cotton cloth and then reextracted with saline. Neat seminal fluid is too viscous and does not permit reliable Gm groupings. Two drops or an equal volume of appropriately diluted anti-Gm sera were added to the diluted seminal fluid sample and permitted to incubate at room temperature for 30 min. Two drops of a 2% suspension of

appropriately sensitized red cells were then added and permitted to incubate at 4°C for 30 min. The suspension was then centrifuged at 1000 *g* for 30 s and the agglutination read macroscopically.

Results

Case 1

The first case is concerned with two-year-old seminal stain evidence found at the rape scene and two "look-alike" suspects. One suspect was arrested and identified by the victim, while the other was arrested on another charge but confessed to this and other rapes. The crime laboratory had determined that both suspects were Group A secretors. Analyses on the seminal stain gave the same results. It would not be expected that isoenzyme typing would produce results.

Gm typing of the suspects' sera (Table 1) indicated that the two suspects differed in that Gm (12) was present in the serum of Suspect 2 but absent in Suspect 1. Subsequent testing of seminal fluid and prepared seminal stains from both suspects showed the same results. The analysis of the stain found at the scene indicated the absence of Gm (12). Although this result might seem to exonerate Suspect 2 and implicate Suspect 1, the interpretation is not necessarily correct. The reason for this ambiguity can be explained as follows.

The Gm antigenic determinants as previously noted are located on immunoglobulin G (IgG) molecules; the IgG class is further divided into four subclasses, IgG_1, IgG_2, IgG_3, and IgG_4. Each subclass carries a different set of determinants; the Gm (1), Gm (2), and Gm (4) determinants are located on IgG_1 molecules and the Gm (10) and Gm (12) determinants are located on IgG_3 molecules. In blood plasma, IgG_1 molecules are about eight times more abundant than IgG_3 molecules and, accordingly, the IgG_1 Gm determinants are more readily detectable. The same situation is presumed to apply to semen.

With this information, we can look more closely at the Gm typing results. When typing for Gm (1), Gm (2), and Gm (4) we are looking at determinants on IgG_1 subclass molecules. That we can detect Gm (4) shows that we could detect other IgG_1 Gm markers were they there and makes valid the negative results for Gm (1) and Gm (2) antigens. When typing for Gm (10) and Gm (12) we are looking at IgG_3 subclass molecules and the same interpretative logic applies. The failure to detect Gm (12) in the evidence stain can have three explanations: (a) the Gm (12) antigen is actually not present, (b) it may be degraded [unlikely since Gm (4) was shown to be present], or (c) it may be present but in a concentration below the limits of detectability of our method. Because we have no independent check on the IgG_3 markers, these alternatives cannot be distinguished. Thus, the only valid results of the Gm typing on the unknown seminal stain are concerned with Gm (1) and Gm (4) and the negative result regarding the Gm (12) determinations must be disregarded. The results of

TABLE 1—*Case 1: results of Gm typing.*

Sample Origin	Whole Blood	Saliva	Seminal Fluid	Prepared Seminal Stains	Seminal Stain Evidence
Suspect 1	−1, −2, 4, −10, −12[a]	−1, −2, 4, −10, −12	−1, −2, 4, −10, −12	−1, −2, 4, −10, −12	...
Suspect 2	−1, −2, 4, −10, 12	no results	4, 12	4, 12	...
Seminal stain evidence	...	...	...	...	−1, 4, −12

[a]The notation "−1" indicates a negative test for Gm (1) and "1" a positive test, and so on.

the serological analysis in this case are inconclusive concerning Gm testing and no absolute conclusion regarding either suspect can be made.

Case 2

The second case involves a rape-homicide brought to my attention because the crime laboratory originally working the case could not differentiate between suspect's and victim's blood using the ABO, phosphoglucomutase (PGM), adenylate kinase (AK), esterase D (EsD), or haptoglobin (Hp) genetic marker systems. Although erythrocyte acid phosphatase (EAP) showed differences, the age of the stain (eight months) precluded conclusive results from being obtained. Since the suspect and victim were of different racial origins, the Gm system offered some potential of making a distinction.

Table 2 summarizes the results of the genetic marker testing conducted on reference blood samples from victim and suspect. The Gm (1,2) type found in the victim's blood occurs in approximately 40% of whites; the Gm (1, −2) phenotype found in the suspect's blood occurs in approximately 99% of blacks [*11, 12*]. The victim and suspect differed additionally in the MN and EAP systems.

The evidence received from the police consisted of the victim's underpants and nightgown and the suspect's underpants; the typing results on these items are also shown in Table 2. The victim's underpants were covered with dried blood, which turned out to be consistent with the victim's blood. The victim's nightgown had several stains of seminal, blood, or mixed seminal and blood origin. Analysis of the mixed blood-semen stains gave results that indicated consistency with the victim's blood. The seminal stain was not contaminated with blood and gave clear-cut results consistent with the Gm phenotype of the suspect. Finally, bloodstains on the suspect's underpants gave Gm and MN typing results consistent with the blood of the victim but not of his own type. Interpretation of the MN results must be made with caution since, at this point, it is not known whether the results were obtained from a person of MN or M'N' (cross-reacting N) type [*10*]. The Gm phenotype of the blood on the suspect's underpants clearly indicates that it was not his own.

This case illustrates two interpretative aspects of Gm typing. First, as with most other antigen markers, mixed stains yield cumulative results. Thus, in this case, assuming the blood is from the victim, it is not possible to specify the semen phenotype in the mixed blood-semen stains; the positive reactions for Gm (1) and Gm (2) mask the semen type. The second point is that in some cases the failure to detect an antigen can be meaningfully interpreted. The example in this case is provided by the semen stain on the nightgown. Because both the Gm (1) and Gm (2) antigenic determinants are located on the same immunoglobulin subclass (IgG_1), both should be detected if both are present. Thus the Gm (1) determinant serves as an internal control on the Gm (2) typing result.

TABLE 2—*Case 2: results of genetic marker typing.*

Sample Origin	Stain Origin	Gm Phenotype	MN Phenotype	EAP Phenotype
	WHOLE BLOOD: TYPING RESULTS			
Victim (white)	...	1, 2	MN	A
Suspect (black)	...	1, −2[a]	N	B
	EVIDENCE: TYPING RESULTS			
Victim's underpants	blood	1, 2	MN	inconclusive
Victim's nightgown	seminal fluid and blood	1, 2	MN	inconclusive
Victim's nightgown	seminal fluid	1, −2	...	...
Suspect's underpants	blood	1, 2	MN	inconclusive

[a]The notation "−2" indicates a negative test for Gm (2) and "1" a positive test for Gm (1).

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