ENZYME IMMUNO ASSAY FOR THE TYPING OF THE Gm(a) AND Gm(f) ALLOTYPES OF HUMAN IgG1 IN SEMEN, VAGINAL SECRETIONS AND OTHER BODY FLUIDS

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INTRODUCTION.

Gm allotypes of human immunoglobulins, which are highly polymorphic and stable, have always been of greatest interest for bloodstains' identification. However, the results obtained from semen stains' analysis by using the classical haemagglutination inhibition method, are often not reliable, due to the low immunoglobulin content of extracts.

Enzyme immuno assay (EIA) procedures known for their sensitivity might be better for Gm typing of semen, provided highly selected antibodies such as monoclonal reagents, can be used. We have previously applied a capture EIA to type Gm(f) in blood stains (FRANCOIS-GERARD and HOSTE, 1987). Its sensitivity was proved to be 1000 times higher than the haemagglutination inhibition method. Since the recent commercialization of several anti-Gm monoclonal reagents (DE LANGE et al., 1989), we now use anti-Gm(a) and anti-Gm(f) to type semen stains. These IgG1 markers are antithetical in Caucasian populations.

MATERIAL and METHODS.

MATERIALS.

-A panel of human sera phenotyped for Gm allotypic markers, obtained from G. De Lange.
-Purified IgG1 and IgG3 of known Gm phenotypes, obtained from Dr F. Skvaril from the WHO/IUIS Immunoglobulin Subcommittee, Berne, Switzerland;
-Semen samples provided by the Urology clinics;
-Vaginal secretions collected with plain sterile cotton wool swabs, obtained from the Gynaecology clinics and from volunteer donors;
-Saliva and nasal mucus samples provided by volunteers; blood and urine samples from blood donors. (Liquid salivas were not boiled, but stored at -20°C until analysis).
-Experimental stains on cotton cloth were air dried and stored at room temperature.

METHODS.

Extraction procedure : Stains (5 mm x 5 mm corresponding to 2 or 3 ul of body fluid) were extracted overnight at 20°C in 300 ul of phosphate buffered saline (PBS) containing 0,3 % (w/v) of bovine serum albumine (BSA) and 1 % (w/v) sodium azide. For older stains, a double amount was extracted for 36 h.

Enzyme immuno assay for Gm : a four steps test as previously described (1):
1.A capture antibody coated: rabbit Ig anti-human IgG (A090 DAKO) 1 : 4000.
2.The sample to be tested, in 11 two-fold dilutions.
3.The mouse monoclonal anti-Gm : anti-Gm(f) (clone TM14 from OXOID) 1:16.000.
anti-Gm(a) (clone 10H1 from G. De Lange) 1:20.000
4.Peroxidase conjugated rabbit anti-mouse Ig (P260 DAKO) 1:250.

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As for other EIA methods, important points are to select a capture antibody, to avoid cross-reactivity by using affinity purified reagents and to optimalize the dilution of each reagent.

The optical densities were measured by means of a spectrophotometer and the results expressed as the corrected optical density value (Δ OD = OD sample -OD blank). In cases of stains' extracts, they were alternatively expressed as the last sample dilution still giving a significant Δ OD value.

The amount of IgG1 in various body fluids was estimated by using a similar EIA procedure, with a monoclonal anti-γ1 (clone NL16 UNIPATH) 1:5000, in step 3.

Prostate specific antigen (PSA or p30) detected by a capture EIA (KAMENEV et al., 1989) was used as a control for the presence of semen.

RESULTS.

The monoclonal anti-Gm(a) and Gm(f) reagent were proved to be specific in this enzyme immunoassay, by testing a panel of 20 sera of various Gm phenotypes, including rare ones.

In order to determine a threshold of significant Δ OD values, the following negative controls were tested : various biological fluids from persons of Gm(a-) or Gm (f-) group : semen, semen stains, sera, blood stains, vaginal secretions, saliva, saliva stains, nasal mucus stains. The following samples of any Gm group were also tested : urine stains, sweat stains, faeces stains. The cutt-off was calculated as the mean + 2s of neat negative controls and found 0.097 (mean = 0.031, s = 0.033, n = 52) for Gm(a) and 0.260 (mean = 0.117, s = 0.076, n = 36) for Gm(f) respectively. .

A purified IgG1 standard allowed us to measure the IgG1 concentration of 55 liquid semen. The mean concentration is 32 $\pm$ 40 ug/ml, i.e.around 100 times less than in whole blood. As in blood, the individual variation is important. The mean IgG1 content of 5 liquid salivas was found to average 3 $\pm$ 6 ug/ml. IgG levels have been reported 1000 times lower in saliva than in blood (WAISSBLUTH and LANGMANN, 1971).

TABLE 1 : EXPERIMENTAL SEMEN STAINS.

Stains (**)	Age (months)	Serum Gm group	Semen Gm group (*) Gm(a)	Gm(f)
1	6.5	a-, f+	0	1/512
2	6.5	a-, f+	0	1/128
3	6.5	a+, f+	1/32	1/16
4	6.5	a-, f+	0	1/16
5	6.5	a-, f+	0	1/512
6	6.5	a+, f-	1/512	0
7	6.5	a-, f+	0	1/64
8	6.5	a-, f+	0	1/128
9	32	a-, f+	0	1/512
10	33	a-, f+	0	1/128
11	36	a+, f-	1/1024	0
12	36	a+, f+	1/256	1/128

(*) last dilution significantly detected by EIA.
(**) 5 mm x 10 mm extracted.

One can see from table 1 that semen stains can be clearly typed using this method.

TABLE 2 : SENSITIVITY OF Gm SPECIFIC DETECTION IN VARIOUS BODY FLUIDS

		Range of Gm(a) or (f) detection (*)		
samples	N	liquid	N	fresh stains(**)
blood	20	1/64,000-1/1,000,000	20	1/1024
semen	55	1/800-1/25,000	10	1/16-1/1024
vag. sec			16	1/16-1/1024
saliva	5	1/256-1/1024	5	1/4 -1/16
nasal muc			10	1/2 -1/256
urine	12	0- (1/16, 1/32)	12	0
sweat			3	0 -1/2

(**) 5 mm x 5 mm extracted -2 to 3 ul

Table 2 shows the sensitivity range of Gm detection in various body fluids, liquid or dried, with the same amount of stain. To summarize, only the positive results for Gm (a) or Gm(f) are given; they were always in agreement with the serum Gm groups.The absence of blood or semen contamination was tested by using the benzidine and the p30 tests.

As for other semen polymorphisms, such as ABO and PGM, the main problem in forensic applications, is the presence of the same marker in vaginal secretions.

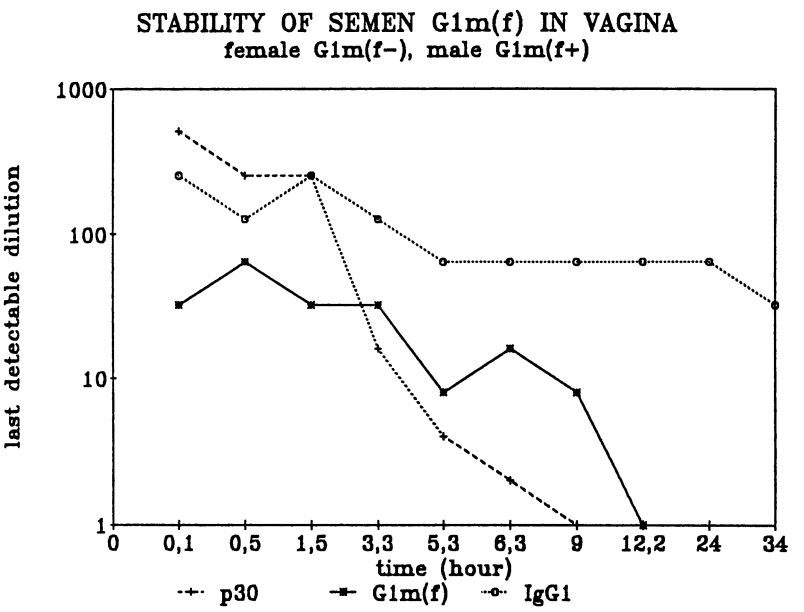
In two other body fluids, the Gm allotypes could also be clearly determined : saliva and nasal mucus. The Gm concentration in nasal mucus was extremely variable : in several samples it was as high as in semen stains.

All the liquid urines tested were negative, except one sample collected from a male donor and which was positive for p30 as well, and one urine from a female donor and which was negative for blood and for p30. This should be investigated further. Stains of all urine samples including these two, were negative or doubtful. Sweat stains did not drive to conclusive results.

Results of 63 stains ageing from 6 months up to 3 years showed the same excellent stability of the Gm(a) and (f) allotypes in dried semen and vaginal secretions as already known of those allotypes in dried blood.

The stability of semen Gm allotype in the vagina, after sexual intercourse could be studied in one case in which the woman was Gm(f-) and the man Gm(f+). They provided a semen sample, a semen free vaginal swab and a timed intervals set of post-coïtal swabs. For each swab, the same extract was tested for Gm(f), IgG1 and p30 content. Fig 1 shows that semen Gm(f+) could be detected in the vagina up to 12 hours after intercourse. The delay for p30 detection was identical.

Fig 1 :



IN CONCLUSION,

This enzymeimmunoassay is a very sensitive and specific method, which can be applied to the typing of Gm antigens in semen and other body fluids.

The possible interference of Gm allotypes of vaginal origin and possibly from saliva or nasal mucus, has to be kept in mind when interpreting the results in sexual assault cases.

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