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/UIS 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.
As for other EIA methods, important points are to select a capture antibody, to avoid cross-reactivity by using affinity purified reagents and to optimize 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 (ΔOD = OD sample - OD blank). In cases of stains' extracts, they were alternatively expressed as the last sample dilution still giving a significant ΔOD value.

The amount of IgG1 in various body fluids was estimated by using a similar EIA procedure, with a monoclonal anti-\( \alpha \) (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 ΔOD 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 ± 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 ± 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.

<table>
<thead>
<tr>
<th>Stains (**)</th>
<th>Age (months)</th>
<th>Serum Gm group</th>
<th>Semen Gm group (*)</th>
<th>Gm(a)</th>
<th>Gm(f)</th>
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<tr>
<td>1</td>
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<td>a-, f+</td>
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<td>1/512</td>
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<td>a-, f+</td>
<td>0</td>
<td>1/128</td>
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<td>6.5</td>
<td>a+, f+</td>
<td>1/32</td>
<td>1/16</td>
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<td>a+, f-</td>
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<td>36</td>
<td>a+, f+</td>
<td>1/256</td>
<td>1/128</td>
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</table>

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