DIFFERENTIAL DETECTION OF A, B AND H ANTIGENS IN SEMEN AND SALIVA STAINS DETECTED BY AN ELISA TECHNIQUE

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INTRODUCTION

In our laboratory we have modified an enzyme linked immunosorbent assay described by Fletcher (1989); and validated it for use in Forensic Samples. As part of the validation exercise a study was made of the differential levels of A, B and H antigens, in order to set a threshold value for the detection of one of the antigens at which the non detection of the others could be taken as their true absence. This is particularly important when grouping weak stains that are often encountered in Forensic work.

METHODS AND MATERIALS

Stains were made from semen and saliva samples from 1) laboratory donors, 2) Criminal cases and 3) a local fertility clinic. A 5mm. square piece of stained material was extracted and serial dilutions made such that the antigenic end point could be observed. The following studies were made:

1) Stains from group A_1, A_2 and B secretors, in respect to the level of H.

2) Comparisions of the levels of A and B in group AB stains.

A direct ELISA method was used where the antigen containing extract was incubated in microtitre plates. The A, B or H specific mouse monoclonal antisera pre-mixed with a conjugate of anti-mouse (μ chain specific) goat polyclonal alkaline phosphatase was added at appropriate concentrations. The enzyme concentration was measured by the addition of p-Nitrophenol phosphate. The colour change which represents the amount of antigen in the sample was meaasured in milli absorbance units (mau) using a Dynetech MR 700 microplate reader connected to an IBM computer.

RESULTS AND DISCUSSION

A TO H AND B TO H DIFFERENTIAL.

The ratio of A to H and B to H showed marked differences in the two body fluids investigated. In saliva the amount of H was low compared to A and B in groups A_1 and A_2 (Fig. 1.) and group B respectively. In semen however it appeared that the H level was high particularly in group A_2 and B (Fig. 2.) and in some A_1.

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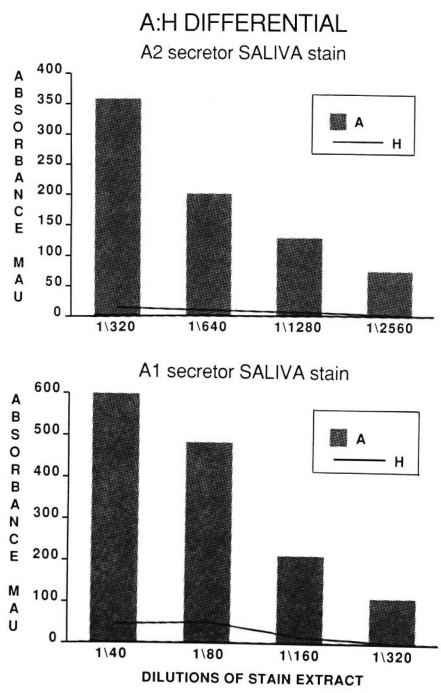

Fig. 1. A and H levels in A_1 and A_2 saliva.

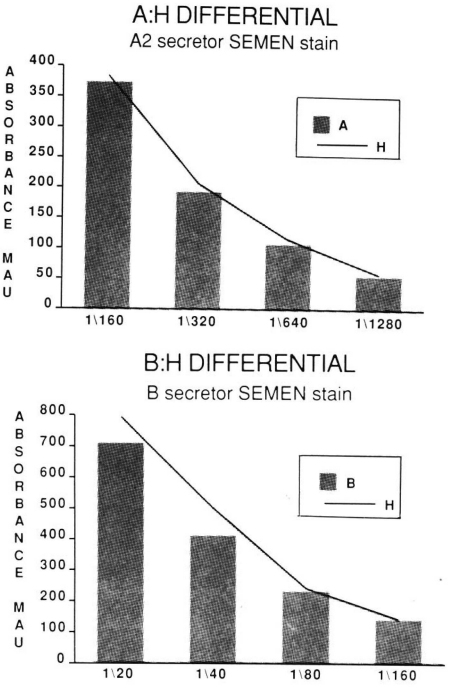

Fig. 2. A or B level compared to H in A_2 and B semen.

The ratios of A to H and B to H were calculated from a number of random semen and saliva stains, expected to contain more A_1 than A_2. The ratio of A to H was small in semen stains as compared to saliva stains (table 1). This indicates that the H level in semen of group A is not entirely related to subtype.

Table 1. A:H and B:H ratio. (The number of stains studied is in parenthesis)

	Semen	Saliva
A sec	1.8(38)	19.3(14)
B sec	1.7(16)	27.7(9)

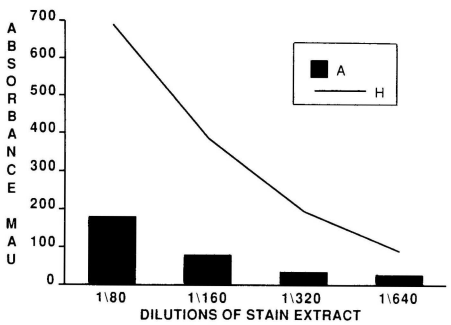

Fig. 3 Extreme A:H differential in a Group A semen.

These results alerted us to the possibility that in weak semen stains of group A or B the H may persist after A or B was lost. An extreme example of this is shown in Fig. 3. Observations of all these samples indicate that a semen stain of group A or B did not loose A or B and retain H at over 300 mau.

Therefore, when a value of over 300 mau was obtained for H with no significant reactions for A or B, then the semen stain could reliably be typed as O. In saliva, the H value was always low compared to A or B, and a value of 100 mau for H in the absence of A and B showed the stain to be group O.

The reason for this differential in semen and saliva H substance could be quantitative, qualitative or both. It is possible that more H substance remains unconverted to A or B in semen than in saliva. It is also possible that the reactions may be due to peripheral core variation (e.g. Type I and Type II) or the branched nature of the H substances forming antigenically distinct structures (Clausen and Hakomori, 1989) conferring differences to the H substance in the two body fluids. These differences could be recognised by the H monoclonal antibody we are using.

COMPARISON OF A AND B IN AB SECRETORS.

Body fluid stains from 35 group AB individuals have been studied. A few of these gave approximately the same amount of A and B, but approximately ¾ of the samples showed a reduction in one or other antigen. The loss was always related to the subtype of A. Extreme examples of these are shown in Fig. 4. and 5.

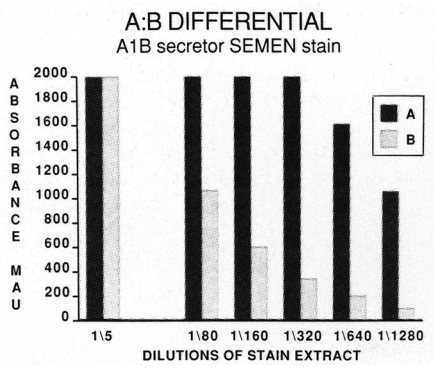

Fig. 4. Semen stain from a group A_1B secretor showing extreme A:B differential.

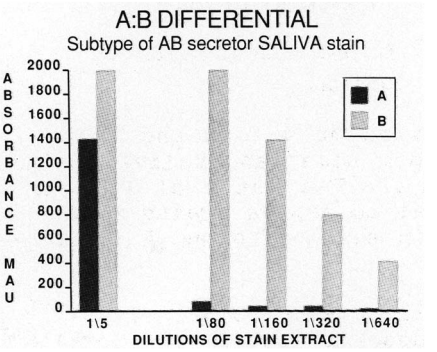

Fig. 5. Saliva stain from a group AB secretor showing extreme B:A differential.

The saliva stain result shown in Fig. 5. comes from a suspect in a rape case. A breast swab from the victim (group O) and seminal staining on her knickers gave reactions for group B only. A blood sample from the suspect was grouped as AB but with very weak reactions for A. This person was a group AB with either a weak A subtype or a super B gene (Yoshida 1983).

Besides these two examples there were many other group AB secretor saliva stains that gave differential values of over 500 mau. The two extreme examples (Figs. 4. and 5.) and the high differential values observed in others suggest that extreme caution is required when excluding group AB when either A or B only is detected. Therefore, until many more group AB samples are studied, a threshold value to exclude the possibility of an AB has been deferred in our laboratory.

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