

Absorption-elution on microplates (AEMP): An improved method for identification of the ABH major glycoproteins in saliva and semen of secretors.

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INTRODUCTION

Recent progress in ABO typing of body fluids and stains have been obtained by highly sensitive methods such as Inhibition of Hemagglutination on Microtiter Plates (HIM) by using monoclonal antibodies (Fiori, 1985), enzyme-immunoassays (EIA) as the ELISA procedures (Inoue and Okada, 1978; Fletcher 1984; Bolton and Thorpe, 1986; Mudd 1986; Mukoyama and Sakai 1986; Takatori and Tsutsubuchi 1986), and EIA-like Mixed Agglutination on Solid Phase (MASP; Fiori and Chiarotti, 1985, Silvestri et al. 1981).

The ELISA procedures are expensive and time-consuming, MASP is tedious and poorly reproducible. Therefore a new method was devised, Absorption-Elution on Microplates (AEMP), that does not require apparatus and is as sensitive (if more) as ELISA (FIORI, 1985). This method is based on the linkage of soluble ABH substances to the bottom of 'U' wells of polystyrene microtiter plates, with subsequent absorption of polyclonal antibodies, elution by heating and detection of the eluted antibodies by microagglutination of indicator red cells.

In the present paper an optimized version of the AEMP is presented.

MATERIALS AND METHOD

Saliva and semen samples from A, B and O secretors and nonsecretors were examined after centrifugation and without any denaturing treatment. Small cloth pieces (2 x 5 mm) stained with saliva and semen were extracted overnight in 0.1-0.2 ml saline.

The coating solution was 0.05 mol/L carbonate/bicarbonate buffer pH 9.6; the diluent for red cells, antibodies and the eluate medium was saline containing 0.2% (w/v) BSA.

The washing solution was saline containing 0.1% gelatin and 0.05% Tween 20; the blocking solution was saline containing 0.2% (w/v) BSA and 0.1% Tween 20.

Samples (50 μ L each) were serially diluted in 24 wells with the coating buffer and incubated overnight at 4°C.

The wells were emptied and washed three times, each of 10 minutes, with the washing solution.

One hundred μL blocking solution was then added to each well and left for 30 min. at room temperature.

The plate was shaken dry and washed again, three times, with saline. Then each well was filled with 50 μL anti-A or anti-B polyclonal antibody (Ortho Diagn.) diluted 1:3 with the diluent. Anti-H from Ulex Europaeus (titre 1/128) was used undiluted. Monoclonal anti-A and anti-B were also assayed (Seraclone, Biotest; Bioclone, Ortho)

After 3 hours incubation at room temperature, the plate was washed twice with washing solution, then rinsed with tap water for 10 minutes, and finally with saline.

Fifty μL diluent was added to each well, the plate was covered and elution carried out for 1 hr in an oven at 60°C. Equal volumes 0.5% indicator red cells were added and sedimentation allowed to occur at room temperature for 3-4 hrs. The plate was then preserved at 4°C to be reexamined.

Macroscopic reading of the agglutination patterns was performed as elsewhere described for 'U' plates (Fiori, 1985). Accordingly, strong agglutination is seen as a small uniform salmon-pink disc covering the entire lower part of the wells, and partial agglutination as rings with increasing thickness and decreasing diameter. Complete inhibition is a smooth round button that forms a tear when the plate is inclinate in semi-vertical position for some minutes.

Haemagglutination Inhibition on 'U' Microplates (HIM) was carried out by using diluted monoclonal anti-A and anti-B (Seraclone, Biotest) and anti-H from Ulex Europaeus according to the method described elsewhere (Fiori 1985).

RESULTS AND DISCUSSION

Liquid samples of saliva and semen of A, B and AB secretors gave highly sensitive results at dilutions ranging from 1×10^5 to 5×10^6 . In O secretors the positive results were usually in the range of 1×10^5 to 6×10^5 .

In Table I some examples are reported. Nonsecretors were in most cases negative. Only in some specimens a few wells showed partial ed irregular agglutinations.

Monoclonal antibodies gave always negative results as in MASP method. Nonspecific agglutinations with heterologous sera were not observed when the whole procedure was carefully followed, especially the washing and blocking steps.

The stain extracts were positive at lower dilutions (in general up to 4×10^4 - 8×10^5) apparently due to the incomplete elution from the stain. But sometimes the results were of high sensitivity. For example a seminal stain from a

A secretor, 5 mm side, extracted with 0.5 ml saline, was 1/2000 with HIM, and 10 μ L of the extract had titer of about 1/500.000 with AEMP.

Negative findings with nonsecretors (which are positive with the conventional absorption-elution tests directly performed on the stain) are presumably due to ABH substances different in molecular size and shape, that probably link to the plate but in such a way that the antibody is hindered from binding or bind in non eluable form.

In forensic casework AEMP can be conveniently used in combination with Hemagglutination Inhibition on 'U' Microplates (HIM) with monoclonal antibodies and with the direct conventional Absorption-Elution (AE) to check the blood group and the secretor status, nonsecretors being positive with AE and often with HIM, usually negative with AEMP.

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Table 1. Absorption-elution on microplates (AEMP) and Hemagglutination Inhibition on microplates (HIM) titer in some samples of saliva and semen.-

Blood group saliva	secretors status	HIM ^o titer*	AEMP titer*
A	S	131072	1048576
A	S	32768	2097152
A	S	131072	1048576
A	S	1048576	4194304
A	S	524288	2097152
A	S	131072	1048576
A	NS	1024	neg
B	S	512	1048576
B	S	65536	524288
B	S	65536	1048576
B	S	32768	524288
B	S	131072	1048576
B	NS	128	neg
AB	S	A 4096	131072
		B 128	131072
AB	S	A 16384	131072
		B 512	131072
O	S	131072	131072
O	S	262144	524288
O	NS	4	neg
O	NS	64	neg
Semen			
A	S	65536	1048576
B	S	131072	2097152
O	S	131072	524288
A	NS	32	neg

^o HIM: Hemagglutination Inhibition on U Microplates; Seraclone A diluted 1:400, Seraclone B diluted 1:200, anti-H diluted 1:50.

* titer: dilution end-points.