A Modified ELISA for the Detection of ABH Antigens in Bloodstains

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Although solid phase ELISA using McAb has been reported for the determination of ABO groups of bloodstains there have been difficulties particularly relating to the stains of group A_2 and especially group AB.

Presented here is an ELISA technique which does not necessitate the extraction of antigens from the stain material or their immobilisation to the solid phase and does appear to overcome some of the previously reported problems.

MATERIALS

McAbs used include anti-A (blend of clones ES9 and LM103/107) and anti-B (clone ES4) purchased from Bioscot Ltd, anti-A (clone A003) donated by Biotest Folex Ltd and anti-H from Fresenius Diagnostics. Anti-mouse IgM alkaline phosphatase came from Sigma Chemical Co.

Over 200 bloodstains were prepared previously from laboratory staff and stored in the laboratory bloodstain file.

TECHNIQUES

1. 3-5mm bloodstain threads attached by one end to polycarbonate sheet using cellulose acetate glue.

2. 50ul of selected dilution of McAb added to each thread and sheets incubated overnight at $4^{\circ}C$ in sealed moist chamber.

3. Excess McAb removed with suction pump and threads washed with jet of ice cold PBS.

4. 50ul of anti mouse IgM alkaline phosphatase conjugate (1/400) added to each thread and sheets incubated at $4^{\circ}C$ for 2 hours in moist chamber.

5. Excess conjugate removed, threads irrigated with ice cold PBS and sheets immersed in tank of PBS for 2 hours at $4^{\circ}C$.

6. Blotted dry, threads cut off and transfered to wells of microplate.

7. Add to each well 100ul substrate (1gm/ml) P-nitrophenyl phosphate in diethanolamine buffer. Plates incubated at $37^{\circ}C$ on rotatest machine for 10 minutes and then 50ul of stopping solution (3M NaOH) added to each well. The threads are discarded, visual examination and OD readings taken.

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Edited by H. F. Polesky and W. R. Mayr

8. OD values greater than 0.1 considered positive provided blanks and negative controls below this value.

Controls: a. substrate only.
b. bloodstain, conjugate, substrate but no McAb.
c. bloodstain, McAb, substrate but no conjugate.
d. known positive and negative bloodstains.

Where the elution test was used this was as described elsewhere (Dodd and Lincoln, Antigen Antibody Reactions Revisited, American Association of Blood Banks 1982, pp223-239).

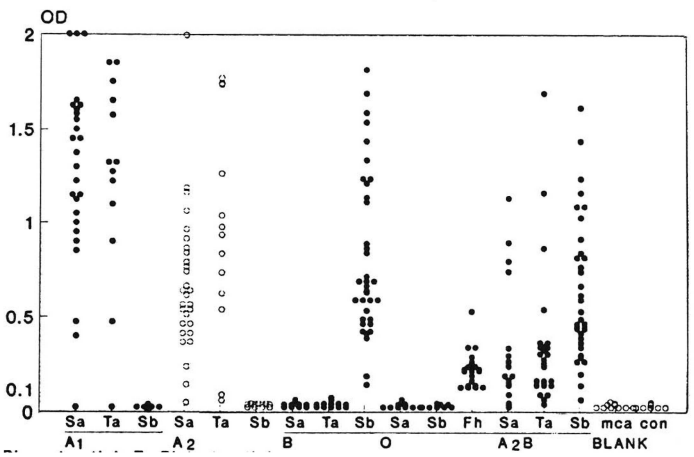
RESULTS

Various dilutions of McAb were used in preliminary experiments using 10 month old A2, B and O bloodstains. Dilutions of 1 in 2 for anti-A, 1 in 4 for anti-B and neat anti-H were selected for further investigations.

The results of ABO grouping of 213 bloodstains ranging in age from under 1 year (28 stains) to over 20 years (29 stains) are shown in Figure 1. All A, B and AB stains were tested with two anti-A reagents and one anti-B whereas the group O stains were tested with one each of anti-A, anti-B and anti-H.

206 stains were correctly typed. 7 produced results in disagreement with records of previous grouping of fresh blood samples. Four of these were 7 years old A_2B stains. (One failed to react with one of the anti-A reagents, two failed to react with either anti-A reagent used and one failed to react with the anti-B). The other three stains showing disagreements, one A_1, one A_2, one A_2B which were 20, 24 and 4 years old respectively all failed to show positive reactions with the relevant anti-A or anti-B reagents used.

Figure 1: The distribution of OD values of bloodstains tested by ELISA



Sa=Bioscot anti-A, Ta=Biotest anti-A
Sb=Bioscot anti-B, Fh=Fresenius anti-H
mca=McAb blank, con=conjugate blank

These seven problem stains were tested by elution and ELISA in parallel tests. Elution was performed using the McAb selected for ELISA and by well tried polyclonal reagents.

In general elution appears to be slightly more sensitive than the ELISA and the selected polyclonal reagents used in elution produced slightly better results than the McAbs. In some instances the relevant ABH antigens could not be detected by any of the techniques used.

CONCLUSION

Modification of the ELISA developed by Fletcher and co-workers involving the omission of extraction and immobilisation and using direct attachment of bloodstained threads to acetate sheets appears to produce a simple sensitive and reliable test system.