DOT BLOT IMMUNOASSAY FOR DETECTION OF HLA ANTIGENS IN FORENSIC STAINS

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ABSTRACT

A new highly sensitive and specific microplate based dot blot immunoassay for detection of HLA antigens in blood and stains is reported. The unique feature of this assay is that the final result is unambiguous since it is a distinct colour reaction. The assay has been used in detection of antigens in fresh and old blood stains with equal success.

INTRODUCTION

Polymorphism of HLA has been widely exploited in organ transplantation and forensic analysis (Berah et al 1970; Newall 1979). Several modified cytotoxicity tests and enzyme immunoassays for detection of HLA antigens in forensic samples have been reported (Newall 1981; Nelson et al 1983; Rittner and Waiyawath 1975 and Bishara et al 1983). Most of these methods either lack the required accuracy or they are too complex to be used routinely. We have developed a highly sensitive and specific microplate dot blot immunoassay (MDBI) for typing HLA in blood and its stains for individualisation, initially using a panel of antisera of high specificity to HLA-A1, A2, A11, A24, A28, B7, B8, Bw52, DR2, DR7 and DR8.

MATERIALS AND METHODS

Sample Preparation and Plan of Study

Blood samples from the volunteers were collected and their HLA phenotypes were ascertained by standard microcytotoxicity test and stains of the same were prepared on cloth. Each stain of the size of 0.5 cm was extracted in 1.0ml of PBS containing 0.1% of Tween20. The extract (50ul) was then used to detect HLA antigens. The extract was serially diluted from 1:100 to 1:1400 to test the sensitivity of the method. To know the applicability of the assay to old samples, the stains were stored both at room temperature and at 4°C for a period

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Advances in Forensic Haemogenetics 4
Edited by Ch. Rittner and P. M. Schneider

of 12 months and tested at the end of every 3rd month. 50 unknown blood stains from old case samples were tested to evaluate its suitabilty actual forensic analysis. Double-blind experiments were repeated to further confirm the results.

Microplate Dot Blot Immunoassay (MDBI)

Blood/stain extracts were loaded on nitrocellulose membrane (NC) using a dot blot apparatus. Discs (3mm dia.) were punched, placed in the wells of microplate and incubated with 200ul of 3% H_2O_2 for 30 min at room temperature to inhibit the endogenous peroxidase. The wells and the discs were blocked twice, first by incubating with 200ul of 2% BSA at 37°C for one hour and secondly with 100ul of 1:500 dilution of antihuman globulins for another hour at 37°C. Wells were washed with phosphate buffer with Tween20 (PBST). 100ul of 1:100 diluted different HLA antisera were placed in their respective wells. The plate was covered with parafilm and incubated for an hour at 37°C. The wells were washed with PBST. In each well 100ul of 1:500 diluted antihuman IgG-peroxidase conjugate was added and incubated for 2 hours at 37°C and washed with PBST. 100ul of substrate mixture containing 0.1% 4-chloronaphthol and 0.002% H O in PBS was placed in each well. Development of purple colour on NC discs within 30 min indicated positive reaction.

RESULTS AND DISCUSSION

Figure 1 shows the result of HLA antigens detection in one of the stains. HLA antigens even in 1000 times diluted extract were accurately detected. The stains stored for a year at 4°C as well as room temperature were correctly typed except 4% of 9 and 12 month old stains stored at room temperature (Table 1). Antigens in 90% of random case samples were successfully identified by MDBI.

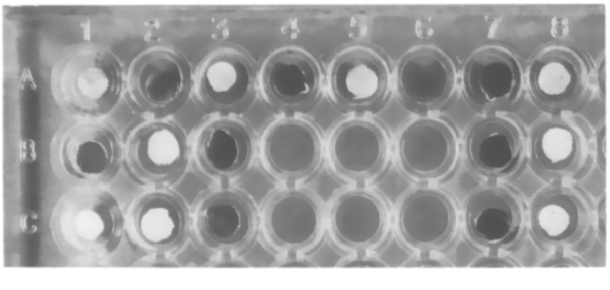

Fig. 1. Photograph showing positive (black) and negative (white) reactions to different HLA antigens obtained in MDBI. Tests performed in wells for different antigens are as follows : A row : 1) A1; (2) A2; (3) A11; (4) A24; (5) A28. B row : 1)B7; (2)B8; (3)Bw52. C row : 1)DR2; (2)DR7; (3)DR8. Column 7 and 8 represent positive and negative controls

Table 1. Effect of storage on the detectability of HLA antigens

Period of Storage	Samples tested	No. of correctly typed stain (%)	
		Stored at room temp	Stored at 4°C
One week	50	100	100
3 months	50	100	100
6 months	50	100	100
9 months	50	96	100
12 months	50	96	100

MDBI combines the best of both micro ELISA and dot blot assay. NC discs hold larger quantity of antigen which enhances the assay's detectability and the end result is a permanent colour reaction on the NC disc. Simultaneous processing of several samples in MDBI using very small volumes of reagents is inherited from micro ELISA. Treatment of sample discs with H_2O_2 eliminate the endogeneous peroxidase interference. False positive reactions initially obtained due to the presence of endogenous immunoglobulins in stain extract has been effectively overcome by introducing a second blocking step with unlabellled antihuman IgG. The criteria for selection of antisera panel in MDBI is the availability of high specificity antisera and their prevelance in Indian population. Enzyme amplification and high quantity of target antigen on disc are the reasons for better sensitivity. Storage studies suggest that the HLA antigens are relatively stable and are best preserved at 4°C. Disintegration of antigen due to microbial contamination and aging may be the reason for marginal decline in detection of some of the experimentally stored and forensic samples. Absence of corresponding antigens tested for in the sample may be the other reason for failure in the detection of 10% of forensic samples. MDBI is highly suitable for forensic and clinical diagnosis of HLA antigens.

REFERENCES

Berah M, Hors J, and Dausset J (1970) A study of HLA antigens in human organs. Transplantation 9, 185-192

Bishara A, Brautbar C, and Bonavida B (1983) Enzyme linked immunosorbent assay for HLA determination on fresh and dried lymphocytes. J Immunol Method 62:265-271

Nelson MS, Turner LL and Reisner EG (1983) A feasibility study of human leucocyte antigen (HLA) typing for dried bloodstains J Forens Sci 28:608-614

Newall PJ (1979) The identification of HLA-A2 and HLA-B5 antigens in dried bloodstains. Can Soc Forens Sci J 12:1-16

Newall PJ (1981) The identification of HLA-A9 in dried stains of blood and body secretions. Can Soc Forens Sci J 14:104-112

Rittner C and Waiyawuth V (1975) HLA typing in dried blood stains. II comperative studies on microcytotoxicity and microcomplement fixation tests. J Immunogenet 2:211-222