# EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

# Detection of Antibodies to HLA 1 Antigens by Enzyme Immunoassay

**S. D. Borozdenkova, A. Yu. Baryshnikov, T. N. Palkina, and F. S. Baranova**

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A modification of the MAILA (monoclonal antibody specific immobilization of lymphocyte antigens) method has been developed for the detection of antibodies to class 1 histocompatibility antigens. Russian biotin-treated monoclonal antibodies IKO-53 (Medbiospektr, Moscow) were used. In a complex with monoclonal antibodies, lymphocyte HLA antigen was found to retain its antigenic properties when stored for a long time. High specificity and sensitivity of the method were demonstrated.

**Key Words:** *antibodies to class 1 histocompatibility antigens; ELISA*

Class 1 histocompatibility antigens (HLA-A, B, C) are highly polymorphic glycoproteins of cell membranes. They are recognized by specific cytotoxic T cells along with viral, tumor, and other antigens, and after processing by antigen-presenting cells may stimulate T cells or induce the production of anti-HLA antibodies as allopeptides [13]. Anti-HLA antibodies are formed as a result of blood transfusion, pregnancy, and transplantation. In the case of an allotransplantation they not only determine the development of superacute and accelerated acute rejection, but also play an essential role in acute and chronic rejection [6,7,9].

At present the principal method of detecting anti-HLA antibodies in the blood serum is the complement-dependent cytotoxic test (CDCT). Despite its great practical significance, the method is not without numerous shortcomings: its sensitivity is low; it does not detect noncomplement-fixing anti-HLA antibodies (IgG2, IgG4, IgA); its specificity is insufficient; live cell panels are necessary [4,9]. These drawbacks are partially overcome by using flow cytofluorometry, a method which is more sensitive than CDCT (approximately 250 times) and, moreover, permits the detection of noncomplement-fixing antibodies [5,7,14]. It has, however, two of the main drawbacks of CDCT: live cells are needed, and not just anti-HLA antibodies, but antibodies to other membranous structures of lymphocytes are detected.

Solid-phase enzyme immunoassay (ELISA) developed recently for the detection of anti-HLA antibodies is no less sensitive than flow cytofluorometry, but is free of its principal shortcomings. Several variants of this method have been proposed [2,8,10,11].

At first we used ELISA with partially purified HLA-A, B, and C obtained by gel filtration of splenocyte membrane lysate on Ultragel Ac34. The main problem we faced was the high level of nonspecific binding, which was evidently due to the presence of admixtures in antigen preparations. Use of affine columns made it possible to prepare an-

Department of Immunology, Research Institute of Transplantology and Artificial Organs, Ministry of Health of Russia; Department of Experimental Diagnosis of Tumors, Cancer Research Center, Russian Academy of Medical Sciences, Moscow (Presented by V. I. Shumakov, Member of the Russian Academy of Medical Sciences)

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tigens of high purity and reduce the level of admixtures. However, this approach is technically difficult and unfit for clinical studies. Later we used a modification of the MAILA method, in which the antigens are isolated from lymphocytes as a complex with monoclonal antibodies (MAb) to the monomorphic determinant of the HLA molecule [11]. The complex was immobilized on the plate using antispecies antibodies or an avidine-biotin system [10].

In this paper we describe our modification of the MAILA method and demonstrate its use for analysis of anti-HLA antibodies by ELISA.

## MATERIALS AND METHODS

Splenocytes or peripheral blood monocytes ($20\times10^6$) were incubated for 1 h at 20°C with biotin-treated MAb to the monomorphic determinant of class 1 HLA (10 μg/ml RPMI-1640). Biotin-treated MAb IKO-53 were obtained from Medbiospektr Research and Manufacturing Center (Moscow). After incubation the cells were washed three times in RPMI-1640, poured into tubes ($10^7$ cells), and stored at -40°C. Directly before measurement of antibodies, the cells were thawed, and 0.1 ml of lysing buffer (50 mM Tris, 150 mM NaCl, 3 mM EDTA, 0.5% NP-40, and 50 mM phenylmethylsulfonyl fluoride, pH 7.2) was added. The cells were incubated for 1 h on ice and then centrifuged for 15 min at 4000 g.

For ELISA Titertek plates were used pretreated with streptavidin, 5 μg/ml buffered normal saline (BNS), 0.05 ml per well, 16 h at 4°C.

Lymphocyte lysate diluted 1:10 in BNS was added to the wells (0.05 ml), incubated 2 h at 37°C, and washed three times, after which the test serum was added in the appropriate dilutions (0.05 ml/well). After a 2-hour incubation at 37°C and washing of the plate, 0.05 ml of anti-FcIgG-specific MAb conjugated with horseradish peroxidase (Cardiology Research Center, Russian Academy of Medical Sciences, Moscow) was added to the wells. After 1 h the plate was washed and o-phenylenediamine was added. The reaction was stopped after 15 min with 2 N $H_2SO_4$ and the optical density was measured at 492 nm using a Titertek Multiscan Plus apparatus. BNS containing 0.1% Tween-20 was used for washing.

## RESULTS

Biotin-treated MAb IKO-53 identical in their specificity to W6/32 were used for the isolation of HLA-A, B, and C [1]. The number of antibodies needed for the treatment of cells was found in special experiments. We demonstrated that lymphocyte HLA-A, B, and C in complex with MAb to the monomorphic determinant of HLA-1 retain their antigenic properties for a long time (at least for 6 months at -40°C). This is a convenient method for storing donor HLA-A, B, and C.

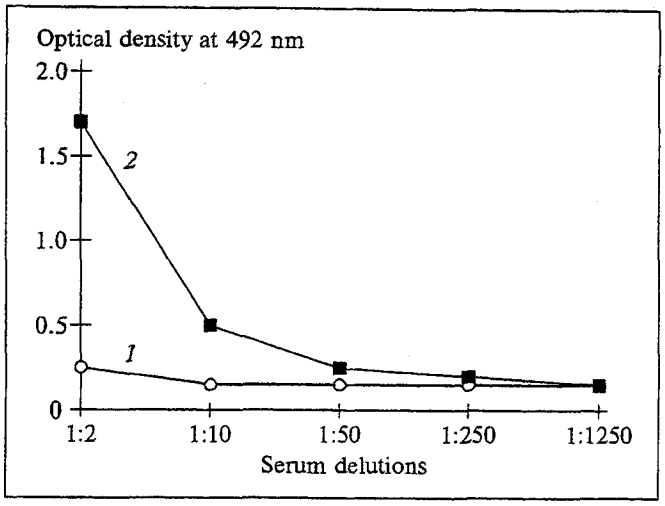


**Fig. 1.** Relationship between optical density of reaction and serum dilution. HLA antigens were isolated from lymphocytes with A3,11; B8,22 phenotype. Pool of negative (*1*) and positive (*2*) sera.

In order to validate the specificity of the method, HLA-A, B, and C were isolated from lymphocytes with HLA phenotypes determined in the lymphocytotoxic test. Corresponding specific alloantisera (Behring) were used. Table 1 shows the results of interactions between monospecific sera and HLA-A, B, and C of different donors. The results indicate a high specificity of the method.

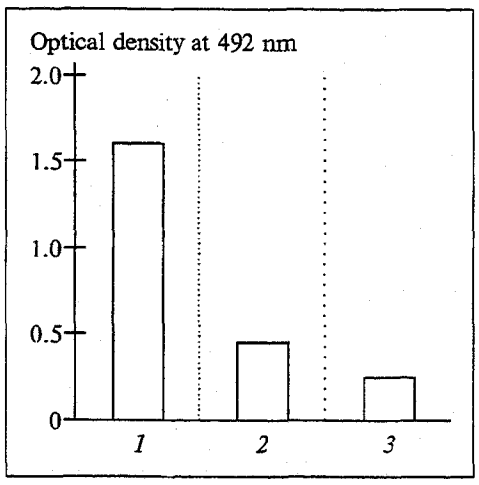


**Fig. 2.** Results of interaction of HLA antigens of cells with A3,11; B8,22 phenotype with pools of positive or negative sera. Proof of the sensitivity of the method. *1*) pool of positive sera; *2*) pool of positive sera adsorbed with platelets; *3*) pool of negative sera.

**TABLE 1.** Interaction of Monospecific Sera to HLA Antigens with Antigens Isolated from Cells with Different Phenotypes

| Cell phenotype | Specificity of alloantisera* | | | | |
|---|---|---|---|---|---|
| | anti-A3 | anti-A2 | anti-A11 | anti-B13 | anti-B14 |
| A2,3; B12,13 | 0.209 | 1.820 | 0.195 | 0.660 | 0.215 |
| A3,11; B22,39 | 1.350 | 0.211 | 0.890 | 0.192 | 0.214 |
| A9,28; B14,22 | 0.198 | 0.213 | 0.223 | 0.202 | 0.960 |
| A1,11; B8,14 | 0.195 | 0.210 | 0.780 | 0.217 | 1.080 |

**Note.** *All values are expressed in optical density units, nm.

In all the cases the optical density of nonspecific interactions did not exceed 0.25, whereas during specific interaction it was as high as 1.8.

Optical density is related to serum dilution (Fig. 1). We found that a 1:2 dilution is the optimal for the majority of sera, with the ratio of the optical density of the test serum to that of the negative control being the maximal. The mean optical density of the negative control obtained from a serum pool from 10 healthy men was equal to 0.22ë0.14. All sera for which the optical density values differed from the negative sera by 4 standard deviations were considered positive.

The specificity of the reaction was confirmed by adsorption of the alloantisera pool by the platelet pool from 35 healthy donors (Fig. 2). 0.3 ml of the serum pool with a high content of anti-HLA antibodies was incubated twice with an equal volume of platelets at room temperature for 2 h. After each incubation the platelets were precipitated for 20 min by centrifuging at 6000 g. Pretreatment of positive sera with platelets was shown to lead to a 70% reduction of the optical density of the reaction.

The MAILA method in various modifications has been widely used in recent years for the detection of anti-HLA antibodies. Our modification is a reliable, highly specific, and sensitive method for the detection of antibodies to class 1 HLA antigens.

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