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ENZYME IMMUNOASSAY — A METHOD OF SEROLOGICAL SURVEY OF MEASLES VACCINATION

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Summary. — Enzyme immunoassay (EIA) for detection of antibodies to measles virus designed in the Moscow Research Institute of Viral Preparations has proved highly sensitive (98%) and specific (100%) as tested in 492 vaccinated children. Comparison of EIA and haemagglutination inhibition (HI) test allowed to determine the cut-off value of the optical density to be equal to 0.1. The serum dilution 1 : 10 was found appropriate for the screening.

Key words: *measles*; *EIA*; *HI*; *postvaccinal immunity*

Successful implementation of the measles eradication projects depends on a number of factors, one of which is the serological surveillance over the immune status of the community to measles. HI is known to be a classical method of epidemiological monitoring of measles. This test helped to study the main regularity of postvaccinal measles immunity (Andzhaparidze *et al.*, 1980; Bolotovsky and Titova, 1981). At the same time, large-scale practical employment of HI is restricted because it needs monkey erythrocytes that are of shortage. EIA has been extensively used in recent years in virological work including measles as it is highly sensitive, specific, and technically simple (Cremer *et al.*, 1985; Neumann *et al.*, 1985).

An economically efficient technology of EIA kit preparation for detection of measles antibodies has been designed in the Research Institute of Viral Preparations of the U.S.S.R. Academy of Medical Science (Vedunova *et al.*, 1986). This EIA system has been shown sensitive and specific in diagnostic use (Vedunova *et al.*, 1986). However, when employed to assay the humoral response in humans vaccinated against measles, it has turned out that under the same performance conditions the interpretation of data yielded false-negative results with the sera revealing low haemagglutination inhibition antibody titres.

The present paper aimed at optimalization of the test conditions employing our EIA system in seroepidemiological studies to assess the immune status of the children vaccinated against measles. The tests were carried out in 492 children aged 6—8 years vaccinated against measles 5 or 6 years ago. Each serum was simultaneously assayed by EIA and HI. EIA was

Table 1. Distribution of EIA and HI titres in the sera of children vaccinated against measles

HI antibody titre	EIA antibody titre														Total
	<10	10	20	40	80	100	200	400	800	1600	3200	6400	12,800	72,900	
<4	12														12
4	3	4	4	1				1							13
8		3	3	5	4	3	4		1						23
16			4	6	1		1	1							13
32					3	2	4	3	2	1					15
64						1	1	1	1	1	2				7
128					1						2	1			4
256										1	3	1	1		6
512												1			1
1024												1			1
2048														1	1
Total	15	7	11	12	9	6	10	6	4	3	7	4	1	1	96

Note. Antibody titres are the reciprocals of limiting dilutions.

Table 2. Comparison of EIA and HI tests in the sera of children vaccinated against measles

Number of sera tested	Number of sera reacting in the tests			
	EIA posit HI posit	EIA negat HI negat	EIA posit HI negat	EIA negat HI posit
396	355 (89.6%)	34 (8.6%)	0	7 (1.8%)

Coincidence of EIA and HI results	98.2% (389/396)
Sensitivity of EIA	98% (355/362)
Specificity of EIA	100%

performed according to a modified procedure described elsewhere (Vedunova *et al.*, 1986). A panel of the following sera was used in each experiment: a serum containing measles antibodies in high and medium titres; a serum with minimal antibody titre (1 : 10) and a serum without antibodies to measles virus. For EIA the sera were tested at 1 : 10 dilution without pretreatment. They were placed into two wells: coated with virus antigen and a control antigen with coated well. The antibody titre of <10 was considered negative, whereas serum-titre of $\geqq 10$ was considered positive. HI was made using a micromethod with 4 haemagglutination units (HU) of measles antigen and 0.5% monkley erythrocyte suspension. The sera were treated with 10% monkey erythrocyte suspension. For standardization of HI a hyperimmune measles serum in a 1 : 512 measles antibody titre was used as working standard. It was kindly supplied by the Tarasevich State Institute of Standardization and Control.

Table 1 shows the results of the titres of antibodies against measles virus as detected by EIA and HI in 96 vaccinated children. As expected, the EIA titres were higher than the HI titres. With increasing serum dilutions the difference between the two tests increased. Thus, for example, within the HI antibody titre range from 1 : 4 to 1 : 16 the EIA titres were 1.5 to 2.5 times higher, whereas among the high HI titres the EIA titres were 10 to 30 times higher. Based on these experiments the starting dilution 1 : 10 was chosen for EIA. This enabled to detect the whole spectrum of antibody titres from the highest to the lowest ones. The cut-off value of optical density (OD) that may vary in different systems was an important factor influencing the sensitivity and specificity of the test. The cut-off OD value for the given assay system was defined as the difference between the ODs as measured against the virus antigen and control antigen ($\triangle$ OD). For determination of a $\triangle$ OD value differentiating between the positive and negative sera, 125 negative sera, i.e. containing no HI, were repeatedly tested. It has been shown that 52% of the sera had a negative $\triangle$ OD, i.e. the OD values against the control antigen were even higher than against the virus antigen. For 48% of sera the $\triangle$ OD ranged from 0.001 to 0.089. The arith-

metic mean of △ OD was 0.028 and thus, allowing for a triple standard deviation, the choice of a cut-off OD value equal to 0.1 was well justified.

Table 2 presents the results of parallel tests in EIA and HI of 396 sera of children vaccinated against measles. It can be seen that EIA and HI results (both the positive and the negative ones) coincided in 98.2% of cases. Seven sera (1.8%) appeared positive in HI at 1 : 4 dilution and negative in EIA at 1 : 10 dilution. Antihaemagglutinin titre of these sera did not exceed 4. The △ OD value ranged from 0.044 to 0.095 in the EIA test of the sera at 1 : 10 dilution. A decrease of the serum dilution tested to 1 : 5 failed to change the EIA results. The sensitivity of the EIA for detection of antibodies to measles virus was 98% and the specificity amounted to 100% which was conform with the data obtained by several investigators at extensive testing with measles EIA systems (Cremer *et al.*, 1985; Neumann *et al.*, 1985) and EIA systems for other viruses (Hornstein *et al.*, 1985; Demmler *et al.*, 1988).

In conclusion, EIA results seem dependent not only on the quality of the conjugates and antigens used for the preparation of immunosorbents, but also on some other factors, such as the dilution of the sera tested and the cut-off OD value of the system. The production design as well as the optimal test conditions and data interpretation criteria allowed the authors to achieve a high specificity and sensitivity of the EIA for the detection of antibodies to measles virus. EIA can be recommended as a highly efficient method for controlling the population immunity level against measles.

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