FOLIA PARASITOLOGICA (PRAHA) 30: 103—106, 1983.

RELATIONSHIP BETWEEN INDIRECT HAEMAGGLUTINATION TEST AND OTHER DIAGNOSTIC TESTS FOR TOXOPLASMOSIS

V. KOZOJED and V. BOZDĚCH

Institute of Parasitology, Czechoslovak Academy of Sciences, Prague, and Institute of Medical Microbiology and Immunology, Faculty of General Medicine, Charles University, Prague

Abstract. The results of indirect haemagglutination test were found to correspond to the results of complement fixation, indirect fluorescence antibody and microprecipitation tests. A possible source of differences in the results is discussed.

The serological diagnosis was first based on the fact that the influenza virus agglutinates the sheep erythrocytes and that the antibodies contained in the serum inhibit this "direct agglutination" (Hirst 1941, McCleland and Hare 1941). After the antigen was bound to the surface of erythrocytes, the agglutination occurs after mixing with the corresponding antibodies (Keogle et al. 1947). In this "indirect" (passive, conditioned) haemagglutination, the adsorption of some protein antigens is much greater if the surface of erythrocytes is changed by some substances, as tannin (Boyden 1951).

In the diagnostics of toxoplasmosis other antigen carriers were also used, as acrylic particles (Siim and Lind 1960), latex (Bozděch and Jíra 1961) or bentonite (Garin and Despeignes 1964). However, the indirect haemagglutination test (IHAT) is usually more sensitive and reliable in the diagnostical practice.

In this paper, the negativity and positivity of IHAT was compared with the negativity and positivity of other tests for toxoplasmosis.

MATERIAL AND METHODS

The dilution of reagents and washing of sensibilized erythrocytes were made with saline with phosphate buffer pH 7.2 (PBS-7.2) of the following composition: 0.15M Na_2HPO_4 380 ml, 0.15M KH_2PO_4 120 ml, 0.15M NaCl 1 000 ml. The erythrocytes were sensibilized with the saline with phosphate buffer pH 6.4 (PBS-6.4) consisting of 0.15M Na_2HPO_4 100 ml, 0.15M KH_2PO_4 210 ml and 0.15M NaCl 310 ml.

The lyophilized, formalinized and tannined sheep erythrocytes used in our experiments were produced by the Institute of Sera and Vaccines, Prague under the name SEVATEST TK-HEM. The antigen was produced by us using the method of Pokorný et al. (1972).

The test was performed in microtitration plates with U-shaped wells.

Takátsy microtitrator with 0.025 ml loops was used for the dilution of sera and dosing of solutions and the method of Lewis and Kessel (1961) was used in further process. Working dilution of antigen in PBS-6.4 was added to the sediment of 1 % erythrocytes. The mixture was incubated for 30 min at 37 °C in water bath and shaken every 10 min. The sensibilized erythrocytes were twice washed in PBS-7.2 with 1 % normal horse or rabbit serum and resuspended in the same solution to the original volume.

A drop of sensibilized erythrocytes was added to a drop of serum diluted by Takátsy microtitrator in a double geometrical titration series (titres 8—16 384). A serum of known titre and negative serum were examined in each series of sera for the control. After shaking for 1 min the plate was left for 2 h at room temperature. The dilutions where the bottom of the well was covered with a homogeneous sediment of agglutinated erythrocytes or where a ring of agglutinated erythrocytes was formed at the

Table 1. Relationship between positivity of indirect haemagglutination test (IHAT) and complement fixation test (CFT) (in percent)

		CFT titre				Total examined
		Negative	8—32	64—128	256 and higher	
IHAT titre	Negative	75.9	22.4	1.4	0.3	694 (= 100 %)
	8—32	37.3	52.3	8.6	1.8	593 (= 100 %)
	64—128	17.9	47.4	27.2	7.5	213 (= 100 %)
	256 and higher	4.4	37.8	44.5	13.3	45 (= 100 %)

Table 2. Relationship between positivity of indirect haemagglutination test (IHAT) and indirect fluorescence antibody test (IFAT) (in percent)

		IFAT titre				Total examined
		Negative	8—32	64—128	256 and higher	
IHAT titre	Negative	57.1	40.9	1.9	0.1	673 (= 100 %)
	8—32	22.7	56.7	16.7	3.9	587 (= 100 %)
	64—128	3.3	38.1	40.5	18.1	210 (= 100 %)
	256 and higher	0.0	29.5	27.3	43.2	44 (= 100 %)

Table 3. Relationship between positivity of indirect haemagglutination test (IHAT) and latex test (LT) (in percent)

		LT titre				Total examined
		Negative	10—40	80—160	320 and higher	
IHAT titre	Negative	61.5	35.9	2.6	0.0	696 (= 100 %)
	8—32	23.9	60.3	14.0	1.8	594 (= 100 %)
	64—128	9.0	46.7	38.6	5.7	210 (= 100 %)
	256 and higher	0.0	29.5	50.0	20.5	44 (= 100 %)

periphery of the sediment were regarded as positive. The dilutions with a conspicuous ring of erythrocytes near the centre of the well or with a compact disc of agglutinated erythrocytes were considered to be negative.

The complement fixation test (CFT) was described elsewhere (Pokorný et al. 1972, Bozděch and Černá 1974), as well as the indirect fluorescence test (IFAT) (Kramář et al. 1964), latex test (LT) (Bozděch 1978) and microprecipitation in agar gel (MPA) (Hübner and Uhlíková 1973). The antigens used for CFT, LT and MPA were produced by the Institute of Sera and Vaccines, Prague, whereas those for IFAT were produced by us after Kramář and Chalupský (1970).

The sera were supplied to our laboratory by the Faculty Hospital 2 with Policlinic and by Infection Department of the Faculty of General Medicine, Bulovka Hospital (headed by Prof .MUDr. K. Kouba, D.Sc.).

RESULTS

The results of IHAT were compared with the results of CFT, IFAT, LT and MPA carried out simultaneously.

Table 1 shows that with increasing positivity of IHAT markedly increases the positivity of CFT in middle (1.4 %—8.6 %—27.2 %—44.5 %) and high titres (0.3 %—1.8 %—7.5 %—13.3 %) and decreases the negativity of CFT (75.9 %—37.3 %—17.9 %—4.4 %).

Table 2 shows that with increasing positivity of IHAT increases the positivity of IFAT in high titres (0.1 %—3.9 %—18.1 %—43.2 %) and partly also in middle titres (1.9 %—16.7 %—40.5 %). With increasing titres of IHAT decrease the negative values of IFAT (57.1 %—22.7 %—3.3 %—0.0 %).

With increasing titres of IHAT increases the occurrence of middle (2.6 %—14.0 %—38.6 %—50.0 %) and high (0.0 %—1.8 %—5.7 %—20.5 %) titres of LT and decreases the occurrence of negative values (61.5 %—23.9 %—9.0 %—0.0 %) (Table 3).

MPA was positive less often (1.7 %) in negative values and in low titres of IHAT (0.6 %) than in middle (7.0 %) and higher (4.1 %) titres (Table 4).

Table 4. Relationship between positivity of indirect haemagglutination test (IHAT) and microprecipitation test (MPA) (in percent)

		MPA results		Total examined
		Negative	Positive	
IHAT titre	Negative	98.3	1.7	289 (= 100 %)
	8—32	99.4	0.6	636 (= 100 %)
	64—128	93.0	7.0	215 (= 100 %)
	256 and higher	95.9	4.1	97 (= 100 %)

DISCUSSION

The differences between the results may be explained by a different onset of the positivity in the test. In the experiments by Jacobs and Lunde (1957) IHAT was positive after 9 days and CFT after 15 days p. i. Kouba et al. (1974) reported a case of laboratory infection where CFT was positive in the titre of 256 two weeks after infection, while at the same time IFAT was positive in the titre of 1 000 and IHAT was

negative. IHAT was positive (in the titre of 1 000) even after 6 months. Due to the different onset of positivity in individual serological tests it can be expected that their results will be identical at a given time. It remains unsolved whether the MPA positivity is always related with the activity of toxoplasmic infection (Hübner and Uhlíková 1969) and to what extent a repeated adding of antigen and serum may become a source of possible errors (Kabat and Mayer 1965).

СРАВНЕНИЕ МЕТОДА НЕПРЯМОЙ ГЕМАГГЛЮТИНАЦИИ С ДРУГИМИ МЕТОДАМИ ДЛЯ ДИАГНОСТИКИ ТОКСОПЛАЗМОЗА

В. Козоед и В. Бездех

Резюме. Обнаружено, что результаты непрямой реакции гемагглютинации отвечают результатам реакции связывания комплемента, непрямой иммунофлуоресценции антител и реакции микропреципитации. Обсуждаются возможные источники различий между этими методами.

REFERENCES

BOYDEN S. V., The adsorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by anti-protein sera. J. exp. Med. 93: 107, 1951.

BOZDĚCH V., Latex reaction with *Toxoplasma* antigen. Folia parasit. (Praha) 25: 109—113, 1978.

—, ČERNÁ Ž., Notes to the technique of complement fixation reaction. Čs. Epidem. 23: 46—52, 1974. (In Czech.)

—, JÍRA J., Latex Agglutinationstest mit dem *Toxoplasma*-antigen. Dtsch. Gesundh. -Wes. 16: 2398—2400, 1961.

GARIN J. P., DESPEIGNES J., Une méthode sérologique de diagnostic de la toxoplasmose. Presse méd. 72: 2317—2321, 1964.

HIRST G. K., The agglutination of red cells by allantoic fluid of chick embryos infected with influenza virus. Science 94: 22, 1941.

HÜBNER J., UHLÍKOVÁ M., Use of the microprecipitation in agar gel method in the diagnosis of toxoplasmosis. J. Hyg. Epidem. Microbiol. Immunol. 13: 514—520, 1969.

—, —, Use of the microprecipitation method in agar gel (MPA) in the diagnostics of toxoplasmosis. III. J. Hyg. Epidem. Microbiol. Immunol. 17: 70—84, 1973.

JACOBS L., LUNDE M. N., A hemagglutination test for toxoplasmosis. J. Parasitol. 43: 308—314, 1957.

KABAT E. A., MAYER M. M., Experimentální imunochemie. (Experimental immunochemistry). Academia, Praha, 602 pp., 1965. (In Czech.)

KEOGLE F. V., NORTH E. A., WARBURTON M. F., Haemagglutinins of the *Haemophilus* group. Nature (Lond.) 160: 63, 1947.

KOUBA K., JÍRA J., HÜBNER J., Toxoplazmóza. Avicenum, Praha, 306 pp., 1974. (In Czech.)

KRAMÁŘ J., ČERNÁ Ž., CHALUPSKÝ J., Immunofluoreszenzreaktionen in der serologischen Diagnostik der Toxoplasmose. Zbl. Bakt. I. Abt. Orig. 193: 523—534, 1964.

—, CHALUPSKÝ J., Some notes to the use of indirect fluorescence reaction in the diagnosis of toxoplasmosis. Čs. Epidem. 19: 286—290, 1970. (In Czech.)

LEWIS W. P., KESSEL J. F., Hemagglutination in the diagnosis of toxoplasmosis and amebiasis. Arch. Ophthal. 66: 471—476, 1961.

McLELAND L. C., HARE A., The adsorption of influenza virus by red cells and a new in vitro method of measuring antibodies for influenza virus. Canad. publ. Hlth. J. 32: 530, 1941.

POKORNÝ J., FRÜHBAUER Z., ČUŘÍK B., ZÁSTĚRA M., A tween-ether preparation of *Toxoplasma gondii* antigen for the complement fixation test. Bull. W.H.O. 46: 127—129, 1972.

SIIM J. C., LIND K., A *Toxoplasma* flocculation test. Acta Path. Microbiol. Scand. 50: 445—446, 1960.

Received 2 June 1981.
Translated by: M. Dašková

V. K., Parasitologický ústav ČSAV, Flemingovo n. 2, 166 32 Praha 6, ČSSR