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INDIRECT SOLID-PHASE IMMUNOSORBENT ASSAY FOR DETECTION OF ARENAVIRUS ANTIGENS AND ANTIBODIES

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Summary. — Indirect enzyme-linked immunosorbent assay (ELISA) and solid phase radioimmunoassay (SPRIA) using either anti-human or anti-mouse IgG labelled with horseradish peroxidase and ^{125}I, respectively, were developed for the detection of Junin, Machupo, Tacaribe, Amapari, Tamiami, Lassa and LCM arenaviruses. Both methods allow high sensitivity detection of arenavirus antigens and antibodies.

Key words: arenavirus; solid phase radioimmunoassay; enzyme-linked immunosorbent assay

Introduction

The current search for highly sensitive and rapid detection of arenavirus antigens and antibodies is aimed at improved laboratory diagnostics. We have recently reported on the application of direct ELISA and SPRIA in detection of arenavirus antigens and of blocking ELISA and SPRIA in detection of arenavirus antibodies (Ivanov *et al.*, 1981*a*, *b*; Rezapkin *et al.*, 1981). Direct ELISA and SPRIA, however, have certain shortcomings: the conjugate should be prepared separately to each virus tested and the blocking test for detection of antibodies is of comparatively low sensitivity, i.e. only 32 times as high as the CF test (Ivanov *et al.*, 1981*a*, *b*; Rezapkin *et al.*, 1981). Therefore, we decided to explore the optimal conditions for indirect ELISA and SPRIA in detection of arenaviruses.

Materials and Methods

Viruses and antigens. Investigations were performed using the following arenaviruses: Amapari, strain An 7063; Junin, strain HJ 15950; LCM, strain Ca 1371; Lassa, strain LP; Machupo, strain c-80/81; Tacaribe, strain Tr 11573; Tamiami, strain CDCW 10777.

Due to the safety of the personnel, Machupo and Junin antigens were formalin inactivated (48 hr at room temperature, formalin diluted 1 : 1000). Inactivated and lyophilized Lassa antigen was purchased from CDC (Atlanta, U.S.A.). The antigens used were blood and 10% suspensions of infected organs of newborn white mice (brain, liver, spleen, kidney), culture fluids of infected Vero cells, blood and sera of patients with LCM infection and with neurological syndromes. Suspensions of organs and blood of non-infected newborn white mice as well as non-infected culture fluids of Vero cells were used as controls.

Antibodies. Immune ascitic fluids (IAFs) of infected adult white mice were used without any pretreatment. IgG for precoating of the plates was isolated from guinea pig immune sera and from immune ascitic fluids of infected white mice (Ivanov *et al.*, 1981). Rabbit anti-mouse IgG ("ICN") and goat anti-human IgG ("Miles") were labelled either with horseradish peroxidase or with ^{125}I, respectively.

The titre of antispecies IgG was at least 1 : 32 in agar gel diffusion and precipitation tests.

Conjugates. Anti-mouse and anti-human IgG horseradish peroxidase ("Sigma", Type VI, RZ = 3.03) conjugates were prepared according to Nakane and Kawaoi (1974) as modified by Mathiesen *et al.* (1978); the ^{125}I (18.7 MBq Na ^{125}I/10 µl, Amersham) labelling was performed by the method of Greenwood and Hunter (1962) and Charles and Parker (1976).

Assays. The indirect ELISA test was based on the direct "double sandwich" method (Ivanov *et al.*, 1981*a*). Polyvinyl (or polystyrene) microtiter plates (Flow, Cooke) were precoated with anti-virus IgG from guinea pig immune serum (anti-mouse conjugate) or with IgG from either IAF or guinea pig immune serum (anti-human conjugate). Seventy-five µl of the appropriate anti-virus IgG, containing 100 µg/ml of protein in 0.1 mol/l phosphate-buffered saline (PBS) pH 7.4, were placed in the bottom of each microtiter well (Mathiesen *et al.*, 1978; Ivanov *et al.*, 1981*b*) and incubated at 37 °C for 3 hr. The unadsorbed globulin was removed, the wells were washed three times with PBS containing 0.5% Tween-20. To each well 200 µl of 1% bovine serum albumin (BSA) in PBS was added and incubated again for 30 min at 37 °C. The unadsorbed BSA was removed, the wells were washed three times and 50 µl of tested or standard antigen diluted in PBS were added. The plate was incubated at 4 °C overnight or 1 hr at 37 °C, the unadsorbed viral antigen was removed, and after six times washing, the wells were filled with 50 µl of tested serum and IAF, respectively, or standard serum (IAF). The plate was incubated at 37 °C for 1 hr. Unbound serum or IAF was removed and the wells were washed five times. Then to each well 50 ul of anti-human or anti-mouse IgG-peroxidase conjugate diluted in PBS that contained 50% foetal calf serum was added, dilutions of 1 : 1280—1 : 2560 were found optimal in preliminary titrations. The plates were incubated at 37 °C for 1 hr, the unbound conjugate was removed, and the wells were washed six times. Freshly prepared solution of ortho-phenylenediamine (0.4 mg/ml)with 0.006% peroxidase in citrate buffer (pH 5.0) prepared by mixing 98.6 parts of 0.1 mol/l citric acid and 101.4 parts of 0.2 mol/l Na_2HPO_4 and 100 µl of this substrate were added to each well. After 50 min of incubation in the dark at room temperature, the reaction was stopped by adding of 50 µl of 2 mol/l H_2SO_4. The results was measured in a double-beam spectrophotometer (Perkin-Elmer) at 492 nm; in the ratio of sample extinction to control extinction (P/N) of ≥ 2.1 was considered positive (Mathiesen *et al.*, 1978).

Indirect SPRIA of antigens and antibodies was performed in the same manner as indirect ELISA with except that soft polyvinyl plates (Flow) were used, washing was performed with 2% BSA in PBS, and BSA was not added after precoating. The anti-human and anti-mouse IgG-^{125}I conjugates were diluted with 1% BSA in PBS to obtain 50 000—100 000 counts per min in 50 µl and incubated for 1.5 hr at 37 °C. All solutions contained sodium azide (1 : 4000). Dried wells were separated and their radioactivity was counted in a gamma-counter (IN-PG 4000). The result was considered positive if the ratio of sample radioactivity to control radioactivity (P/N) was ≥ 2.1.

Indirect immunofluorescence for the detection of antigens and antibodies was performed by the method of Wulff *et al.* (1978).

Results

High sensitivity and specificity of indirect ELISA and SPRIA depend on optimal doses (dilutions) of standard antigen (for the detection of antibodies) and standard IAF (for the detection of antigens). Fig. 1 shows the results of cross-titration of the LCM virus antigen and IAF to LCM virus. It can be seen that the titres of the LCM virus antigen (CF titre 64) were 32 000 (SPRIA) and 16 000 (ELISA) against standard IAF (CF titre 32) in dilutions 1 : 256—1 : 1024. Low dilutions of standard IAF (1 : 8) resulted in a considerable nonspecific binding with normal antigen (data not shown)

thus giving negative results. This nonspecific background was reduced and reached a plateau when standard IAF was used in dilutions of 1 : 256 to 1 : 1024. For the detection of antibodies (Fig. 1) the standard antigen could be used in a wide range of dilutions (from 1 : 8 to 1 : 256) giving antibody titres 128 000 (SPRIA) and 64 000 (ELISA), respectively. The use of low dilutions of the standard antigen (from 1 : 8 to 1 : 64) was observed to result in an increased nonspecific binding with normal ascitic fluid (control) leading to an insignificant reduction of P/N ratio but did not affect the range of antibody titres. Therefore, in subsequent studies standard IAFs (CF titres 16—64) were used in dilutions of 1 : 200—1 : 1000, and standard antigens (CF titres 16—256) in dilutions of 1 : 50—1 : 200. Such dilutions of standard IAFs and antigens allowed maximal elimination of nonspecific background. For clear-cut interpretation of the results as well as for evaluation of the quality of ingredients used in the test, it is necessary to use control antigens and non-immune ascitic fluids or sera in both indirect ELISA and SPRIA. P/N ratio is calculated for the control with the maximum background.

Kinetics of appearence of LCM virus in blood and organs after inoculation of mice (Table 1) have shown that ELISA and SPRIA detect antigens earlier than CF test. The main quantity of antigen was found in brain and liver. Table 2 shows data on detection of antibodies to various arenaviruses.

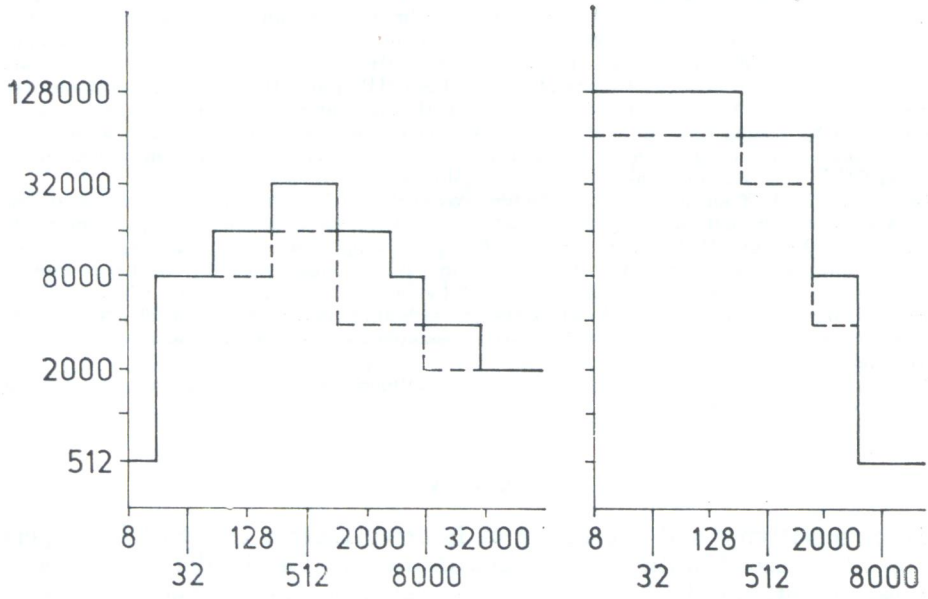

Fig. 1.
Cross-titration of standard LCM antigen and IAF by indirect ELISA and SPRIA
In the left — abscissa: IAF dilution reciprocals; ordinate: antigen titre (reciprocals)
In the right — abscissa: antigen dilution reciprocals; ordinate: titre of IAF (reciprocals)
Solid lines: SPRIA; interrupted lines: ELISA

Table 1. Comparative sensitivity of ELISA, SPRIA and CFT for LCM antigen detection in organs of infected suckling mice

Sample tested	Method	Antigen detected on day						
		1	2	3	4	5	7	8
Blood	CFT	0	0	0	2[b]	2	2	2
	ELISA, SPRIA	0	4	16	64	64	256	256
Brain[a])	CFT	0	2	2	8	8	16	16
	ELISA, SPRIA	8	128	256	1024	1024	2048	2048
Spleen[a])	CFT	0	0	0	2	4	16	16
	ELISA, SPRIA	0	32	128	512	512	1024	1024
Liver[a])	CFT	0	4	16	32	64	64	64
	ELISA, SPRIA	2	512	1024	2048	4096	8192	8192
Kidney[a])	CFT	0	0	0	0	4	8	8
	ELISA, SPRIA	0	2	16	32	256	512	512

[a]) 10% suspension
[b]) dilution reciprocals

As can be seen from Table 2, indirect ELISA and SPRIA strongly exceed sensitivity of traditional serological tests — CF and immunofluorescence, which is very important for investigation of late sera (convalescent T and A sera were tested 8 years after the onset of Bolivian haemorrhagic fever). The results of comparative sensitivity of direct ELISA and SPRIA (blocking test) and indirect methods for detection of antibodies are shown in Table 3. Sensitivity of direct and indirect ELISA and SPRIA for detection of antigens is practically identical (data not shown).

Discussion

Application of the indirect ELISA and SPRIA for the detection of arenavirus antigens and antibodies and their comparison with other serological tests have been described herein for the first time. The methods showed

Table 2. Detection of arenavirus antibodies by indirect ELISA, SPRIA, CF test and immunofluorescence

Method[a])	Titre							
	IAF						Convalescent sera from BHF patients[b])	
	LCM	Tacaribe	Junin	Machupo	Amapari	Tamiami	T.	A.
SPRIA	131 072*	32 768	65 536	32 768	16 384	32 768	1 280	640
ELISA	65 536	16 384	32 768	16 384	16 384	32 768	640	320
CF	64	64	32	16	32	16	<4	<4
IIF	256	1 024	256	128	256	128	4	8

* Dilution reciprocals
[a]) In indirect ELISA and SPRIA materials were tested from the dilutions of 1 : 8, in CF and IIF — from 1 : 4. IIF = in direct immunofluorescence.
[b]) Bolivian haemorrhagic fever sera were tested with Machupo virus antigen.

Table 3. Comparative sensitivity of direct and indirect ELISA and SPRIA for detection of antibodies to arenaviruses

IAF to viruses	Titres of homologous antibodies			
	Direct methods (blocking)		Indirect methods	
	SPRIA	ELISA	SPRIA	ELISA
LCM	2 048*	2 048	13 1072	65 536
Tacaribe	1 024	1 024	1 6384	16 384
Junin	1 024	2 048	3 2768	32 768
Machupo	2 048	1 024	1 6384	16 384
Amapari	1 024	512	1 6384	16 384

* Dilution reciprocals

practically identical sensitivity which exceeded that of CF test 100—200 times (for the detection of antigens) and 500—2000 times (for the detection of antibodies). The methods were also highly specific and reproducible. Important advantage of indirect ELISA and SPRIA was the possibility to detect arenavirus antigens directly in human and animal blood without additional enrichment by isolation attempts or cell cultures.

Comparison of direct ELISA and SPRIA (Ivanov *et al.*, 1981*a*, *b*; Rezapkin *et al.*, 1981) with indirect variants of the methods for the detection of antigens shows a higher sensitivity of the latter. The sensitivity of indirect methods for the detection of antibodies is 10—50 times higher than that of the blocking test. Moreover, indirect methods allow the detection of arenavirus antigens and antibodies using a single antispecies conjugate. Summing up, these advantages of indirect ELISA and SPRIA render them useful for investigations of theoretical and applied nature.

References

Casey, H. (1965): Standardized diagnostic complement fixation method and adaptation to microtest. *Publ. Hlth. Monogr.* **74**, 1—31.

Charles, W., and Parker (1976): *Radioimmunoassay of biologically active compounds.* Prentice-Hall Inc., Englewood Cleffs, New Jersey, U.S.A. pp. 64—111.

Donets, M. A., Rezapkin, G. V., Ivanov, A. P., and Tkachenko, E. A. (1982): Immunosorbent assays for diagnosis of Crimean-Congo haemorrhagic fever. *Am. J. trop. Med. Hyg.* **31**, 156—162.

Greenwood, F. C., and Hunter, W. M. (1962): Preparation of iodine-131-labelled human growth hormone of high specific activity. *Nature (Lond.)* **194**, 495.

Ivanov, A. P., Bashkirtsev, V. N., and Tkachenko, E. A. (1981*a*): Immunoenzyme method in diagnostics of arenavirus infections (in Russian). *Vop. Virus.* **26**, 200—203.

Ivanov, A. P., Bashkirtsev, V. N., and Tkachenko, E. A. (1981*b*): Enzyme-linked immunosorbent assay for detection of arenaviruses. *Arch. Virol.* **67**, 71—74.

Mathiesen, L. S., Feinstone, S. M., Wong, D. C., Skinhoe, J. P., and Purcell, R. H. (1978): Enzyme-linked immunosorbent assay for detection of hepatitis A antigen in sera: comparison with solid-phase radioimmunoassay, immune electron microscopy and immune adherence-hemagglutination assay. *J. clin. Microbiol.* **7**, 184—193.

Nakane, P. K., and Kawaoi, A. (1974): Peroxidase-labelled antibody: a new method of conjugation. *J. Histochem. Cytochem.* **22**, 1084—1090.

Ouchterlony, O. (1958): Diffusion in gel methods for immunological analysis. *Progr. Allergy* 1—78.
Rezapkin, G. V., Tkachenko, E. A., Ivanov, A. P., Bashkirtsev, V. N., and Dzagurova, T. K. (1981): Determination of arenavirus antigens and antibodies by solid phase radioimmunoanalysis (in Russian). *Vop. Virus.* **26**, 459—462.
Trent, D. W., Harvey, C. L., Qureshi, A., and Le Staurgeon, D. (1976): Solid-phase radioimmunoassay for antibodies to flavivirus structural and nonstructural proteins. *Infect. Immun.* **13**, 1325—1333.
Wulff, H., Lange, J., and Webb, P. A. (1978): Interrelationship among arenoviruses measured by indirect immunofluorescence. *Intervirology* **9**, 344—350.