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Development and application of ELISA for the detection of IgG antibodies to lymphocytic choriomeningitis virus

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Summary. – Lymphocytic choriomeningitis virus (LCMV) is a neglected human pathogen, which can cause severe illnesses in humans. The most vulnerable are the human foetus and immunosuppressed individuals. Since there is no commercially available enzyme-linked immunosorbent assay (ELISA) for the diagnosis of anti-LCMV antibodies in human sera, we developed a sandwich ELISA method detecting anti-nucleoprotein IgG antibodies, using a specific monoclonal anti-nucleoprotein antibody and cells persistently infected with LCMV strain MX as antigen. In the present study we show standardization of this ELISA protocol, determination of its clinical specificity and sensitivity and its application on 30 clinical samples from multiorgan donors. Comparison of these results to the indirect immunofluorescence antibody test (IFA) demonstrates that ELISA is more sensitive. The developed ELISA assay provides a fast, simple and efficient tool for the clinical detection of anti-nucleoprotein antibodies in human sera.

Keywords: lymphocytic choriomeningitis virus; ELISA; human serum; IgG antibodies

Introduction

Lymphocytic choriomeningitis virus, a prototype arenavirus, is a rodent-borne pathogen carried mostly by house mouse (*Mus musculus*), but it has also been detected in other rodent species, such as hamsters, rats, and guinea pigs. Persistently infected rodents shed the virus in urine, faeces, milk, saliva, and semen (Jamieson *et al*., 2006). Humans become infected through the contact with contaminated material or by inhalation of aerosolized viral particles. In immunocompetent adults, infections are mostly asymptomatic or mild, with flu-like symptoms, but diseases of the CNS, such as aseptic meningitis, can also occur (Barton and Mets, 2001). In contrast to the self-limited acquired postnatal LCMV infection, prenatal infection leads to an increased risk of spontaneous abortion, severe CNS defects (hydrocephalus, microcephaly), and chorioretinitis of the foetus (Bonthius *et al*., 2007). Moreover, recently reported transmissions of LCMV through infected solid organ transplants revealed a risk to immunocompromised individuals. Six clusters of organ transplant-associated LCMV and LCMV-like arenavirus transmissions have been documented in the USA and Australia before April 2013. Of 24 recipients (including kidney, liver, lung, and cornea recipients), 15 died of multisystem organ failure. Survivors of these infections were treated with oral ribavirin and reduction of immunosuppressive therapy. Interestingly, LCMV could not always be detected in donors by RT-PCR or serology testing (Fischer *et al*., 2006; CDC, 2008; Palacios *et al*., 2008; MacNeil *et al*., 2012; Schafer *et al*., 2014). The most commonly used serological tests for detec-

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Abbreviations: Ab = antibody; ELISA = enzyme-linked immunosorbent assay; IFA = indirect immunofluorescence antibody test; LCMV = lymphocytic choriomeningitis virus; NP = nucleoprotein of LCMV

tion of specific anti-LCMV IgM or IgG antibodies are ELISA and indirect immunofluorescence assay (IFA) (OIE, 2006). Complement fixation test is insensitive and should not be used (Mets *et al.*, 2000). PCR can be utilized for the detection of viral RNA in the serum or cerebrospinal fluid (OIE, 2006). In terms of congenital infection, several authors have noted that LCMV is an underreported and underdiagnosed teratogen, whose true incidence is not defined (Jamieson *et al.*, 2006). More than 70 cases of children with congenital LCMV infection have been reported worldwide (Barton *et al.*, 2002; Bonthius *et al.*, 2007). Serological studies indicate that approximately 5% of the human population in the United States has LCMV antibodies (Park *et al.*, 1997a). In a study from an inland city in Argentina, an overall LCMV seroprevalence was 3.3% (Riera *et al.*, 2005). A seropositivity rate of 4% was detected among inhabitants of Nova Scotia (Marrie and Saron, 1998), 1.7% in the Community of Madrid in Spain (Lledó *et al.*, 2003), 3.5% in the inner city of Birmingham (Park *et al.*, 1997b). The prevalence of LCMV antibodies on the Croatian island of Vir was found to be 36% (detected by IFA) and 13% (by complement fixation) (Dobec *et al.*, 2006). The study from 1973 proved that 9.1% of the rural population in southern Germany had neutralizing antibodies (Ackermann, 1973). Of the serum samples of nearly 500 forestry workers from Trentino (Italy) tested by IFA, 12 (2.5%) were positive for specific LCMV IgG (Kallio-Kokko *et al.*, 2006). Immunoprecipitation analysis of 56 human sera in Slovakia revealed 37.5% seropositivity for anti-nucleoprotein (anti-NP) IgG (Reiserová *et al.*, 1999).

Since there is no commercially available ELISA kit for the detection of LCMV-specific antibodies in human sera, we developed a sensitive method using an anti-NP monoclonal antibody and cells persistently infected with the LCM virus. In this paper, we outline this ELISA protocol, determine its clinical sensitivity and specificity, and describe its application for the detection of LCMV IgG antibody seroprevalence in a group of multiorgan donors in comparison to the immunofluorescence antibody assay.

Materials and Methods

Cell culture and virus. BHK21 cells were infected with lymphocytic choriomeningitis virus strain MX (designated BHK/MX) by filtered cell free extract as described earlier (van der Zeijst *et al.*, 1983). Noninfected BHK21 cells cultured in parallel were used as control. The cells were grown in Dulbecco modified Eagle medium (DMEM) with 10% FCS, 2 mmol/l L-glutamine, and 160 μg/ml gentamicin and maintained at 37°C in a humidified 5% CO_2 atmosphere.

Collection of samples. Sera samples were collected by MVDr. Ján Rosocha, CSc. from the Faculty of Medicine, Pavol Jozef Šafárik University and Louis Pasteur University Hospital Associated Tissue Bank in Košice and by MUDr. Ľuboslav Beňa, PhD. from the Department of Transplantation of the LP UH in Košice, Slovakia. Sera used for the determination of the cut-off value were collected by MUDr. Daniel Kuba, PhD. from the National Transplantation Organization, Slovakia. Anti-LCMV antibody-positive KH serum from a mammary carcinoma patient was described previously (Závada *et al.*, 1972).

Antibodies. Mouse monoclonal antibodies M16 and M87 detect different epitopes of LCMV NP (Pastorekova *et al.*, 1992; Tomaskova *et al.*, 2011). M16 was labelled with Alexa Fluor 488 using Molecular Probes' Alexa Fluor® 488 antibody labelling kit (Invitrogen, UK) according to the manufacturer's instructions. Secondary antibodies used were goat anti-human IgG (Fc-specific)-peroxidase antibody, goat anti-mouse IgG (Fc-specific)-peroxidase antibody (both from Sigma, USA) and goat anti-human IgG (H+L)/Alexa Fluor 568 (Invitrogen).

Determination of nucleoprotein concentration in BHK/MX protein extracts. For protein extraction, BHK/MX and BHK21 cells were lysed in ice-cold lysis buffer (50 mmol/l Tris pH 7.5, 150 mmol/l NaCl, 1% Triton X-100, 0.1% sodium deoxycholate) containing inhibitors of proteases (Roche Applied Science, Germany). Antigen (NP of LCMV) concentration was set indirectly by the ELISA method where 96-well high binding plate (Greiner Bio-One, Austria) was coated with BHK/MX and BHK21 protein extracts. After incubation at 37°C overnight, the plates were washed three times with 0.1% Tween 20 in PBS (Tween-PBS). Then, each well of the plates was blocked for 1.5 hr with 10% nonfat dry milk in 0.1% Tween 20 in PBS (200 μl per well) and washed 3 times with Tween-PBS. After this step, plates were incubated for 1 hr with purified M87 antibody in blocking buffer at concentration 250 ng/well. The plates were then washed and incubated for 1 hr with goat anti-mouse IgG (Fc-specific)-peroxidase antibody (Sigma) diluted 1:10,000 in blocking buffer. Peroxidase activity was detected by colorimetric reaction using o-Phenylenediamine, which was stopped by 50 μl of 1 mol/l H_2SO_4. Optical density (OD) was measured at 492 nm by the Synergy HT reader (BioTech Instruments, USA). Adjusted optical density was calculated by subtracting the OD of the negative antigen-coated wells and was set as OD_{NP}.

Enzyme-linked immunosorbent assay with human sera. Detection of nucleoprotein specific serum antibodies was carried out by sandwich ELISA. To obtain the best signal to noise ratio, different protocols were used. The maximum differences in optical density values between positive and negative sera were achieved by following this protocol: 96-well high binding plates were coated with NP-specific monoclonal antibody M87 at the final concentration of 250 ng per well diluted in 0.05 mol/l sodium carbonate-bicarbonate buffer (pH 9.6). After incubation at 4°C overnight, the plates were washed three times with 0.1% Tween 20 in PBS. Then, each well of the plates was blocked for 1.5 hr with 200 μl of 10% milk in Tween PBS and washed 3 times. Subsequently, plates were incubated for 1 hr with protein extracts (BHK/MX and BHK21) in blocking buffer. The adjusted optical density of nucleoprotein in BHK/MX protein extracts set by the ELISA method described above was 0.36. The

plates were then washed and incubated with human serum samples diluted 1:40 in PBS in a total volume of 50 µl per well. In the case of determining anti-NP antibody titers, sera were serially diluted in PBS. Each serum was analysed in duplicates. After 1 hr incubation, the plates were washed and subsequently incubated for 45 min with goat anti-human IgG (Fc-specific)-peroxidase Ab (Sigma) diluted 1:35,000 in blocking buffer. Peroxidase activity was detected by colorimetric reaction using o-Phenylenediamine, which was stopped by 50 µl of 1 mol/l H_2SO_4. Optical density was measured at 492 nm by the Synergy HT reader. Adjusted OD was calculated by subtracting the OD of the negative antigen-coated wells.

Cut-off value determination. To find suitable internal positive and negative controls, human sera were analysed by immunoprecipitation and subsequent immunoblotting with NP-specific monoclonal antibody (described below). 25 sera, which had been proved negative with the immunoprecipitation test, were subsequently analysed in ELISA and the adjusted OD values obtained were then expressed as percent positivity (PP) of the average of 5 internal positive controls (selected based on the immunoprecipitation results). PP was calculated as follows: the mean of the adjusted OD value from duplicate samples divided by the mean value of the adjusted OD values of the five positive controls multiplied by 100. The ELISA cut-off value, which served as the threshold between positive and negative serum samples, was determined as the mean PP value obtained with these 25 samples plus three standard deviations.

Indirect detection of anti-NP antibodies in human sera by immunoprecipitation. BHK/MX and BHK lysates (200 µg of proteins per sample) were precleared with 60 µl of 50% suspension of Protein G-Sepharose (PGS; GE Healthcare, Sweden). Eight µl of human serum were incubated with 192 µl of 10% FCS in PBS for 1 hr at room temperature. The mixture was further added to precleared cell lysates and immunocomplexes were allowed to form on rotating shaker at 4°C overnight. Immunocomplexes were then incubated with 80 µl of 50% suspension of Protein G-Sepharose in PBS for 3 hr at 4°C. Beads were washed six times with PBS, immunoprecipitated proteins were subjected to SDS-PAGE and transferred onto a polyvinylidene difluoride membrane (Immobilon, Millipore, USA). Membranes were probed with specific anti-NP monoclonal antibody M87 in medium, washed with washing buffer (0.2% Nonidet P-40 in PBS), and finally incubated with secondary goat anti-mouse-peroxidase Ab for 1 hr. After an additional washing step, immunoblots were visualised using the ECL detection system.

Indirect immunofluorescence antibody test. To prepare antigen for the IFA, mixture of BHK/MX and BHK21 (1:1) cells were grown on glass coverslips. After removing the medium and washing with PBS, the cells were fixed with ice-cold methanol at -20°C for 5 min. Nonspecific binding was blocked by incubation with 3% bovine serum albumin (BSA) in PBS for 1 hr at room temperature. Subsequently, the cells were incubated with human serum diluted 1:40 in 1% BSA for 1 hr at room temperature. After washing five times with 0.1% Tween 20 for 5 min, the cells were incubated with anti-human/Alexa Fluor 568 secondary antibody and M16/Alexa Fluor 488 antibody (diluted 1:1,700 and 1:190 in 1% BSA respectively) for 1 hr at 37°C. After washing, cells were mounted on slides with Fluoroshield mounting medium with DAPI (Abcam, UK) and analysed by confocal microscope LSM 510 META (Zeiss, Germany).

Calculations of clinical sensitivity and specificity of ELISA and IFA. As already mentioned, there is no commercially available and routinely used ELISA test for the detection of LCMV-specific antibodies, which could be considered as a "gold standard". Therefore we decided to validate our new ELISA test and standard IFA against immunoprecipitation, which in our hands served as the "gold standard" method.

Clinical sensitivity was calculated as: [number of true positive sera/(number of true positive sera+number of false negative sera)]*100%.

Clinical specificity was calculated as: [number of true negative sera/(number of true negative sera+number of false negative sera)]*100%.

Results

ELISA protocol optimization

To obtain maximum differences in the OD values of the positive and negative sera, different ELISA formats and various titration protocols for the antibody and antigen were analysed. The best results were achieved when instead of coating the plates with NP containing cell lysate, the monoclonal NP-specific Ab M87 was used (Fig. 1a). To determine the appropriate concentration of purified M87 antibody and NP containing BHK/MX protein extracts pre-

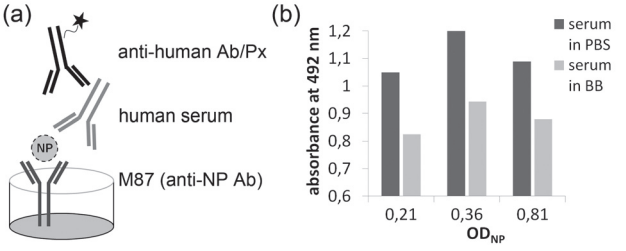

Fig. 1

Development of ELISA

(a) ELISA scheme. Wells were coated with monoclonal antibody M87 (specific to nucleoprotein (NP) of LCMV). Nucleoprotein present in BHK/MX cell lysates served as antigen. Anti-nucleoprotein IgG antibodies in human sera were detected by peroxidase-labelled anti-human antibody. (b) Anti-NP IgG-positive human serum KH ELISA results. The wells were coated with 250 ng of purified M87 antibody, followed by incubation with BHK/MX protein extracts with ODs corresponding to NP concentrations of 0.21, 0.36 and 0.81. BHK21 and antigen-positive BHK/MX lysate concentrations were equivalent. KH serum was diluted to 1:40 in PBS or in blocking buffer (BB; 10% milk in 0.2% Tween-20 in PBS). Adjusted optical densities were calculated by subtraction of the appropriate unspecific background at 492 nm.

pared from BHK21 cells persistently infected with LCMV strain MX, we tested four different concentrations of M87 (200, 250, 300, and 350 ng/well) diluted in carbonate-bicarbonate buffer and three concentrations of BHK/MX protein extracts, in which ODs corresponding to the amount of NP (OD_{NP}) were 0.21, 0.36 and 0.81. Concentrations of BHK21 protein extracts coincided with concentrations of antigen-positive BHK/MX lysates. Positive control human serum KH was diluted in blocking buffer (10% milk in Tween-PBS) or in PBS and tested in duplicate for each Ab-Ag combination. Comparison of blocking buffers (10% milk in 0.1% Tween 20 in PBS and 10% FCS in Tween-PBS) showed that 10% milk was as effective as 10% FCS. Analysis of anti-human-peroxidase secondary antibody incubation times proved that shortened incubation (45 min instead of 1 hr) led to reduced background signal. The adjusted OD was calculated by subtracting the OD of the BHK21-protein extract-coated wells from that of the corresponding BHK/MX wells. The observed optical densities (Fig. 1b) showed that the best signal to noise ratio was provided with OD_{NP} = 0.36 and serum diluted in PBS. Since differences between M87 concentrations did not significantly alter the results (data not shown), we decided to use 250 ng of antibody per well in the subsequent assays.

Determination of cut-off value

To differentiate between positive and negative sera in ELISA, we had to determine the cut-off value of absorbance. For this purpose we decided to use another anti-NP IgG antibody detecting method – immunoprecipitation. IgG antibodies in human sera (8 µl) were bound to protein G and after incubation with NP-containing BHK/MX lysate, nucleoprotein was detected by Western blot using specific monoclonal antibody M87. We thus indirectly confirmed the presence of anti-NP IgGs in the sera. Based on the immunoprecipitation results (Fig. 2), we selected 25 negative and 5 positive sera, which we used for the determination of the ELISA cut-off value that served as the threshold between positive and negative serum samples. The OD average value which resulted from ELISA testing of negative sera was 0.009. The average of adjusted ODs of 5 internal positive controls was 1.028. Subsequently, values were changed to percent positivity (PP). The cut-off value was determined as the mean percent positivity obtained with 25 negative samples plus three standard deviations. Using this method, the cut-off value was determined to be 4.28%, which represents OD at 0.044 and was used for subsequent ELISA analyses.

Moreover, we titrated all 5 sera, which were used as positive controls in the cut-off determination and found that anti-NP IgG titers in these sera were 800, 1,600, 5,000, 10,000, and 15,000.

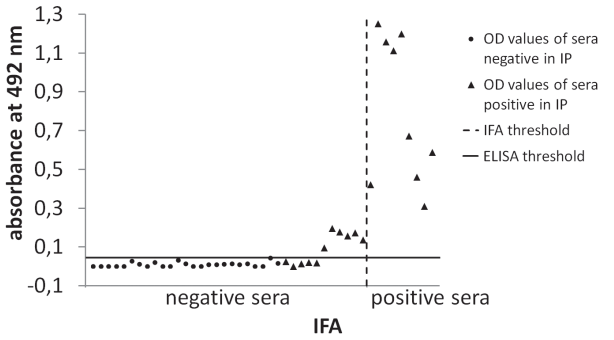

Fig. 3

Testing of 45 sera by ELISA, IFA and "gold standard" immunoprecipitation

Human sera (diluted 1:40 in PBS) were tested by the developed ELISA, standard IFA and 8 µl of each serum were analysed also by immunoprecipitation (IP). Threshold OD value for ELISA was 0.044. The results were used for clinical specificity and sensitivity calculations of the ELISA and IFA methods (Table 1).

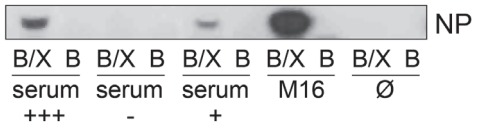

Fig. 2

Immunoprecipitation with human sera

BHK/MX (B/X) and BHK21 (B) cell lysates were incubated with human sera, followed by the binding of the immunocomplexes to Protein G-Sepharose (PGS). Subsequently, the presence of anti-NP IgG antibodies was detected with anti-NP monoclonal antibody M87 by Western blot. Cell lysates incubated with anti-NP monoclonal antibody M16 and PGS and cell lysates incubated only with PGS served as controls. Using this method, we were able to differentiate positive (strongly positive serum +++, weakly positive serum +) and negative (–) sera.

Table 1. Calculation of clinical sensitivity and specificity of ELISA and IFA with immunoprecipitation as "gold standard" (20 sera positive and 25 negative)

Clinical sensitivity			
ELISA	true positive	15	[15/(15+5)]*100 = **75%**
	false negative	5	
IFA	true positive	9	[9/(9+11)]*100 = **45%**
	false negative	11	
Clinical specificity			
ELISA	false positive	0	[25/(25+0)]*100 = **100%**
	true negative	25	
IFA	false positive	0	[25/(25+0)]*100 = **100%**
	true negative	25	

Determination of clinical ELISA sensitivity and specificity and comparison with IFA

After optimization of the ELISA method, we determined its clinical sensitivity and specificity. For this purpose, 25 negative and 20 positive sera (based on the immunoprecipitation results) were tested by a new ELISA test. To compare the developed ELISA to another standard method, we analysed the same sera with IFA (Fig. 3). As shown in Table 1, clinical sensitivity of our ELISA is 75%, since we were able to detect 15 positive sera out of 20 and clinical specificity is 100%, since we detected as negative all sera, which were found to be negative by immunoprecipitation. On the other hand, clinical sensitivity of IFA is 45% (we detected 9 sera as positive out of 20) and specificity is 100%.

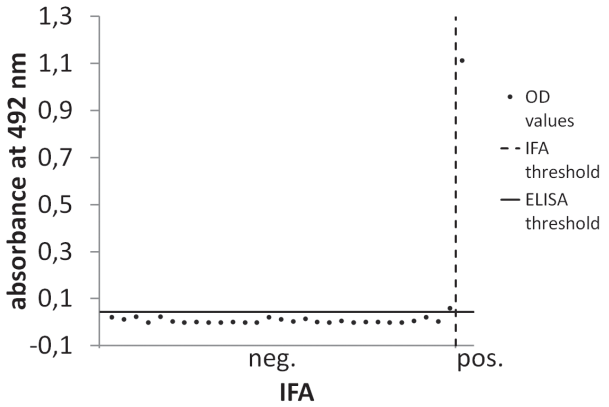

Fig. 5

Testing of multiorgan donor sera
30 sera were analysed by the newly-established ELISA and standard IFA. Threshold OD value for ELISA was 0.044. Results were used for the calculation of the total coincidence rate of the two tests.

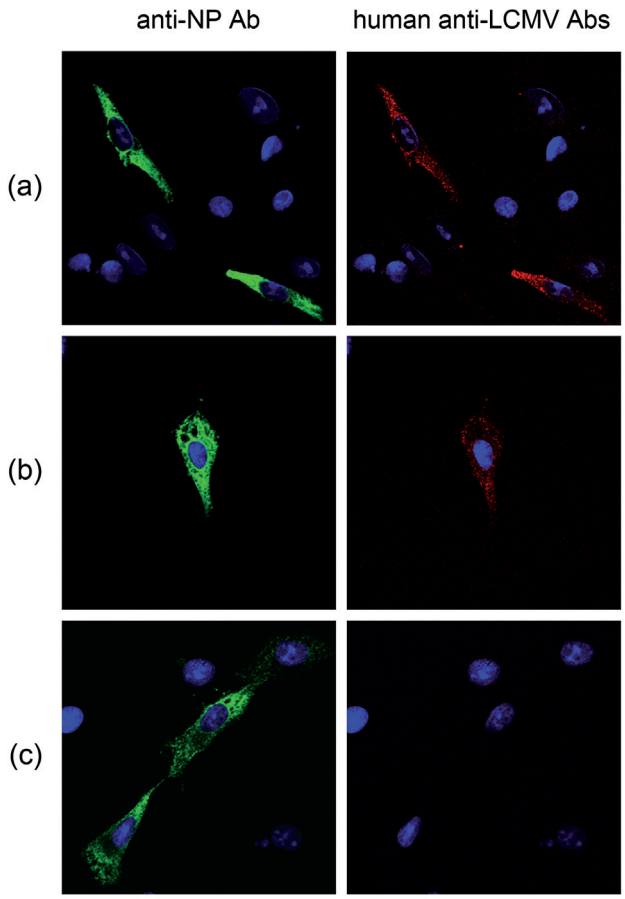

Fig. 4

Indirect immunofluorescence assay with human sera
Mixture of BHK21 cells and BHK21 cells persistently infected with LCMV strain MX was incubated with human sera diluted to 1:40. Anti-LCMV IgG antibodies were visualised by anti-human Ab labelled with Alexa Fluor 568 (red). To simultaneously identify cells infected with the virus, nucleoprotein was detected by monoclonal antibody M16 labelled with Alexa Fluor 488 (green). (a) IFA with positive human serum KH. (b) IFA with strongly positive serum (+++; titer of anti-NP IgG 1,600) according to ELISA results. (c) IFA with weakly positive serum (+; titer of anti-NP IgG 40) according to ELISA results.

Testing of multiorgan donor sera

Furthermore, we used our ELISA for the detection of anti-NP antibodies in 30 sera collected from multiorgan donors. Based on the obtained adjusted OD values, calculated percent positivity, and comparison to the cut-off value, we designated 2 sera as positive and 28 as negative. Positive sera were subsequently titrated and the antibody titer of a strongly positive serum was estimated 1,600 and of a weakly positive serum 40.

The same sera were then analysed with IFA. Representative samples of IFA, including the positive serum KH (Fig. 4a) are shown in Fig. 4. Only 1 out of 30 clinical samples was found positive using the IFA method (Fig. 4b). One weakly positive serum in ELISA (anti-NP IgG titer 40) was negative in IFA

Table 2. ELISA and IFA results comparison

		IFA		
		positive	**negative**	**total**
ELISA	**positive**	1	1	2
	negative	0	28	28
	total	1	29	30

Compared to IFA, the total coincidence rate (= detection results in the same number/total samples): [(1+28)/30]*100 = 96.67%.

(Fig. 4c). Comparison of the ELISA to the IFA results showed a total coincidence rate of 96.67% (Fig. 5; Table 2).

Discussion

Lymphocytic choriomeningitis virus is a human pathogen, which, as has been recently shown, possesses a great risk to several groups of people. Congenital infection may lead to spontaneous abortion (Bonthius *et al*., 2007), and previously documented cases of transplant-associated infections of immunocompromised individuals showed fatal outcomes in most patients (Fischer *et al*., 2006; CDC, 2008; Palacios *et al*., 2008; MacNeil *et al*., 2012; Schafer *et al*., 2014). In the case of congenital infection, the virus may not be detected after the infant is born, but specific IgGs are present in the mother's and infant's sera. The most common methods for the detection of specific anti-LCMV antibodies in sera are ELISA and IFA (OIE, 2006). The complement fixation test is relatively insensitive. Since there are no commercial ELISA kits for the detection of such antibodies available for routine use, we developed a method serving this purpose. For the ELISA tests, the antigen is usually prepared from cells infected with LCMV (Marrie and Saron, 1998; Riera *et al*., 2005). During such preparation of the antigen, handling of infectious LCMV is required, which is necessary to perform in a biosafety containment facility. In our ELISA, we used nucleoprotein-containing protein extracts from MX-infected BHK21 cells as antigen. The advantage of the LCMV strain MX is that it causes persistent infection of cells, and therefore provides cell lysates containing large amounts of antigen. Moreover, it does not form distinct virions released into the medium, and its transmission to uninfected cells in normoxia is mediated only by direct cell-cell contact (Reiserová *et al*., 1999; Laposova *et al*., 2013). This makes the handling of MX (and MX-infected cells) safer than handling other strains of LCMV. Also, ELISA with recombinant LCMV-NP as antigen has been previously reported by several groups (Homberger *et al.,* 1995; Takimoto *et al.*, 2008). In this case, either crude extracts from insect cells infected with recombinant baculovirus (Homberger *et al*., 1995) or LCMV-NP obtained by purification with high molar urea were used as antigen (Takimoto *et al*., 2008). According to published data, the ELISA assay using purified antigen was more sensitive in the detection of anti-LCMV antibodies. In our assay, we used an NP-capturing monoclonal antibody for the purification of LCMV-NP directly in the multiwell plate. Preparation of antigen by this method is more convenient and less time consuming in comparison to preparation of purified recombinant LCMV-NP. The use of the monoclonal anti-NP antibody in the first step also increases ELISA specificity and leads to a reduced background signal. By our ELISA assay, we detect specific anti-NP antibodies in acute, as well as persistent infection because nucleoprotein is highly produced in both, in contrast to the viral glycoprotein, the production of which is limited in the persistent state (Francis and Southern, 1988). To distinguish positive and negative sera, we had to set a cut-off value. According to literature, there are many methods for determination of the ELISA threshold. We decided to use calculations based on the results of immunoprecipitation, since it is a very sensitive method, and it allowed us to identify anti-NP antibody-positive and -negative sera by Western blot. Subsequently, the sera were tested by ELISA, and their absorbances were used for the cut-off value calculation where we set stringent conditions to avoid false positive results. After determining the threshold, we calculated clinical sensitivity and specificity of the newly-developed ELISA and standard IFA. Results show, that clinical specificity of both methods is 100%, but clinical sensitivity of ELISA is 30% higher than that of IFA. Next, we analysed sera from multiorgan donors since this is an interesting group of samples based on the recently described LCMV transmission through organ transplants (Fischer *et al*., 2006; CDC, 2008; Palacios *et al*., 2008; MacNeil *et al*., 2012; Schafer *et al*., 2014). We identified 2 of those sera as positive by our new ELISA method. Comparison of these results to the IFA method showed that the newly-established ELISA is more sensitive, since a weakly positive serum detected by ELISA (titer of anti-NP IgG 40) was negative by IFA. With this method, we were able to detect only the serum with anti-NP IgG titer 1,600. Moreover, in our experience, the testing of human sera by the IFA method is accompanied by high background, which is significantly reduced in ELISA.

In summary, our ELISA assay is very sensitive and enables the detection of NP-specific antibodies, which can be used not only for determining the prevalence of LCMV-specific antibodies in human populations for scientific purposes, but also for the diagnostic testing of LCMV infection.

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