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Evaluation of a new serological technique for detecting rabies virus antibodies following vaccination

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ABSTRACT

Two major techniques are currently used to estimate rabies virus antibody values: neutralization assays, such as the rapid fluorescent focus inhibition test (RFFIT), and enzyme-linked immunosorbent assays (ELISAs). The RFFIT is considered the gold standard assay and has been used to assess the titer of rabies virus neutralizing antibodies for more than three decades. In the late 1970s, ELISA began to be used to estimate the level of rabies virus antibody and has recently been used by some laboratories as an alternate screening test for animal sera. Although the ELISA appears simpler, safer and more efficient, the assay is less sensitive in detecting low values of rabies virus neutralizing antibodies than neutralization tests. This study was designed to evaluate a new ELISA-based method for detecting rabies virus binding antibody. This new technique uses electro-chemi-luminescence labels and carbon electrode plates to detect binding events. In this comparative study, the RFFIT and the new ELISA-based technique were used to evaluate the level of rabies virus antibodies in human and animal serum samples. By using a conservative approximation of 0.15 IU/ml as a cutoff point, the new ELISA-based technique demonstrated a sensitivity of 100% and a specificity of 95% for human samples and for experimental animal samples. The sensitivity and specificity for field animal samples was 96% and 95%, respectively. The preliminary results from this study appear promising and demonstrate a higher sensitivity than traditional ELISA methods.

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1. Introduction

Rabies serological tests are essential for evaluating the immune response to rabies virus antigens. The presence of antibodies directed against rabies virus envelope glycoprotein is considered an ideal indicator of immune response after vaccination. Since Webster and Dawson devised the Mouse Neutralization Test (MNT) in 1935 [1], several different methods have been developed to better detect rabies virus antibody. The rapid fluorescent foci inhibition test (RFFIT) was developed as one method for the measurement of rabies virus neutralizing antibodies [2]. A microtest, adapted from the RFFIT, for use in micro-plates was developed to quantify rabies virus neutralizing antibodies in human sera [3]. The fluorescent antibody virus neutralization (FAVN) test was also evaluated for use in animal sera [4]. Briggs et al. compared the RFFIT and the FAVN test with animal sera and now these two methods serve as the gold standards for rabies virus neutralizing antibody quantification [5]. Alternatively, the enzyme-linked immunosorbent assay (ELISA) was first used in rabies serological test by Atanasiu et al. [6] and now is used as a screening test in some laboratories [7]. Compared with the MNT, the RFFIT has apparent advantages, such as eliminating the use of animals and faster results. However, the RFFIT requires trained personnel and appropriate laboratory equipment for cell culture and manipulating live rabies virus. In addition, the RFFIT remains a complex and time consuming test, cell condition and sample quality can influence the test results. The RFFIT is very sensitive to cytotoxicity in serum samples with poor quality such as wildlife sera collected under field conditions and may produce false-positive results if read incorrectly. Furthermore, the observation of fluorescing cells in the RFFIT is somewhat subjective, which can cause variance of the endpoint titer between different observations for the same sample.

The ELISA is a new generation of serological test used to estimate the level of rabies virus antibodies and has recently been used by some laboratories as an alternate screening test for animal sera. Conventional ELISA tests appear simpler, safer, and more efficient [8] but may have lower sensitivity than neutralization tests [9–12], and the concordance between the ELISA and neutralization assays have not been satisfactory.

The objective of this study was to evaluate the performance of a new technique called electro-chemi-luminescence (ECL) in determining anti-rabies glycoprotein antibodies in human and animal

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serum samples. The ECL assay is based on the Meso Scale Discovery (MSD) technology which utilizes a sulfo-tag-label that emits light upon electrochemical stimulation. MSD carbon electrode plates supply a platform for the development of sandwich immunoassays and have a 10 times greater binding capacity than polystyrene used in conventional ELISA. When a MSD plate was loaded, the MSD plate reader applies an electrical current to the plate-associate electrodes and causes the label bound to the electrode surface to emit light. The MSD instrument measures intensity of the emitted light to afford a quantitative measure of the amount of the target protein. Previous studies demonstrated that the ECL assay exhibits a wide dynamic range and high sensitivity for detecting low concentrations of antibodies in human serum samples [13,14]. In this study, the RFFIT and the ECL test were used to evaluate the level of rabies virus antibodies in human and animal serum samples. This report is the first about the ECL test being used for detecting the level of rabies virus antibody.

2. Materials and methods

2.1. Serum samples

The samples used in this study included human sera, human cerebrospinal fluids (CSF), and animal sera. One hundred twenty serum samples from vaccinated and naive humans collected under CDC-IRB Protocol #5506, along with 22 serum samples and 14 CSF samples from clinical specimens submitted for human rabies diagnosis were tested. In addition, 88 dog serum samples, 92 experimental raccoon and 167 field raccoon serum samples were tested. All sera and CSF samples were heat inactivated at 56 °C for 30 min prior to testing and stored at −20 °C. Test samples from vaccinated humans and animals were blinded at the time of the RFFIT and the ECL test.

2.2. Reference serum

Each test included a positive and a negative control sample from vaccinated or unvaccinated subjects. The reference serum standard used in the RFFIT for determining anti-rabies neutralizing antibody levels in human and animal serum samples was the U.S. Standard Rabies Immune Globulin (SRIG), NIH Lot R-3, with an activity of 59 IU. A stock suspension was prepared to yield a solution containing 2 IU/ml. This stock was dispensed in aliquots of 0.5 ml and stored at −20 °C. Both the SRIG and human serum samples that tested positive by the RFFIT with 2 IU/ml of anti-rabies neutralizing antibody were used as positive control in the ECL test. Positive reference samples for laboratory and field raccoons were selected from samples that tested positive by the RFFIT, with a neutralizing antibody level of 2 IU/ml. Negative control sera were obtained from unvaccinated human and animal that tested negative by the RFFIT.

2.3. Production of virus and purification of envelop glycoprotein

An Evelyn-Rokitnicki-Abelseth (ERA) strain of rabies virus was propagated in a culture of BSR (a BHK-derived cell clone) cells. Rabies virus was collected and concentrated from supernatant. Rabies glycoprotein was isolated and purified as previously described [15–17].

2.4. The RFFIT

The RFFIT was performed according to the technique described by Smith et al. [2] to determine anti-rabies neutralizing antibody level in human and animal sera. In brief, serial five-fold dilutions of test sera were made and incubated with a constant amount of challenge virus-CVS 11 (50 FFD_{50}/0.1 ml). The serial dilutions of SRIG (2 IU/ml) were used as a positive control reference and included in parallel in each test. After 90 min incubation, mouse neuroblastoma (MNA) cells in Eagle's minimum essential medium with 10% fetal bovine serum (MEM-10) were added to each serum-virus mixture. The serum-virus-cell cultures were incubated for 20 h in a CO2 incubator at 37 °C. The cultures were then removed from the incubator, washed with phosphate buffered saline (PBS), fixed with acetone and stained with FITC anti-rabies monoclonal globulin. Immunofluorescence staining was used as an indicator of viral growth. The slides were observed under a fluorescence microscope for the presence of fluorescing cells. Twenty microscopic fields (160–200×) were read for each serum dilution and the number of fields containing fluorescing cells was recorded. A reduction of 50% or more in the number of fields containing fluorescing cells indicated that there were enough antibodies present to neutralize the virus. The serum neutralization endpoint titer was defined as the dilution factor of the highest serum dilution in which there is a 50% reduction in the number of fluorescing foci. The United States Advisory Committee on Immunization Practices (ACIP) defines complete virus neutralization at a 1:5 serum dilution by the RFFIT as an adequate immune response, which is equivalent to approximately 0.10 IU/ml [18].

2.5. The ECL test

Standard carbon-electrode Multi-array 96-well plates (Cat#: L15XA-3, Meso Scale Discovery, Gaithersburg, Maryland, USA) were coated with 30 μl/well of 4 μg/ml purified rabies virus glycoprotein and were placed at 4 °C overnight. After washing with 150 μl/well of diluted Tween20 (Cat#: P1379, Sigma–Aldrich, Saint Louis, MO, USA) three times, the plates were blocked with 150 μl/well of blocking buffer and incubated on a plate shaker for 1 h at room temperature. After removing the blocking buffer, serum samples were directly added into the wells and diluted 1:5 in sample buffer. Each sample was tested at a single dilution in duplicate. After 1 h incubation at room temperature on a plate shaker, serum samples were removed and the plates were washed three times with 150 μl/well of washing buffer. Pre-diluted 25 μl of 0.5 μg/ml Biotinylated anti-species IgG (anti-human IgG, Cat. No. 16-10-06; anti-raccoon IgG, Cat. No. 37-00-06; anti-dog IgG, Cat. No. 16-19-06; Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD, USA) and sulfo-tag-streptavidin (Cat#: R32AD-5, Meso Scale Discovery, Gaithersburg, MD, USA) was added to each well of the plate. The plates were incubated at room temperature on a plate shaker for 1 h and then washed three times with 150 μl/well of washing buffer. Immediately before reading, 150 μl of reading buffer (1×) was added to each well of the plate and the plates were read on a MSD Sector Imager (Cat#: R31QQ-2, Meso Scale Discovery, Gaithersburg, MD, USA). Serial five-fold dilutions of positive control reference sera starting with a 1:5 dilution were included in parallel in each test along with a negative control.

3. Results

3.1. Assessment of the ECL test for human serum and CSF samples

Titration of the 120 human sera with the ECL test and the RFFIT is shown in Fig. 1. The cutoff values of 5.19 (log_{10} ECL value) for the ECL test and -0.82 (log_{10} 0.15 IU/ml) for the RFFIT were used to distinguish positive and negative samples. The ECL cutoff value is the mean of the log transformed ECL values of negative samples at 1:5 dilution plus two standard deviations. The log transformed ECL value of SRIG at a 1:5 dilution is 6.07 and the log transformed ECL value of SRIG at a 1:125 dilution is 5.25, which is very close to the ECL cutoff value. The actual ECL value of the reference serum SRIG

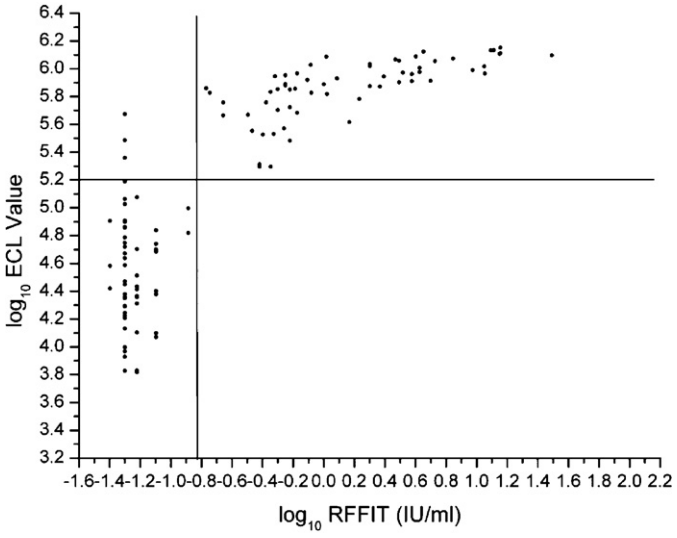

Fig. 1. Comparison between the RFFIT and the ECL test with human sera.

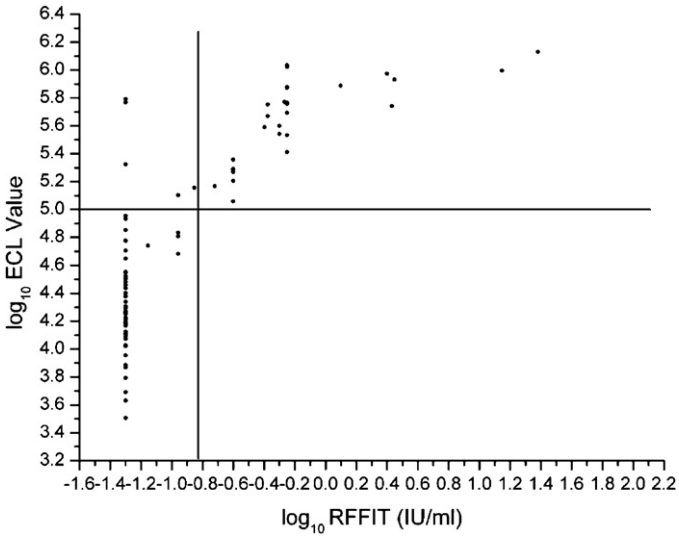

Fig. 2. Comparison between the RFFIT and the ECL test with raccoon sera.

Table 1
Diagnostic sensitivity and negative cutoff value of the ECL test established with sera from vaccinated and unvaccinated human.

Range of log ECL reading of negative samples	3.8175–5.1899
Mean of log ECL reading of negative samples	4.4881
Standard deviation (SD)	0.3526
Log ECL reading of SRIG	5.2516
Negative cutoff[a]	5.1933
Range of log ECL reading of positive samples	5.2959–6.1531
Diagnostic sensitivity	64/64 (100%)

[a] The negative cutoff value is the mean of log ECL reading of negative samples + 2 SD.
The diagnostic sensitivity is established by using the value of negative cutoff of 5.1933.

at a 1:5 dilution is 1,173,697, which is 28 times the average ECL value of negative serum samples (41,805). Establishing the cutoff value for the ECL test is explained in Table 1.

Of the 120 human sera, 64 that tested positive by using the RFFIT were collected from vaccinated individuals and also positive when tested using the ECL test. Among the 56 sera that tested negative by using the RFFIT, 53 were negative and 3 were positive by using the ECL test. One of the sera that tested negative by both the RFFIT and the ECL test was collected from a previously (31 years ago) vaccinated individual. The other negative sera were collected from rabies vaccine naïve persons. The ECL test resulted in 100% sensitivity and 95% specificity using a cutoff value of 0.15 IU/ml for the RFFIT (Table 2).

Thirty six samples (22 sera and 14 CSF) from patients suspected of rabies were analyzed by the RFFIT and the ECL test. All serum samples that tested negative ($n = 18$) or positive ($n = 4$) by using the RFFIT were also negative or positive by using the ECL test, respectively. Of 14 CSF samples, one tested positive and 13 tested negative by using the RFFIT. The ECL test gave the same results as RFFIT for the CSF samples. The sensitivity and specificity of the ECL test in human rabies diagnosis were both 100% in this comparison. The four human samples positive by using the RFFIT and the ECL test were from confirmed cases of human rabies. Vaccination histories obtained on human clinical specimens were in direct correspondence to the results obtained by RFFIT in relation to standard used in our laboratory.

3.2. Assessment of the ECL test for raccoon serum samples

The comparison between the RFFIT and the ECL test with the raccoon sera collected under laboratory condition is shown in Fig. 2. The cutoff values −0.82 ($\log_{10}$ 0.15 IU/ml) for the RFFIT was also used to distinguish positive and negative raccoon samples. An ECL cutoff value of 4.98 is the mean of the log transformed ECL values of negative samples at 1:5 dilution plus two standard deviations. The ECL value of a 1:5 dilution of a reference raccoon serum sample with 2 IU/ml neutralizing rabies virus antibody is 572,160, which is more than 21 times the average ECL value of negative serum samples (26,788).

Among the 92 experimental raccoon serum samples, 32 samples collected from vaccinated raccoons were positive by using both the RFFIT and the ECL test. The remaining 60 samples (58 from unvaccinated and 2 from vaccinated raccoons) tested negative by using the RFFIT, of which 57 negative and 3 positive when tested by the ECL test. Using 0.15 IU/ml as a cutoff point, the ECL test demonstrated the same sensitivity (100%) and specificity (95%) for raccoon serum samples collected under laboratory conditions as for human samples (Table 3).

The sensitivity and specificity of the ECL test in detecting anti-rabies antibodies for raccoon serum samples collected under field conditions were also evaluated. Three raccoon serum samples with cytotoxicity present using RFFIT were excluded. For 167 samples tested by using both methods, the ECL test demonstrated a sensitivity of 96% and a specificity of 95% (Table 4). The 26 positive

Table 2
Cross classification of the ECL test results by the RFFIT results for the human serum samples.

RFFIT 0.15 IU/ml cut-off	ECL test		
	Negative	Positive	Total
Negative	53	3	56
Positive	0	64	64
Total	53	67	120

Table 3
Cross classification of the ECL test results by the RFFIT results for the experimental raccoon serum samples.

RFFIT 0.15 IU/ml cut-off	ECL test		
	Negative	Positive	Total
Negative	57	3	60
Positive	0	32	32
Total	57	35	92

Table 4
Cross classification of the ECL test results by the RFFIT results for the field raccoon serum samples.

RFFIT 0.15 IU/ml cut-off	ECL test		
	Negative	Positive	Total
Negative	135	6	141
Positive	1	25	26
Total	136	31	167

Table 5
Cross classification of the ECL test results by the RFFIT results for the dog serum samples.

RFFIT 0.15 IU/ml cut-off	ECL test		
	Negative	Positive	Total
Negative	47	2	49
Positive	0	39	39
Total	47	41	88

samples tested by the RFFIT were collected from the oral rabies vaccination (ORV) area, of which 25 were positive and 1 negative tested by the ECL test. Among the 135 positive samples tested by both the RFFIT and the ECL test, 75 were collected from the ORV area and 60 from the non-ORV area. The 6 samples that were negative by the RFFIT and positive by the ECL test were collected from the ORV area.

3.3. Assessment of the ECL test for dog serum samples

A panel of laboratory dog serum samples was tested using both the RFFIT and the ECL test. The results are shown in Fig. 3. A lower log transformed RFFIT cutoff value (-1.0, $\log_{10}$ 0.10 IU/ml) was used for evaluating the test results. The ECL cutoff value for dog sera is 5.19, which is the mean of the log transformed ECL values of negative samples at 1:5 dilution plus two standard deviations. The ECL value of a 1:5 dilution of a reference dog serum sample with 2 IU/ml neutralizing rabies virus antibody is 790,616, which is 22 times the average ECL value of negative serum samples (36,627).

Among the 88 dog serum samples collected under laboratory conditions, 47 samples that collected from unvaccinated dogs were negative by using both the RFFIT and the ECL test. Of the 41 samples that tested positive by the ECL test, 39 were positive and 2 were negative when tested by the RFFIT. Of the two dog sera with discrepant results, one was collected from an unvaccinated dog; another was from a vaccinated dog on day 15 after vaccination. Using 0.10 IU/ml as a cutoff point, the ECL test demonstrated a sensitivity of 100% and a specificity of 96% for dog serum samples (Table 5).

4. Discussion

This is the first study that evaluates the performance of the ECL test, a new ELISA-based technique, for detecting anti-rabies glycoprotein antibodies in human and animal serum samples collected under different conditions. By using electro-chemi-luminescent labels and carbon electrode plate, the ECL test demonstrated ultra-sensitive detection of antibodies. The assay developed in this study was able to detect rabies virus antibodies level in a 1:5 dilution of serum samples. Therefore, the new method is able to capture very low level of antibodies in test sera.

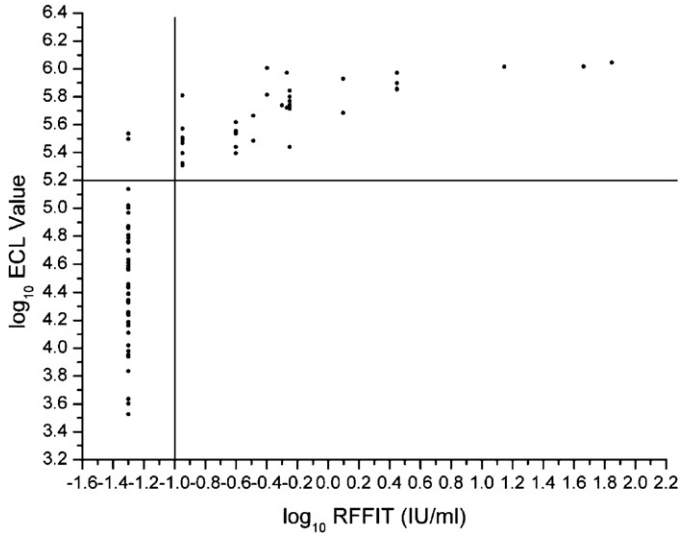

Fig. 3. Comparison between the RFFIT and the ECL test with dog sera.

Results from the ECL test for 120 unvaccinated and vaccinated human serum samples had a100% sensitivity by using the RFFIT as the gold standard and 0.15 IU/ml as a cutoff point. The ECL test was also evaluated for diagnosis of human rabies cases and demonstrated a 100% concordance with the RFFIT by using 36 serum and CSF samples from patients suspected of rabies.

Performance of the ECL test for experimental animal samples was similar to that for human samples. The ECL test demonstrated a sensitivity of 100% in detecting rabies virus antibodies in animal serum samples collected under laboratory conditions.

In the United States, an oral vaccination (ORV) program has been established since 1997 in an attempt to prevent the westward spread of raccoon rabies [19]. Serological testing is one method used to monitor anti-rabies antibody in the animals collected from areas with an ongoing ORV program. The baits distributed contain a vaccinia-rabies glycoprotein (VRG) vaccine, which induces antibodies against the envelope G protein [20,21]. Hence, the ECL test that uses G protein coated plates has a theoretically good correlation with the RFFIT. The ECL test for 167 vaccinated and unvaccinated field raccoons resulted in a very high concordance with the results of those tested with the RFFIT. A sensitivity of 96% was produced by the ECL test by using 0.15 IU/ml as the RFFIT cutoff, although the quality of field raccoon sera was relatively poor compared with experimental animal sera. The CDC receives thousands of serum samples from raccoons each year for sero-surveillance of ORV zones. Processing these samples by RFFIT is time consuming. A high throughput method for assessing anti-rabies antibody levels is needed to improve and expand processing of this large number of sera. Although simpler, safer, and more rapid, the traditional ELISA methods have not been widely accepted for determining rabies antibody levels because of a lack of sensitivity [9–12]. Apparent false-negative results are one major problem that prevents broader application of traditional ELISA in detecting anti-rabies antibody levels. Although traditional ELISA and the RFFIT have shown concordance under optimal serum conditions, they did not correlate well when using serum collected under field conditions [22]. The ultra-sensitivity of the ECL test overcomes the sensitivity limitation of the traditional ELISA for detecting low level rabies virus antibodies. The elimination of cell culture with the ECL test also reduces concerns of false-positive results seen with the RFFIT when testing cytotoxic serum samples [7,17].

The MSD Sector imager is very sensitive to the signal generated by binding events. Shorter incubations may cause lower ECL reading. Total volume and dilution of the serum are also important for the MSD Sector imager to generate a consistent reading. The selection of positive controls is another consideration for the ECL test. Serum samples from the same species with a value of 2 IU/ml anti-rabies antibody is a good choice for establishing a threshold of the ECL reading to distinguish negative samples from positive. Positive samples with very high titers should not be used for positive

controls with the ECL test because the signal generated at a 1:5 dilution may exceed the reading saturation of the MSD Sector imager.

The application of the ECL assay depends on the testing purposes. To evaluate the effectiveness of oral vaccination, the ECL assay appears to have a promising potential to distinguish seropositive samples from negative samples. Our ECL test results reliably demonstrated herd immune response in raccoons after oral vaccination with a sensitivity of 96% and a specificity of 95% using 0.15 IU/ml as a cutoff. At this level, additional testing would likely not be done since the focus is on determining the proportion of the population with detectable rabies virus antibody levels.

The ECL test also appears to be an alternative choice for detecting rabies virus antibodies in human and laboratory animals. Using 0.15 IU/ml as a cutoff, the ECL test demonstrated a sensitivity of 100% for both the human and laboratory raccoon serum samples. The results of laboratory dog serum samples demonstrated a sensitivity of 100% and a specificity of 96%, with a threshold of 0.10 IU/ml.

The high sensitivity is a major advantage of the ECL test compared with a traditional ELISA. However, the MSD imager (reader) is fairly expensive, although the ECL test costs approximately one third of the RFFIT in terms of reagents and other related supplies. The application of the ECL test with a wide range of animals, such as skunks and bats is limited at present, by whether the anti-species antibody is available commercially.

For clinical diagnostic purposes, the ECL test is able to produce a faster result. However, it would likely be necessary to retest ECL positive samples to distinguish rabies virus neutralizing antibodies from antibodies without detectable neutralizing function.

The ECL assay is still a binding test, not a neutralizing test, with the inherent limitation of all ELISA methods versus a functional test. Compared with the RFFIT, the ECL test generated few false positive results in the human and animal samples. However, protection against rabies in vivo is complex and rabies virus neutralization is a partial contributor in protection [23]. When a test is aimed at determining protective levels of antibodies, it is important for a serological assay to detect non-neutralizing antibodies. The ECL test provides valuable results for further investigation of those individuals that do not have rabies neutralizing antibody but may have other specific antibodies against rabies virus.

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References

[1] Webster LT, Dawson JR. Early diagnosis of rabies by mouse inoculation. Measurement of humoral immunity to rabies by mouse protection test. Proc Soc Exp Biol Med 1935;32:570–3.
[2] Smith JS, Yager PA, Baer GM. A rapid reproducible test for determing rabies neutralizing antibody. Bull WHO 1973;48:535–41.
[3] Zalan E, Wilson C, Pukitis D. A microtest for the quantitation of rabies virus neutralizing antibodies. J Biol Stand 1979;7(3):213–20.
[4] Cliquet F, Aubert M, Sagne L. Development of a fluorescent antibody virus neutralization test (FAVN) for the quantitation of rabies neutralizing antibody. J Immunol Methods 1998;212(1):79–87.
[5] Briggs DJ, Smith JS, Mueller FL, Schwenke J, Davis RD, Gordon CR, et al. A comparison of two serological methods for detecting the immune response after rabies vaccination in dogs and cats being exported to rabies-free areas. Biologicals 1998;26:347–55.
[6] Atanasiu P, Savy V, Perrin P. Epreuve immunoenzymatique pour la détection rapide des anticorps anti-rabiques. Ann Microbiol (Paris) 1977;128A(4):489–98.
[7] Cliquet F, Sagne L, Schereffer JL, Aubert MFA. ELISA test for rabies antibody titration in orally vaccinated foxes sample in the fields. Vaccine 2000;18(28):3272–9.
[8] Feyssaguet M, Dacheux L, Audry L, Compoint A, Morize JL, Blanchard I, et al. Multicenter comparative study of a new ELISA, Platella Rabies II, for the detection and titration of anti-rabies glycoprotein antibodies and comparison with the rapid fluorescent focus inhibition test (RFFIT) on human samples from vaccinated and non-vaccinated people. Vaccine 2007;25:2244–51.
[9] Cliquet F, McElhinney LM, Servat A. Development of a qualitative indirect ELISA for the measurement of rabies virus-specific antibodies from vaccinated dogs and cats. J Virol Methods 2004;117:1–8.
[10] Servat A, Cliquet F. Collaborative study to evaluate a new ELISA test to monitor the effectiveness of rabies vaccination in domestic carnivores. Virus Res 2006;120:17–27.
[11] Welch RJ, Anderson BL, Litwin CM. An evaluation of two commercially available ELISAs and one in-house reference laboratory ELISA for the determination of human anti-rabies virus antibodies. J Med Microbiol 2009;58:806–10.
[12] Knoop EV, Freuling C, Kliemt J, Selhorst T, Conraths F, Muller T. Evaluation of a commercial rabies ELISA as a replacement for serum neutralization assays as part of the pet travel scheme and oral vaccination campaigns of foxes. Berl Munch Tierarztl Wochenschr 2010;123:278–85.
[13] Marchese RD, Puchalski D, Miller P, Antonello J, Hammond O, Green T, et al. Optimization and validation of a multiplex, electrochemiluminescence-based detection assay for the quantitation of immunoglobulin G serotype-specific antipneumococcal antibodies in human serum. Clin Vaccine Immunol 2009;16(3):387–96.
[14] Cludts I, Meager A, Thorpe R, Wadhwa M. Detection of neutralizing interleukin-17 antibodies in autoimmune polyendocrinopathy syndrome-1 (APS-1) patients using a novel non-cell based electrochemiluminescence assay. Cytokine 2010;50(2):129–37.
[15] Atanasiu P, Tsang H, Perrin P, Favre S, Sisman J. Extraction d'un antigene soluble (Glycoprotein) par le Triton X-100 a partir d'un vccine antirabique de culture tissulaire de premier expant Resultats d'immunisation et pouvoir protecteur. Ann Microbiol (Inst Pasteur) 1974;125B:539–57.
[16] Cox JH, Dietzschold B, Schneider LG. Rabies virus glycoprotein II. Biological and serological characterization. Infect Immun 1977;16(3):754–9.
[17] Barton LD, Campbell JB. Measurement of rabies-specific antibodies in carnivores by an enzyme-linked immunosorbent assay. J Wildl Dis 1988;24(2):246–58.
[18] CDC. Human rabies prevention – United States: recommendations of the advisory committee on immunization practices. MMWR Morb Mort Wkly Rep 2008;57(RR03):1–26, 28.
[19] Slate D, Rupprecht CE, Rooney DD, Lein DH, Chipman RB. Status of rabies vaccination in wild carnivores in the United States. Virus Res 2005;111:68–76.
[20] Kieny MP, Lathe R, Drillien R, Spehner S, Skory D, Schmitt T, et al. Expression of rabies virus glycoprotein from a recombinant vaccinia virus. Nature 1984;321:163.
[21] Hanlon CA, Niezgoda M, Hamir AN, Schumacher C, Koprowski H, Rupprecht CE. First North American field release of a vaccinia-rabies glycoprotein recombinant virus. J Wildl Dis 1998;34(2):228–39.
[22] Bahloul C, Taieb D, Kaabi B, Diouani MF, Hadjahmed SB, Chtourou Y, et al. Comparative evaluation of specific ELISA and RFFIT antibody assays in the assessment of dog immunity against rabies. Epidemiol Infect 2005;133:749–57.
[23] Moore SM, Hanlon CA. Rabies-specific antibodies measuring surrogates of protection against a fatal disease. PLoS Negl Trop Dis 2010;4(3):e595.