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1. An inter-laboratory comparison to evaluate the technical performance of rabies diagnosis Lateral Flow Assays

Alexandre Servat¹, Emmanuelle Robardet¹, Florence Cliquet¹

¹French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Nancy Laboratory for Rabies and Wildlife, OIE Reference Laboratory for Rabies, European Union Reference Laboratory for Rabies, European Union Reference Laboratory for Rabies Serology, Technopôle Agricole et Vétérinaire, Domaine de Pixérécourt, CS 40009, 54220 Malzéville – France.

Corresponding author:

Alexandre Servat, Anses, Nancy Laboratory for Rabies and Wildlife, Technopôle Agricole et Vétérinaire, Domaine de Pixérécourt, CS 40009, 54220 Malzéville – France. Email : alexandre.servat@anses.fr

Abstract

As in previous years, the European Union Reference Laboratory (EURL) for rabies organised in 2018 an Inter-laboratory trial (ILT) on rabies diagnosis. Contrarily to past years, the 2018 ILT did not aim to evaluate the performance of participating laboratories, but the technical performance of new rapid tests. Two lateral Flow Assays (LFA), namely the Anigen® and the CDIA™ Rabies Virus Antigen Rapid Test” (commercialized by Bionote and Creative Diagnostics Cie respectively), were evaluated together with the Fluorescent Antibody Test (FAT). One panel of virus samples (including RABV as well as EBLV1a, EBLV-1b, and EBLV2 strains) was sent to participating laboratories to compare results obtained with these different techniques.

The study revealed that the FAT provided a good agreement toward expected results for both negative/positive samples (99.1%). The Anigen® test produced similar results to the FAT, with only one false negative result (0.5%) reported by all participants and a concordance of 100% for all but one sample demonstrating a good inter-laboratory reproducibility of the Anigen® batch. The CDIA™ test produced reproducible results for Rabies Virus (RABV) samples only. However, it hardly detected the Bokeloh Bat Lyssavirus (BBLV) and the European Bat Lyssaviruses types 1b and 2 (EBLV-1b and EBLV-2) in most laboratories resulting in a moderate inter-laboratory concordance (58.4% to 82.7%) for these lyssaviruses.

The two LFAs provided reliable and reproducible results on all RABV samples (100%) but lead to heterogeneous performances with other lyssaviruses leading to different levels of diagnostic/analytical sensitivity, specificity. The study confirmed that LFAs should be used

with caution and that their validation are of utmost importance before any use in laboratories.

Keywords

Rabies, diagnosis, rapid tests, inter-laboratory comparison

1. Introduction

The effective surveillance capacity is a key element for rabies prevention and control. These capacities encompass a continuous collection of samples, the use of recommended diagnosis techniques, the interpretation of results and the dissemination of information to relevant public and veterinary health authorities taking specific control measures. Rabies surveillance is of utmost importance in parts of the world where the disease is still neglected. The absence or the lack of surveillance contribute directly to the underreporting of rabies cases in both animals and humans and to the biased burden of the disease in some countries. Surveillance activities remain also an important tool for countries that remained free of rabies or that have succeeded in eliminating the rabies virus (RABV) so that to detect effectively any infected animals in a timely manner. Rabies re-emergence from bordering infected countries (De Benedictis et al., 2008) or from introduction of rabid animals illegally imported (Cliquet et al., 2014) still represent serious threats for any rabies free territories.

Rabies diagnosis is generally carried-out using reference techniques that are recognised as gold standards methods. The most widely used primary test for the detection of rabies antigens is the direct Fluorescent Antibody Test (FAT) (Dean et al., 1996), which is recommended by both OIE and WHO. More recently, the Direct Rapid Immunohistochemistry Test (dRIT) (Lembo et al., 2006; “The direct rapid immunohistochemistry test for the detection of lyssaviruses antigens, chapter 12,” 2018) has also been recognized by the OIE (OIE - World Organisation for Animal Health, 2018) and the WHO (*Laboratory techniques in rabies*, 2018) as a possible alternative to DFA due to its similar sensitivity and specificity, more especially in laboratories that do not have access to fluorescent microscopes. Virus isolation on cell culture (“Virus isolation in cell culture: the rabies tissue culture infection test, chapter 9,” 2018), or Rapid Tissue Culture Infection Test (RTCIT), is also frequently used as a confirmatory test in case of FAT or dRIT inconclusive results. This *in-vitro* method has widely replaced virus isolations on animals while being less expensive, more rapid and as sensitive as the Mouse Inoculation Test (“Virus isolation in animals: the mouse inoculation test, chapter 8,” 2018). Molecular methods (conventional RT-

PCR, real time RT-PCR) have gained increasing interest since the past decades and are now recognized by OIE and WHO as possible alternatives to antigen detection methods for passive and active surveillance (OIE - World Organisation for Animal Health, 2018; World Health Organization, 2018). Immunochromatographic techniques (Koczula and Gallotta, 2016), also known as lateral flow assays (LFAs) are the last generation of methods that have raised some interest from the rabies scientific community (OIE - World Organisation for Animal Health, 2018). These tests are devices able to detect an analyte at the point of care and do not require cold chain to store the devices, making them particularly appropriate for field studies. They are quite rapid, low cost and one-step tests. Test results and validation can be easily visualized by the naked-eye (test line and control line) as it is generally done with the well-known pregnancy test for the detection of human gonadotropin hormone in urine (Butler et al., 2001).

There is an increasing number of LFAs for rabies diagnosis that are becoming available on the market. Contrarily to veterinary medicines such as vaccines, marketing veterinary diagnostic tests/kits is not necessarily subjected to mandatory regulatory approval process in many parts of the world. This questions the confidence that can be placed in these new diagnostic tests. Fortunately, this lack of approval, of control before commercialization is partially compensated by the cooperation and validation studies performed by reference laboratories (OIE, European Union). Inter-laboratory tests are one of the means to get a clearer picture of the performance of such new rapid tests.

The objective of the present study was to evaluate the inter-laboratory performance of two commercial LFAs for the detection of rabies antigen in brain material, and to compare it with the one of the FAT, which is considered as a standard technique for rabies diagnosis. The evaluation consisted in an international inter-laboratory comparison (ILC) in which various rabies National Reference Laboratories (NRLs) in the European Union have participated.

2. Materials and methods

2.1. Participating laboratories

Twenty-two rabies National Reference Laboratories (NRLs) from Member States were invited to take part in the inter-laboratory trials dedicated to for rabies diagnosis rapid techniques. Participation was voluntary. These laboratories were randomly coded from L1 to L22. These 22 laboratories were located in the following countries (Table 1): Austria, Belgium, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Italy, Latvia, Lithuania, Luxembourg, Netherlands, Poland, Romania, Slovenia, Slovak Republic, Spain and United Kingdom.

2.2. Composition of the panel

Panels consisted in lyophilized brain material samples. The infected brain batches used in this study were produced by experimental infection of mice (intracerebral route) to reproduce as much as possible standard conditions of a diagnosis process. Experimental procedures were evaluated favorably by the ANSES/ENVA/UPEC Ethic Committee (N° of approval 17-076) and were authorized by the French Ministry of Higher Education and Research, DG Research and Innovation. All animal experimentations were conducted according to the national and European directives for animal experimentation (*Off J Eur Union*, 2010). Virus production procedures were stopped when animals harbored symptoms suggestive of rabies (stage 4 of clinical signs) (Bruckner et al., 2003) to collect a maximum amount of virus. For each batch of virus, brains were excised after the death of animals then pooled, mixed, homogenized, aliquoted into 1 milliliter tubes and then freeze-dried. The panel was finally constituted of 10 blindly coded samples. Nine samples corresponded to freeze-dried homogenized brains infected with various rabies virus species circulating in Europe or close to Europe. One sample consisted in one freeze-dried brain originated from an uninfected lamb. Details of the rabies variants included in this inter-laboratory test are listed in Table 2. The evaluation of the homogeneity of each sample was undertaken for each batch by analysing in duplicate 10 randomly chosen samples by FAT, RTCIT, RT-PCR and Real Time RT-PCR. The analysis was performed after lyophilisation when all the samples were under their final form (BBLV 07-16; EBLV-1a 12-14; EBLV-1b 10-14; EBLV-2 05-16; RABV 10-13 / 17-15 / 12-15 / 11-16 and 15-18 for the positive batches, and Negative 13-18 for the negative batch). All the batches were considered homogeneous, as all the results were concordant to the expectations.

For each panel, all items were coded randomly. The code was constituted by the date of the inter-laboratory test campaign, the identification of the laboratory and the unique specific code of the item.

2.3. Panel testing

2.3.1. The Fluorescent Antibody Test

Laboratories were invited to test the panel samples with the FAT (Fluorescent Antibody test) using their own routine protocol. Expected FAT results were a binomial response “positive” or “negative”.

2.3.2. Lateral Flow assays

The Anigen® rapid rabies antigen Test kit, produced and commercialized by Bionote (South Korea), is a chromatographic immunoassay for the qualitative detection of rabies virus antigen in canine, bovine, raccoon dog's secretions of saliva, and brain homogenates. The kit is based on a sandwich format where the rabies antigen is captured by free gold conjugated detector monoclonal antibodies and revealed using purified anti-IgG antibodies attached to the test zone of a nitrocellulose membrane.

The CDIA™ Rabies Virus Antigen Rapid Test kit produced and commercialized by Creative Diagnostics (USA), is a rapid assay based on colloidal gold linked antibody-binding technology that allows detecting rabies virus antigen in canine and cat's saliva. The assay utilizes an anti-rabies virus monoclonal antibody to identify the rabies virus antigen, and is based on a double-antibody sandwich format on a nitrocellulose membrane.

Two cassettes of 10 rapid test kits (Anigen® batch N°1801DD024 and "CDIA™ Rabies Virus Antigen Rapid Test" batch N°20180601) were provided to participants. A standard operating procedure (SOP) was supplied with the LFA kits. This SOP detailed the different steps to carry out for each of the LFAs (Anigen® and CDIA™ Rabies Virus Antigen Rapid Test) and gave indications for validation and interpretation of the results. All samples were shipped at the same date and were requested to be stored at +4°C upon reception until analysis.

Basically, lyophilized samples were rehydrated with 1 ml of sterile water and mixed properly. After thirty minutes, the appropriate amount of rehydrated brain was collected to carry out the FAT and the remaining brain sample was diluted in 6 ml of sterile PBS and properly mixed using a vortex. For each LFA, a swab (provided in the kit) was impregnated to collect the diluted brain homogenate. The soaked swab was then transferred into the assay diluent (provided in the kit) and mixed about 10 seconds for a proper extraction/draining of the swab. Four drops (five drops for the CDIA™ test) of the extracted sample were added into the sample hole of the device using a dropper provided in each kit.

For both LFAs, the interpretation was carried-out after 10 and 15 minute migrating for the Anigen® and the CDIA™ rapid tests, respectively. The test was considered valid when a coloured band appeared in the control zone « C ». The presence of a coloured band in the test zone « T » indicated a positive result. The absence of a coloured band in the test zone « T » indicated a negative result. Expected results were a binomial response "positive" or "negative".

2.4. Data analysis

The sensitivity and specificity were estimated for each test (FAT, Anigen® and the CDIA™ rapid tests) by comparing their results with the ones expected for each sample.

Reproducibility and repeatability measure the precision of an assay, which is the degree of dispersion of results for repeated testing of one sample. Reproducibility provides a measurement of the variability between analyses conducted by different operators at different laboratories on identical materials. Given that rapid tests are qualitative assays, the method of Langton et al. (Langton et al., 2002) was used to estimate the inter-laboratory concordance, which can be considered as an analogous quantity of reproducibility for qualitative data. The more appropriate way to determine inter-laboratory concordance is simply to enumerate all possible between laboratory pairings in the data.

The agreement between FAT and rapid tests results was estimated for each participant. For each laboratory, results obtained with FAT and immunochromatographic test were compared using the Kappa statistic test (Cohen J, 1960). The overall percentage of agreement was computed as the proportion of concordant results (positive + negative concordant test results) over all test results with corresponding binomial exact 95% confidence intervals. Cohen's Kappa values were calculated as well as 95% confidence intervals and interpreted using a commonly cited scale (Landis and Koch, 1977a).

3. Results

Twenty-two laboratories (100%) responded favourably and agreed to participate in the ILC. All of them sent back results for the three techniques.

3.1. The fluorescent antibody test

The FAT did not produce discordant result on the negative sample among the 22 participants (Tables 3 and 6).

Only two false negative results (1%) were reported by participants on 198 positive samples resulting in a concordance (sensitivity) of 99% (95% CI: 96.4-99.9%). These discordant results were obtained by laboratories L5 and L7 on the EBLV-1b sample.

All RABV samples were properly detected using the FAT (concordance of 100%).

3.2. The Anigen® rapid test

The Anigen® rapid test did not produce discordant result on the negative sample among the 22 participants (Tables 4 and 6).

Only one false negative result was reported by participants on 198 positive samples resulting in a concordance (sensitivity) of 99.5% (95% CI: 97.2-100%). This discordant result was obtained by laboratory L5 on the EBLV-1b sample.

All RABV samples were properly detected by the Anigen® rapid test (concordance of 100%).

For the Anigen® test, a concordance of 100% was observed for all but one sample (EBLV-1b). For the latter, a concordance of 91% was calculated. A concordance of 100% was calculated for the negative sample.

3.3. The CDIA™ rapid test

The CDIA™ rapid test did not produce discordant results on the negative sample among the 22 participants (Tables 5 and 6).

A total of forty-five false negative results were reported by laboratories on the 198 positive samples resulting in a concordance (sensitivity) of 77.3% (95% CI: 70.8 -82.9%). These discordant results (table 6) were obtained on the BBLV (20/22), EBLV-2 (19/22) and EBLV-1b samples (6/22).

All RABV samples and the EBLV-1a were properly detected by the CDIA™ rapid test (concordance of 100%).

For the CDIA™ rapid test, a concordance of 100% was observed for the negative sample and for all RABV strains and EBLV-1a but three positive samples (BBLV, EBLV-1b and EBLV-2). A concordance of 58.4%, 75.3% and 82.7% was calculated for the samples EBLV1-b, EBLV-2 and BBLV, respectively.

3.4. Agreement between FAT and rapid test results

When comparing FAT and Anigen® rapid test results, a perfect agreement (Cohen's kappa of 1) was observed for all but one laboratory. For laboratory L7, the EBLV-1b sample was detected negative by FAT whereas it was properly diagnosed using the Anigen® test resulting in a lower agreement between the 2 tests (Kappa = 0.615; 95% CI: -0.045 – 1).

The CDIA™ rapid test failed to detect the BBLV and the EBLV-2 strains for 19 and 20 laboratories respectively. For six more laboratories, the CDIA™ rapid test failed to detect the EBLV-1b strain as well. This inability to detect some lyssaviruses resulted in a kappa value that did not exceed 0.412 (95% CI: -0.145 – 1) for about 81% of laboratories corresponding to a moderate agreement between the FAT and the CDIA™ test. The best agreement (Kappa = 0.615; 95% CI: -0.045 – 1) was obtained for laboratories L12, L15 and L17.

4. Discussion

This work is part of the mandate assigned to European Union Reference Laboratories (EURLs) (*Commission Regulation (EC) No 737/2008 of 28 July 2008 designating the Community reference laboratories for rabies*, n.d.). Following its appointment as EURL for rabies, ANSES Nancy organizes annual inter-laboratory ring trials on rabies diagnosis. The objective of the present study was to evaluate the inter-laboratory performance of two immunochromatographic tests (Lateral Flow Assays) and the FAT, which is considered as a standard technique for rabies diagnosis. Beside this, the study offered the opportunity to some of the participating European National Reference Laboratories to carry out a lateral flow test intended for rabies diagnosis. Nowadays, many different LFAs for rabies diagnosis are commercialized and can be easily purchased on various e-commerce platforms. Some of them fade from sight just as quickly as they appeared on the market. These LFAs are intended to rabies diagnosis on brain material and/or saliva samples. For the present study, we selected two products, namely the Anigen® and the CDIA™ Rabies Virus Antigen Rapid Test (commercialized by Bionote and Creative Diagnostics Cie, respectively). While the Anigen® rapid test has already been evaluated in numerous studies (Certoma et al., 2018; Gury Dohmen et al., 2018; Kang et al., 2007; Servat et al., 2012), the CDIA™ rapid test was included in only one evaluation study together with the Anigen® Test (Eggerbauer et al., 2016). Most of time, these studies are focusing on determining the diagnostic performance of LFAs using different sets of naturally or experimentally infected samples coming from different laboratories. We decided to carry out the present ILC to evaluate the reproducibility of the two selected LFAs handled by different laboratories testing blindly the same panel of coded samples.

The FAT results obtained by laboratories on the panel of 10 samples (9 positives and one negative) were quite satisfactory. Only two laboratories (9%) obtained discrepant results, failing to detect the sample containing the EBLV-1b variant. This corroborates the results of the overview study on reference diagnosis techniques demonstrating the possible occurrence of discordant results of FAT when testing bat strains (Robardet et al., 2011).

More than 99% of the whole samples (n=218/220) were properly diagnosed by the participants.

The Anigen® rapid test results were quite similar to the FAT with only one laboratory (4.5%) that misdiagnosed the sample containing the EBLV-1b variant. About 99.5% of the whole samples (n=219/220) were properly diagnosed by the participants. A concordance of 100% was observed for all but one sample (91% for the EBLV- 1b sample) demonstrating an excellent inter-laboratory reproducibility of the Anigen® batch tested in this trial. The comparison of results between FAT and the Anigen® rapid test demonstrated a perfect agreement for 21 laboratories.

The overall performance of the CDIA™ rapid test was globally and significantly less satisfactory than the Anigen® rapid test. Whereas the kit perfectly detected all RABV strains (even the diluted GS strain) contained in five samples of the panel, it hardly detected bat lyssaviruses except EBLV-1a providing 100% of agreement. Variants EBLV-2 and BBLV were misdiagnosed in 19 and 20 laboratories respectively. In addition, whereas it perfectly detected the EBLV-1 sub lineage “a” in all laboratories, the rapid kit surprisingly failed to detect sub lineage “b” of EBLV-1 in six laboratories. As a result, the inter-laboratory reproducibility of the CDIA™ rapid test was perfect for RABV, but ranged from 58.4% to 82.7% for BBLV, EBLV-2 and EBLV-1b. The agreement with FAT results was moderate for most participants. However, the performance of the CDIA™ rapid test was consistent with its intended use i.e. detection of RABV antigen in dogs and cats.

Bat lyssaviruses EBLV-1, EBLV-2 and BBLV included in the panel are all phylogroup I viruses that also encompasses RABV. Full genome sequence analyses and antigenic cartography have demonstrated that BBLV is most closely related to EBLV-2 (Nolden et al., 2014) rather EBLV-1. The poor detection of both BBLV and EBLV-2 by the CDIA™ rapid test is therefore consistent with this previous observation. The reason why EBLV-1b was partially misdiagnosed when EBLV-1a was properly detected in all laboratories remains puzzling considering the close relatedness of both variants. This difference may be simply due to heterogeneous limits of detection between strips, or misinterpretation of faded coloured bands. These results suggest that monoclonal antibodies used in the CDIA™ rapid test have a limited detection capacity towards lyssaviruses of phylogroup I. On the contrary, monoclonal antibodies used in the Anigen® rapid test seem to have a broad spectrum detection for this phylogroup.

In this study, the Anigen® rapid test provided the best performance on the panel containing different viral strains: almost perfect agreement with the FAT, good inter-laboratory reproducibility. However, the results obtained in this trial must be considered with caution, as

they do not necessarily reflect what would be observed with field samples infected with RABV or other lyssaviruses. It was not feasible to undertake a trial using unpooled field samples as it is quite difficult to obtain specimens originating from naturally infected animals while insuring the homogeneity of samples for 22 participants. Hence, the ILC was performed on a reduced panel comprising samples obtained from pooled brains of experimentally infected mice that are generally highly susceptible to rabies through the intracerebral route. The performance of the two kits may probably differ if tested on a larger set of field samples with different viral loads. Moreover, it must be emphasized that in the present study participants were asked to dilute samples 1/6 instead of 1/10 as it is usually recommended in product leaflet so that to enhance the sensitivity of LFAs and improve the performance of the evaluated kits. The present study confirms that the performance of LFAs may be quite heterogeneous: two different kits may lead to different diagnostic results even on experimental infected samples containing high viral loads. It also demonstrates that some products may achieve more than their intended use and can detect not only RABV but also other lyssaviruses such as EBLV-1, EBLV-2, BBLV (Anigen®). On the contrary, some kits such as the CDIA™ are less pan-reactive and must be considered for the detection of RABV solely, as stated in the leaflet of the product. In the present ILC, both kits demonstrated a fairly good inter-laboratory reproducibility for RABV infected samples (intended-use) revealing a good homogeneity of tested batches.

Nevertheless, despite the good performance and reproducibility of the Anigen® rapid test in this specific situation, caution must be taken when using LFAs for rabies diagnosis. In a previous study (Eggerbauer et al., 2016), it was demonstrated that different LFAs may produce highly variable performances (diagnostic/analytical sensitivity, specificity) on different sets of samples, some kits being even totally incapable of detecting any rabies species in naturally or experimentally infected samples. However, in several other studies (Certoma et al., 2018; Gury Dohmen et al., 2018; Kang et al., 2007; Léchenne et al., 2016; Servat et al., 2012), the Anigen® performed quite satisfactorily and was considered as a promising tool for field use, where the test could be helpful for rapid rabies preliminary diagnostic results. Obviously, a lot still need to be done to improve the quality of LFAs to ensure they can detect, at least, any RABV in any part of the world. These tests could be probably refined as regards their sensitivity. In a previous study (Léchenne et al., 2016), authors have modified the test procedure by omitting a dilution step and placing the brain sample directly into the buffer vial provided with the kit. This modification, which led to a better sensitivity of the test and the higher intensity of the test band, indicates that improvements can be done. The selection of broad monoclonal/polyclonal antibodies is also critical to get products able to detect all RABV variants, and eventually other lyssavirus

species. Because veterinary diagnostic tests escape to approval process in many part of the world before being released, considerable attention should be paid before any use of rabies LFAs.

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Conflict of interest

None

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Table 1: List of participating laboratories

Laboratory name	Country
Institute for Veterinary Disease Control	AUSTRIA
SCIENSANO - NRC/NRL for rabies	BELGIUM
Laboratory for Rabies and general virology	CROATIA (Republic of)
Animal Health Laboratory - Virology Section	CYPRUS
State Veterinary Institute Prague/ SVÚ Praha	CZECH REPUBLIC
DTU National Veterinary laboratory	DENMARK
Estonian Veterinary and Food Laboratory	ESTONIA
Finnish Food Safety Authority Evira	FINLAND
Anses, Nancy Laboratory for Rabies and Wildlife	FRANCE
Friedrich-Loeffler-Institut, Institute of Molecular Virology and Cell Biology	GERMANY
Athens Veterinary Center, Virology Laboratory	GREECE
Istituto Zooprofilattico Sperimentale delle Venezie	ITALY
Institute of Food Safety, Animal Health and Environment BIOR	LATVIA
National Food and Veterinary Risk Assessment Institute	LITHUANIA
Laboratoire de Médecine Vétérinaire de l'Etat (LMVE)	LUXEMBOURG
Wageningen Bioveterinary Research	NETHERLANDS
National Veterinary Research Institute	POLAND
Institute for Diagnosis and Animal Health	ROMANIA
State Veterinary and Food Institute - Veterinary Institute Zvolen	SLOVAKIA
University of Ljubljana, Veterinary Faculty	SLOVENIA
Centro Nacional de Microbiología. Instituto de Salud Carlos III	SPAIN
APHA Weybridge	UNITED KINGDOM

Table 2: Composition of the panel of the inter-laboratory test for rabies diagnosis.

ID	Batch name	Passaged on	Strain Origin	Species	Country	Year of isolation	Original species
1	BBLV 07-16	Mouse	127900	BBLV	France	2012	<i>Myotis nattereri</i>
2	EBLV-1a 12-14	Mouse	122938	EBLV1	France	2002	<i>Eptesicus serotinus</i>
3	EBLV-1b 10-14	Mouse	123008	EBLV1	France	2002	<i>Eptesicus serotinus</i>
4	EBLV-2 05-16	Mouse	RV1787	EBLV2	United Kingdom	2004	<i>Myotis daubentonii</i>
5	RABV 10-13	Mouse	37-12	RABV	Rep. North Macedonia	2011	<i>Vulpes vulpes</i>
6	RABV 17-15	Mouse	124155	RABV	Morocco	2004	<i>Canis lupus familiaris</i>
7	RABV 12-15	Mouse	201020958	RABV	Spain	2010	<i>Canis lupus familiaris</i>
8	GS7 11-16	Mouse	GS7	RABV	France	1986	<i>Vulpes vulpes</i>
9	GS7 15-18 (1/30)	Mouse	GS7	RABV	France	1986	<i>Vulpes vulpes</i>
10	Negative 13-18	/	/	/	France	/	<i>Ovis aries</i>

Table 3: FAT results obtained for each strain

	n Concordant/ total	Concordant (%)	95% Interval confidence (%)
Negative samples	22/22	100	[84.6 – 100]
Positive samples	196/198	99	[96.4 – 99.9]
Positive and Negative samples	218/220	99.1	[96.8 – 99.9]
BBLV	22/22	100	[84.6 – 100]
EBLV-1a	22/22	100	[84.6 – 100]
EBLV-1b	20/22	90.9	[70.8 – 98.9]
EBLV-2	22/22	100	[84.6 – 100]
RABV Rep. North Macedonia	22/22	100	[84.6 – 100]
RABV Morocco	22/22	100	[84.6 – 100]
RABV Spain	22/22	100	[84.6 – 100]
GS-7	22/22	100	[84.6 – 100]
GS-7 1/30	22/22	100	[84.6 – 100]

Table 4: Anigen® rapid test results obtained for each strain

	n Concordant/ total	Concordant (%)	95% Interval confidence (%)
Negative samples	22/22	100	[84.6 – 100]
Positive samples	197/198	99.5	[97.2 – 100]
Positive and Negative samples	219/220	99.6	[97.5 – 100]
BBLV	22/22	100	[84.6 – 100]
EBLV-1a	22/22	100	[84.6 – 100]
EBLV-1b	21/22	95.5	[77.2 – 99.9]
EBLV-2	22/22	100	[84.6 – 100]
RABV Rep. North Macedonia	22/22	100	[84.6 – 100]
RABV Morocco	22/22	100	[84.6 – 100]
RABV Spain	22/22	100	[84.6 – 100]
GS-7	22/22	100	[84.6 – 100]
GS-7 1/30	22/22	100	[84.6 – 100]

Table 5: CDIA™ rapid test results obtained for each strain

	n Concordant/ total	Concordant (%)	95% Interval confidence (%)
Negative samples	22/22	100	[84.6 – 100]
Positive samples	153/198	77.3	[70.8 – 82.9]
Positive and Negative samples	175/220	79.6	[73.6 – 84.7]
BBLV	2/22	9.1	[11.2 – 29.2]
EBLV-1a	22/22	100	[84.6 – 100]
EBLV-1b	16/22	72.7	[49.8 – 89.3]
EBLV-2	3/22	13.6	[2.9 – 34.9]
RABV Rep. North Macedonia	22/22	100	[84.6 – 100]
RABV Morocco	22/22	100	[84.6 – 100]
RABV Spain	22/22	100	[84.6 – 100]
GS-7	22/22	100	[84.6 – 100]
GS-7 1/30	22/22	100	[84.6 – 100]

Table 6: Summary of diagnostic results obtained by participants using the FAT, the Anigen® and the CDIA™ rapid tests. Shaded “+” or “-“ signs indicate a discordant result (false positive or false negative)

Samples		BBLV	EBLV-1a	EBLV-1b	EBLV-2	RABV Macedonia	RABV Morocco	RABV Spain	RABV GS-7	RABV GS-7 1/30	Negative
Lab 1	FAT	+	+	+	+	+	+	+	+	+	-
	Anigen®	+	+	+	+	+	+	+	+	+	-
	CDIA™	-	+	+	-	+	+	+	+	+	-
Lab 2	FAT	+	+	+	+	+	+	+	+	+	-
	Anigen®	+	+	+	+	+	+	+	+	+	-
	CDIA™	-	+	-	-	+	+	+	+	+	-
Lab 3	FAT	+	+	+	+	+	+	+	+	+	-
	Anigen®	+	+	+	+	+	+	+	+	+	-
	CDIA™	+	+	-	-	+	+	+	+	+	-
Lab 4	FAT	+	+	+	+	+	+	+	+	+	-
	Anigen®	+	+	+	+	+	+	+	+	+	-
	CDIA™	-	+	+	-	+	+	+	+	+	-
Lab 5	FAT	+	+	-	+	+	+	+	+	+	-
	Anigen®	+	+	-	+	+	+	+	+	+	-
	CDIA™	-	+	-	-	+	+	+	+	+	-
Lab 6	FAT	+	+	+	+	+	+	+	+	+	-
	Anigen®	+	+	+	+	+	+	+	+	+	-
	CDIA™	-	+	-	-	+	+	+	+	+	-
Lab 7	FAT	+	+	-	+	+	+	+	+	+	-
	Anigen®	+	+	+	+	+	+	+	+	+	-
	CDIA™	-	+	+	+	+	+	+	+	+	-
Lab 8	FAT	+	+	+	+	+	+	+	+	+	-
	Anigen®	+	+	+	+	+	+	+	+	+	-
	CDIA™	-	+	+	-	+	+	+	+	+	-
Lab 9	FAT	+	+	+	+	+	+	+	+	+	-
	Anigen®	+	+	+	+	+	+	+	+	+	-
	CDIA™	-	+	+	-	+	+	+	+	+	-
Lab 10	FAT	+	+	+	+	+	+	+	+	+	-
	Anigen®	+	+	+	+	+	+	+	+	+	-
	CDIA™	-	+	+	-	+	+	+	+	+	-
Lab 11	FAT	+	+	+	+	+	+	+	+	+	-
	Anigen®	+	+	+	+	+	+	+	+	+	-
	CDIA™	-	+	+	-	+	+	+	+	+	-
Lab 12	FAT	+	+	+	+	+	+	+	+	+	-
	Anigen®	+	+	+	+	+	+	+	+	+	-
	CDIA™	-	+	+	+	+	+	+	+	+	-
Lab 13	FAT	+	+	+	+	+	+	+	+	+	-
	Anigen®	+	+	+	+	+	+	+	+	+	-
	CDIA™	-	+	+	-	+	+	+	+	+	-
Lab 14	FAT	+	+	+	+	+	+	+	+	+	-
	Anigen®	+	+	+	+	+	+	+	+	+	-
	CDIA™	-	+	+	-	+	+	+	+	+	-
Lab 15	FAT	+	+	+	+	+	+	+	+	+	-
	Anigen®	+	+	+	+	+	+	+	+	+	-
	CDIA™	+	+	+	-	+	+	+	+	+	-
Lab 16	FAT	+	+	+	+	+	+	+	+	+	-
	Anigen®	+	+	+	+	+	+	+	+	+	-
	CDIA™	-	+	+	-	+	+	+	+	+	-
Lab 17	FAT	+	+	+	+	+	+	+	+	+	-
	Anigen®	+	+	+	+	+	+	+	+	+	-
	CDIA™	-	+	+	+	+	+	+	+	+	-
Lab 18	FAT	+	+	+	+	+	+	+	+	+	-
	Anigen®	+	+	+	+	+	+	+	+	+	-
	CDIA™	-	+	+	-	+	+	+	+	+	-
Lab 19	FAT	+	+	+	+	+	+	+	+	+	-
	Anigen®	+	+	+	+	+	+	+	+	+	-
	CDIA™	-	+	-	-	+	+	+	+	+	-
Lab 20	FAT	+	+	+	+	+	+	+	+	+	-
	Anigen®	+	+	+	+	+	+	+	+	+	-
	CDIA™	-	+	+	-	+	+	+	+	+	-
Lab 21	FAT	+	+	+	+	+	+	+	+	+	-
	Anigen®	+	+	+	+	+	+	+	+	+	-
	CDIA™	-	+	+	-	+	+	+	+	+	-
Lab 22	FAT	+	+	+	+	+	+	+	+	+	-
	Anigen®	+	+	+	+	+	+	+	+	+	-
	CDIA™	-	+	-	-	+	+	+	+	+	-

