anses

Nancy Laboratory for Rabies and Wildlife

UNIT : Lyssavirus

European Union Reference Laboratory

for Rabies

PROFICIENCY TEST REPORT
Diag-11-2021-V0-EN

PROFICIENCY TEST FOR RABIES DIAGNOSIS

Session : 11-2021

Final report - Version 0

STUDY CODE : Diag-11-2021

	Name, First name	Function	Date	Signature
APPROVAL	ROBARDET Emmanuelle	Coordinator	30/08/2021	
AUTHORIZATION FOR DISTRIBUTION	MONCHATRE-LEROY Elodie	LRFSN Director	30/08/2021	

French Agency for Food, Environmental and Occupational Health Safety -
Nancy Laboratory for Rabies and Wildlife -
Technopole Agricole et Vétérinaire, Bâtiment H, CS 40 009, 54220 Malzéville - France
Tél +33.3.83.29.89.50 – Fax : +33.3.83.29.89.58

Table of contents

1. INTRODUCTION

An inter-laboratory trial dedicated to rabies diagnosis was organized by the European Union Reference Laboratory (EURL) for Rabies. The objective was to assess the rabies diagnosis performance of laboratories based on recommended techniques as the Fluorescent Antibody Test (FAT) (OIE, 2018; WHO, 2018), the Rabies Tissue Culture Infection test (RTCIT) (OIE, 2018; WHO, 2018) and the biological molecular techniques represented by the conventional (OIE, 2018; WHO, 2018) and the Real Time RT-PCR (OIE, 2018; WHO, 2018). This work was undertaken in the frame of the Commission Regulation (EU) No 415/2013 of 6 May 2013 laying down additional responsibilities and tasks of the EURL for Rabies and amending Regulation (EC) No 737/2008 designating the EURL for Rabies.

2. GENERAL INFORMATION

2.1 IDENTIFICATION OF COORDINATOR AND STAFF INVOLVED IN THE STUDY

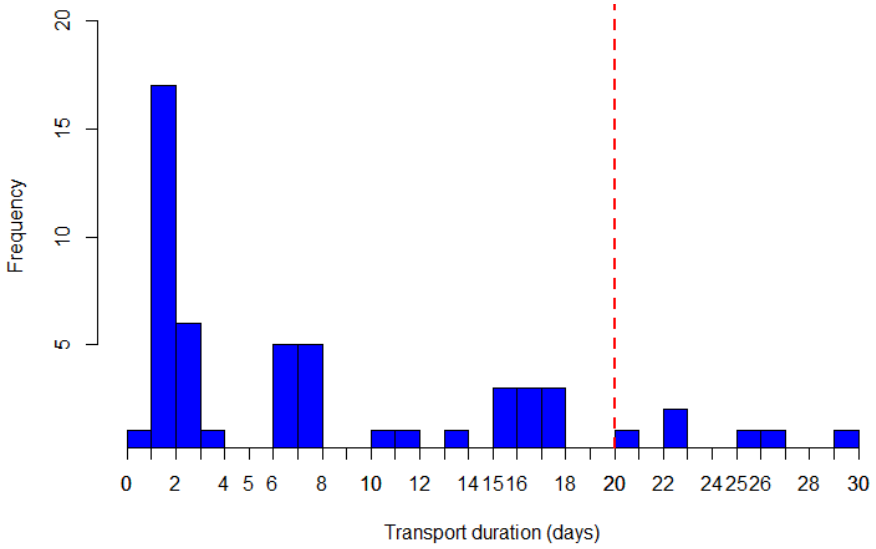
- **Director of the Laboratory**: E. Monchâtre-Leroy
- **EURL director and proficiency test Coordinator**: E. Robardet
- **Quality Manager**: F. Rizzo
- **Technical Staff**: J. Rieder and E. Longueval

2.2 INSTRUCTION TO PARTICIPANTS

The call for participation in the proficiency test for rabies diagnosis was sent to laboratories by e-mail in December 2020. At this step it was asked to participants to fill and sign the Anses Mutual Transfer Agreement, to frame work the exchange of strains and their utilisation and to take measure to have licence importation of samples ready for the transport, if requested, in accordance with the national custom clearance regulation.

A panel of 10 coded samples to be tested was sent to participating laboratories by a specialized carrier. Each sample contained 1ml of lyophilised homogenate of brains (animal origin) and was susceptible to be infected by a RABV, EBLV-1, EBLV-2, BBLV, DUVV or ABLV strain. The shipment of the panel to the laboratories started on May 31st 2021 at 4°C temperature by an international agreed carrier and under UN3373 conditions as specified by international regulation for the shipment of biological samples. All the panels were received by the participating laboratories during the time range where stability was ensured (20 days) except for six laboratories (L12; L26; L41; L44; L50; L56). Globally, 34% of the panels were received within 2 days, 47% within 6 days; 70% within 13 days and 89% within 20 days (Figure 1). Before its use, it was asked to laboratories to store the panel at 4°C as soon as received. Web links were sent to participants to fill online the acknowledgment (checking the satisfactory condition of reception of the samples) and the result forms.

Figure 1: Transport duration of panels for the 2021 session. *Data based on 53 laboratories, according to their date reception statements, except for 4 laboratories for which carrier data was used.*



Laboratories were requested to provide a unique diagnosis conclusion per sample according their own rabies diagnosis procedure to mimic as much as possible routine diagnosis condition of participating laboratories. This diagnosis was the unique performance evaluation criteria of this proficiency test. The techniques considered to test the samples and for which the panel was validated were the following (s): FAT (Fluorescent Antibody test) and/or RTCIT (Rabies Tissue Culture Infection test) and/or conventional RT-PCR and/or Real Time RT-PCR. Expected diagnosis conclusion was a binomial response “positive” or “negative”.

Instruction to participants (FO-PQEILA-EXE-R_INFORMATION-4) was sent in the same time than the call for participation sent in December. This document explained among others that the participant will not be evaluated in case of one or more missing value in the column positive or negative of the result form, in case of reception of the panel out of the limit of evaluated stability, or in case of result reception after the fixed deadline without prior to EURL agreement. The deadline for result reception was fixed on July 5th 2021.

2.3 REGISTERED PARTICIPATING LABORATORIES

In 2021, fifty-eight laboratories were registered in the inter-laboratory test for rabies diagnosis. Registered laboratories includes 25 National Reference Laboratories (NRLs) from the European Union (EU) and 33 laboratories from third countries (including 11 laboratories participating under EC fundings).

Forty-seven laboratories were registered for the FAT, 31 for the RTCIT, 32 for the conventional RT-PCR and 36 for the Real Time RT-PCR (Table 1). Proportion of registration for the different tests was 98% for the FAT, 53% for the RTCIT, 55% for the conventional RT-PCR and 62% for the Real Time RT-PCR.

Table 1: Number of laboratories registered as participants for the different techniques covered by 2021 rabies diagnosis inter-laboratory test.

	European Union NRLs	Third country laboratories participating under EC fundings	Third country laboratories participating not under EC fundings	**Total**
FAT	24	11	22	**57**
RTCIT	15	6	10	**31**
Conventional RT-PCR	16	4	12	**32**
Real Time RT- PCR	22	2	12	**36**
Total Laboratories	25	11	22	**58**

3. PROFICIENCY TEST ITEMS

3.1 PREPARATION OF THE PROFICIENCY TEST ITEMS

The virus batches used in this study were produced by intra-cerebral inoculation of mice to reproduce as much as possible standard conditions of an animal infection. Viruses were produced according to the animal experimentation directives issued by the French Ethic Committee (Ethic committee approval number 17-076 and ministery agreement number #11772-2017101311312783). For each batch of virus, brains were excised after the death of the animals then mixed, homogenized, aliquoted into 1ml tubes and then freeze-dried. The positive samples of the panel were finally constituted of 7 blindly coded samples of freeze-dried homogenized brains infected with various rabies virus species.

The strains involved in the inter-laboratory test were:

- RABV Greece: a classical rabies virus (RABV) fox strain isolated in Greece in 2012.
- CVS 27: a RABV fixed strain.
- RABV dog Es: a RABV dog strain isolated in Spain in 2010.
- BBLV: a Bokeloh bat lyssavirus strain isolated in France in 2012.
- EBLV-1b: an European bat lyssavirus type 1 isolated in France in 2002.
- DUVV: a Duvenhage strain isolated in South Africa in 1971.
- Vaccinal Strain: a strain of vaccine induced case isolated in Latvia in 2014.

The material used as negative batch was negative brains of carnivores from French wild origin. An additional sample was also included in the panel test of each participant to avoid collusion. This sample differed from one laboratory to another and was originated from previous virus batches. These samples named “MIX” were not included in the evaluation process of the laboratories.

3.2 IDENTIFICATION OF THE PROFICIENCY TEST ITEMS

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. Each item was dully labelled.

3.3 HOMOGENEITY

The evaluation of the homogeneity was undertaken for each positive batch by analysing in duplicate 10 randomly chosen samples.

- For all of them: FAT, RTCIT, Real Time RT-PCR SYBR Green was performed.
- For 3 samples, conventional RT-PCR and rapid genotyping by Taqman Real Time RT-PCR was also performed.
- For 1 sample, strain typing by sequencing was perfomed.

The evaluation of the homogeneity was undertaken for each negative batch by analysing in duplicate 10 randomly chosen samples by the following techniques: FAT, RTCIT, Real Time RT-PCR SYBR Green and conventional RT-PCR.

The analysis was performed after lyophilisation when all the samples were under their final form for the positive batches (RABV Greece, CVS27, RABV Dog Esp, BBLV, EBLV-1b, DUVV, Vaccinal Strain) and for the negative batches. All the batches were declared homogeneous as all the results were concordant to the expectations (positive samples were all found positives and negative samples were all found negatives whatever the technique used).

3.4 STABILITY

Stability of the panel was evaluated just before sending the panel under 4 different conditions:

- At day 0 (D0)
- After one week at Room Temperature (RT) (D6 and not D7 due to calendar constrains)
- After two weeks at RT (D14)
- After three weeks at RT (D21)

The samples were analysed with the FAT, the RTCIT and the Real Time RT-PCR SYBR Green to ensure that positive samples were positive and that negative samples were negative. The transport conditions requested to the specialised carrier being at +4°C, conditions tested were in consequence considered extreme situations. In the laboratory, lyophilised samples stored at +4°C have indeed been shown stable for 5 years.

Under the 4 conditions, all the positives samples were tested as positives and all negatives samples were tested as negatives.

Stability of the panel was consequently declared for 20 days of transport duration even with thawed ice-blocks conditions.

4. LABORATORY PERFORMANCE EVALUATION

It was requested to laboratories to test the panel as usually done in their laboratory, by using their current own protocol to declare a rabies diagnosis conclusion for each sample. The rabies diagnosis conclusion per sample had to be expressed as a binomial response “positive” or “negative”.

For the rabies diagnosis performance evaluation, correct results on the overall diagnosis conclusion were required for each sample meaning that the evaluation was considered as “satisfactory” when the participating laboratory declared the rabies diagnosis conclusion corresponding to the determined status of the samples (positive or negative) while the evaluation was considered “unsatisfactory” when at least discordant result occurs.

A performance score was attributed to the laboratories according to the number of discordant results as followed:

No discrepant results: Score 1 = Satisfactory performance
1 discrepant result : Score -1 = Unsatisfactory performance
2 discrepant results: Score -2 = Unsatisfactory performance
3 discrepant results: Score -3 = Unsatisfactory performance
4 discrepant results: Score -4 = Unsatisfactory performance
5 discrepant results: Score -5 = Unsatisfactory performance
6 discrepant results: Score -6 = Unsatisfactory performance
7 discrepant results: Score -7 = Unsatisfactory performance
8 discrepant results: Score -8 = Unsatisfactory performance
9 discrepant results: Score -9 = Unsatisfactory performance

5. RESULTS

Fifty laboratories provided answer to the overall rabies diagnosis assessment (Table 2 and table 4).

Six laboratories experienced long transport duration out of the range where stability was declared (>20 days). As stated in the proficiency test instruction FO-PQEILA-EXE-R_INFORMATION-4, the performance of those laboratories was not evaluated (**L12; L26; L41; L44; L50; L56**).

On the 44 evaluated laboratories, two laboratories (**L09** and **L31**) (5% of evaluated laboratories) provided at least one discrepant result: a false positive diagnosis conclusion on a negative sample (**L09** and **L31**) and a false negative conclusion on a CVS 27 positive sample (**L31**).

Table 2: Overall diagnosis conclusion results per strain

	n laboratories with discrepant results/ total laboratories	% of laboratories with discrepant results and 95 CI
Total	2/44	4.5 [0.6 – 15.5]
Negative samples	2/44	4.5 [0.6 – 15.5]
Positive samples	1/44	2.3 [0.1 – 12.0]
RABV samples	1/44	2.3 [0.1 – 12.0]
CVS 27	1/44	2.3 [0.1 – 12.0]
RABV Greece	0/44	0 [0.0 – 8.0]
RABV Dog Es	0/44	0 [0.0 – 8.0]
Vaccinal strain	0/44	0 [0.0 – 8.0]
EBLV-1	0/43	0 [0.0 – 8.2]
BBLV	0/42	0 [0.0 – 8.4]
DUVV	0/43	0 [0.0 – 8.2]

Individual laboratory scores for rabies diagnosis and corresponding performance evaluations are indicated in the table 3.

Table 3: Rabies diagnosis performance evaluation per laboratory. NA: Not applicable.

Lab code	Score	Evaluation
L02	1	Satisfactory
L03	1	Satisfactory
L05	1	Satisfactory
L06	1	Satisfactory
L07	1	Satisfactory
L08	1	Satisfactory
L09	**-1**	**Unsatisfactory**
L10	1	Satisfactory
L11	1	Satisfactory
L12	NA	Not evaluated
L13	1	Satisfactory
L14	1	Satisfactory
L15	1	Satisfactory
L16	1	Satisfactory
L17	1	Satisfactory
L18	1	Satisfactory
L20	1	Satisfactory
L21	1	Satisfactory
L22	1	Satisfactory
L23	1	Satisfactory
L24	1	Satisfactory
L25	1	Satisfactory
L26	NA	Not evaluated
L27	1	Satisfactory
L28	1	Satisfactory
L29	1	Satisfactory
L30	1	Satisfactory
L31	**-2**	**Unsatisfactory**
L32	1	Satisfactory
L33	1	Satisfactory
L34	1	Satisfactory
L35	1	Satisfactory
L36	1	Satisfactory
L37	1	Satisfactory
L38	1	Satisfactory
L40	1	Satisfactory

Lab code	Score	Evaluation
L41	NA	Not evaluated
L42	1	Satisfactory
L44	NA	Not evaluated
L46	1	Satisfactory
L48	1	Satisfactory
L49	1	Satisfactory
L50	NA	Not evaluated
L51	1	Satisfactory
L52	1	Satisfactory
L54	1	Satisfactory
L55	1	Satisfactory
L56	NA	Not evaluated
L57	1	Satisfactory
L58	1	Satisfactory

Table 4: Results of the overall diagnosis conclusion. Coded laboratories written in red are those that did not provide satisfactory results.
Pink box: positive result. Green box: negative result. Grey box because result not considered: the participant is not evaluated as the stability of the samples is not ensured due to transport duration >20 days or Mix samples (included to avoid collusion).

Lab code	FAT	RTCIT	RT-PCR	RT-qPCR	Negative 1		Negative 2		RABV Greece		CVS 27		RABV Dog Es		BBLV		EBLV-1b		DUVV		Vaccinal strain		MIX	
L02	x	x	x	x	21060294	Negative	21060201	Negative	21060298	Positive	21060208	Positive	21060252	Positive	21060265	Positive	21060238	Positive	21060291	Positive	21060217	Positive	21060268	Positive
L03	x	x	x	x	21060374	Negative	21060370	Negative	21060337	Positive	21060329	Positive	21060324	Positive	21060394	Positive	21060372	Positive	21060338	Positive	21060382	Positive	21060375	Positive
L05	x				21060500	Negative	21060504	Negative	21060505	Positive	21060517	Positive	21060501	Positive	21060547	Positive	21060553	Positive	21060502	Positive	21060511	Positive	21060546	Positive
L06	x	x	x	x	21060686	Negative	21060650	Negative	21060684	Positive	21060685	Positive	21060636	Positive	21060680	Positive	21060623	Positive	21060663	Positive	21060600	Positive	21060639	Positive
L07	x	x	x		21060780	Negative	21060794	Negative	21060742	Positive	21060787	Positive	21060704	Positive	21060719	Positive	21060751	Positive	21060706	Positive	21060736	Positive	21060735	Positive
L08	x	x	x	x	21060859	Negative	21060867	Negative	21060817	Positive	21060869	Positive	21060816	Positive	21060805	Positive	21060874	Positive	21060833	Positive	21060824	Positive	21060889	Positive
L09					21060972	Negative	21060960	Positive	21060986	Positive	21060903	Positive	21060988	Positive	21060973	Positive	21060919	Positive	21060902	Positive	21060953	Positive	21060916	Positive
L10	x	x		x	21061016	Negative	21061075	Negative	21061045	Positive	21061091	Positive	21061030	Positive	21061062	Positive	21061007	Positive	21061020	Positive	21061073	Positive	21061074	Positive
L11	x	x	x		21061156	Negative	21061135	Negative	21061120	Positive	21061164	Positive	21061174	Positive	21061147	Positive	21061121	Positive	21061130	Positive	21061110	Positive	21061145	Positive
L12	x				21061242	Negative	21061296	Negative	21061264	Positive	21061200	Positive	21061212	Positive	21061222	Positive	21061201	Positive	21061209	Positive	21061241	Positive	21061275	Positive
L13	x	x	x		21061322	Negative	21061309	Negative	21061308	Positive	21061389	Positive	21061380	Positive	21061335	Positive	21061355	Positive	21061388	Positive	21061360	Positive	21061313	Positive
L14	x	x	x	x	21061464	Negative	21061462	Negative	21061455	Positive	21061478	Positive	21061477	Positive	21061479	Positive	21061409	Positive	21061400	Positive	21061433	Positive	21061443	Positive
L15	x	x	x	x	21061566	Negative	21061559	Negative	21061540	Positive	21061546	Positive	21061508	Positive	21061501	Positive	21061582	Positive	21061591	Positive	21061524	Positive	21061533	Positive
L16	x		x	x	21061674	Negative	21061640	Negative	21061699	Positive	21061612	Positive	21061679	Positive	21061600	Positive	21061689	Positive	21061675	Positive	21061666	Positive	21061620	Positive
L17	x		x	x	21061706	Negative	21061772	Negative	21061710	Positive	21061757	Positive	21061785	Positive	21061766	Positive	21061717	Positive	21061764	Positive	21061761	Positive	21061746	Positive
L18	x	x	x	x	21061870	Negative	21061867	Negative	21061817	Positive	21061864	Positive	21061884	Positive	21061892	Positive	21061874	Positive	21061863	Positive	21061810	Positive	21061813	Positive
L20	x	x	x	x	21062046	Negative	21062025	Negative	21062088	Positive	21062091	Positive	21062045	Positive	21062087	Positive	21062097	Positive	21062071	Positive	21062019	Positive	21062048	Positive
L21					21062120	Negative	21062165	Negative	21062108	Positive	21062185	Positive	21062100	Positive	21062152	Positive	21062198	Positive	21062109	Positive	21062174	Positive	21062129	Positive
L22	x				21062234	Negative	21062200	Negative	21062266	Positive	21062219	Positive	21062283	Positive	21062289	Positive	21062212	Positive	21062215	Positive	21062205	Positive	21062260	Positive
L23	x				21062306	Negative	21062386	Negative	21062388	Positive	21062371	Positive	21062387	Positive	21062394	Positive	21062398	Positive	21062374	Positive	21062305	Positive	21062382	Positive
L24	x			x	21062407	Negative	21062455	Negative	21062495	Positive	21062456	Positive	21062430	Positive	21062452	Positive	21062437	Positive	21062400	Positive	21062449	Positive	21062458	Positive
L25	x				21062533	Negative	21062550	Negative	21062593	Positive	21062521	Positive	21062553	Positive	21062560	Positive	21062583	Positive	21062515	Positive	21062523	Positive	21062580	Positive
L26	x		x		21062653	Negative	21062682	Negative	21062625	Positive	21062600	Positive	21062648	Positive	21062614	Positive	21062622	Positive	21062601	Positive	21062681	Positive	21062616	Positive
L27					21062757	Negative	21062788	Negative	21062767	Positive	21062786	Positive	21062700	Positive	21062795	Positive	21062797	Positive	21062777	Positive	21062734	Positive	21062784	Positive
L28	x		x	x	21062857	Negative	21062843	Negative	21062882	Positive	21062875	Positive	21062801	Positive	21062807	Positive	21062871	Positive	21062841	Positive	21062814	Positive	21062874	Positive
L29	x	x	x	x	21062928	Negative	21062967	Negative	21062974	Positive	21062930	Positive	21062951	Positive	21062921	Positive	21062979	Positive	21062992	Positive	21062957	Positive	21062948	Positive
L30	x		x		21063078	Negative	21063059	Negative	21063086	Positive	21063047	Positive	21063093	Positive	21063087	Positive	21063019	Positive	21063010	Positive	21063084	Positive	21063041	Positive
L31	x		x	x	21063140	Positive	21063168	Negative	21063177	Positive	21063188	Negative	21063146	Positive	21063122	Positive	21063160	Positive	21063115	Positive	21063112	Positive	21063141	Positive
L32	x	x		x	21063253	Negative	21063244	Negative	21063297	Positive	21063293	Positive	21063243	Positive	21063291	Positive	21063206	Positive	21063299	Positive	21063233	Positive	21063210	Positive
L33	x	x	x	x	21063321	Negative	21063353	Negative	21063350	Positive	21063306	Positive	21063311	Positive	21063326	Positive	21063307	Positive	21063378	Positive	21063301	Positive	21063394	Positive
L34					21063427	Negative	21063490	Negative	21063430	Positive	21063431	Positive	21063433	Positive	21063489	Positive	21063446	Positive	21063432	Positive	21063428	Positive	21063474	Positive
L35	x	x	x	x	21063516	Negative	21063511	Negative	21063545	Positive	21063578	Positive	21063523	Positive	21063591	Positive	21063557	Positive	21063504	Positive	21063515	Positive	21063532	Positive
L36	x			x	21063691	Negative	21063656	Negative	21063685	Positive	21063606	Positive	21063692	Positive	21063617	Positive	21063673	Positive	21063679	Positive	21063675	Positive	21063609	Positive
L37					21063722	Negative	21063711	Negative	21063749	Positive	21063783	Positive	21063790	Positive	21063719	Positive	21063717	Positive	21063737	Positive	21063733	Positive	21063725	Positive
L38	x		x		21063890	Negative	21063810	Negative	21063836	Positive	21063814	Positive	21063830	Positive	21063843	Positive	21063852	Positive	21063837	Positive	21063842	Positive	21063804	Positive
L40					21064005	Negative	21064093	Negative	21064063	Positive	21064042	Positive	21064008	Positive	21064062	Positive	21064097	Positive	21064047	Positive	21064034	Positive	21064051	Positive
L41	x	x	x		21064122	Negative	21064184	Negative	21064154	Positive	21064141	Positive	21064126	Positive	21064159	Positive	21064123	Positive	21064110	Positive	21064158	Positive	21064116	Positive
L42	x	x	x		21064224	Negative	21064227	Negative	21064286	Positive	21064294	Positive	21064237	Positive	21064232	Positive	21064277	Positive	21064210	Positive	21064226	Positive	21064207	Positive
L44	x	x			21064400	Negative	21064426	Negative	21064445	Positive	21064490	Positive	21064497	Positive	21064471	Positive	21064452	Positive	21064447	Positive	21064449	Positive	21064428	Positive
L46	x	x	x	x	21064630	Negative	21064674	Negative	21064605	Positive	21064683	Positive	21064648	Positive	21064626	Positive	21064600	Positive	21064644	Positive	21064618	Positive	21064615	Positive
L48	x			x	21064892	Negative	21064872	Negative	21064854	Positive	21064851	Positive	21064868	Positive	21064800	Positive	21064841	Positive	21064875	Positive	21064805	Positive	21064888	Positive
L49	x			x	21064990	Negative	21064993	Negative	21064980	Positive	21064944	Positive	21064951	Positive	21064932	Positive	21064992	Positive	21064949	Positive	21064947	Positive	21064938	Positive
L50	x				21065065	Negative	21065098	Negative	21065011	Positive	21065048	Positive	21065068	Positive	21065099	Negative	21065047	Positive	21065078	Positive	21065072	Positive	21065040	Positive
L51	x	x	x	x	21065198	Negative	21065169	Negative	21065143	Positive	21065128	Positive	21065195	Positive	21065125	Positive	21065130	Positive	21065158	Positive	21065131	Positive	21065172	Positive
L52	x	x	x	x	21065224	Negative	21065231	Negative	21065207	Positive	21065299	Positive	21065213	Positive	21065294	Positive	21065240	Positive	21065225	Positive	21065212	Positive	21065264	Positive
L54	x	x	x	x	21065464	Negative	21065451	Negative	21065443	Positive	21065496	Positive	21065436	Positive	21065444	Positive	21065400	Positive	21065481	Positive	21065494	Positive	21065435	Positive
L55	x		x	x	21065542	Negative	21065510	Negative	21065505	Positive	21065524	Positive	21065535	Positive	21065570	Positive	21065563	Positive	21065504	Positive	21065554	Positive	21065507	Positive
L56	x		x	x	21065675	Negative	21065647	Positive	21065697	Positive	21065695	Positive	21065694	Positive	21065610	Negative	21065668	Positive	21065676	Positive	21065679	Positive	21065682	Positive
L57	x		x	x	21065767	Negative	21065773	Negative	21065714	Positive	21065777	Positive	21065749	Positive	21065742	Positive	21065778	Positive	21065701	Positive	21065768	Positive	21065723	Positive
L58					21065890	Negative	21065898	Negative	21065894	Positive	21065865	Positive	21065808	Positive	21065889	Positive	21065882	Positive	21065884	Positive	21065873	Positive	21065802	Positive

6. FOLLOW-UP OF INDIVIDUAL PERFORMANCES ON OVERALL DIAGNOSIS CONCLUSION

Within the last three sessions, where overall diagnosis conclusion evaluation was assessed, the number of laboratories presenting an unsatisfactory evaluation vary from 1 to 2 per year (1 laboratory in 2017; 2 in 2019 and 2 in 2021). Unsatisfactory evaluation was an isolated event as none of the evaluated laboratories repeated this event. Laboratories with unsatisfactory evaluation in 2021 are laboratories that did not participate the last two years (Table 5).

Table 5: Performance evaluation evolution of participating laboratories in the 2021 session. S: Satisfactory evaluation; UNS: Unsatisfactory evaluation.

Laboratory code 2021	2017	2019	2021
L02		S	S
L03	S	S	S
L05	S	S	S
L06	S	S	S
L07			S
L08	S	S	S
L09			**UNS**
L10		S	S
L11	S	S	S
L13	S	S	S
L14	S	S	S
L15	S	S	S
L16	S	S	S
L17	S	S	S
L18	S	S	S
L20	S	S	S
L21	S	S	S
L22	S	S	S
L23	S	S	S
L24	S		S
L25			S
L27	S	S	S
L28	S	S	S
L29	S	S	S
L30	S	S	S
L31			**UNS**
L32	S	S	S
L33	S	S	S
L34			S
L35	S	S	S

Laboratory code 2021	2017	2019	2021
L36	S	S	S
L37	S	S	S
L38	S		S
L40	S	S	S
L42	S		S
L46	S		S
L48	S	S	S
L49	S	S	S
L51	S	S	S
L52	S	S	S
L54	S	S	S
L55	S	S	S
L57	S	S	S
L58	S	S	S

7. REFERENCES

OIE, 2018, Manual of standards for diagnostic tests and vaccines, Rabies, Chapter 3.1.17. 35 p. *http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.01.17_RABIES.pdf*

World Health Organization, Rupprecht, Charles E, Fooks, Anthony R & Abela-Ridder, Bernadette. (2018). Laboratory techniques in rabies, volume 1, 5th ed. World Health Organization. *https://apps.who.int/iris/handle/10665/310836. Licence: CC BY-NC-SA 3.0 IGO. 289 p.*

8. ACKNOWLEGMENTS

This study was funded by the European Commission and by ANSES.

We would like to thank the ANSES staff involved in this study for carrying out the technical work:

- panel constitution and result reception: J. Rieder and E. Longueval;
- experiment on mice and rabies diagnosis: V. Brogat, S. Kempff and E. Litaize under the management of A. Servat;
- molecular biology: M. Badre-Biarnais, C. Peytavin and J.L. Schereffer under the management of E. Picard-Meyer.

We would like also to thank scientists who kindly provided the strains used for the trial.