Maisons-Alfort Laboratory
For Animal Health

Virology Unit

European Union Reference Laboratory
for equine diseases

INTERLABORATORY PROFICIENCY TEST FINAL REPORT ILPT / AHL / VIRO / VSV / 2014 / 1

PT-VSV/2014/RT-PCR

Date of ILPT: November 2014

Final Report - Version 1

ILPT CODE : VSV / 2014 / RT-PCR

	Surname, First Name	Position	Date	Signature
REDACTOR	Romey Aurore	Technical responsible and Coordinator	December 16th 2014	
APPROVAL (authorization for distribution)	Zientara Stephan	Head of EU-RL	December 16th 2014	

French agency for food, environnemental and occupational health safety – Maisons-Alfort Laboratory for animal health
23, avenue du Général de Gaulle – 94 706 Maisons-Alfort Cedex – Tél : +33 (0)1 49 77 13 00 - Télécopie : + 33 (0)1 49 77 26 95 - www.anses.fr

PT-VSV/2014/RT-PCR

INTERLABORATORY PROFICIENCY TESTING CONTRIBUTORS	
Name, First name	**Function**
ROMEY Aurore	Technical responsible
ROMEY Aurore	Data treatment responsible
BAKKALI-KASSIMI Labib	Quality control correspondent

LIST OF PARTICIPANTS INVOLVED IN ORGANISATION OF THE ILPT	
ROMEY Aurore	Samples preparation
RELMY Anthony	Homogeneity test

CONTENTS

1. INTRODUCTION

Objectives of this PT were (i) to consolidate European Laboratory Network for the diagnosis of vesicular stomatitis virus (VSV), (ii) to increase inventory of RT-PCR methods available for detection and characterization of VSV and to evaluate their performances in order to improve molecular diagnosis of VSV, (iii) to evaluate the real-time RT-PCR method available on the EURL for Equine diseases website, for VSV detection and typing.

2. GENERAL INFORMATION

2.1 PARTICIPANTS

14 European laboratories and the EU-RL participated to this PT:

- AGES Institute for Veterinary Disease Control Moedling, Austria
- CODA CERVA, Unit for Vesicular and Exotic diseases, Belgium
- European Union Reference Laboratory for equine diseases, Anses, France
- FLI, Friedrich Loeffler Institute, Germany
- IAH, The Pirbright Institute, United Kingdom
- INIAV, Instituto Nacional de Investigação Agrária e Veterinária, Laboratório de Virologia, Portugal
- Institute for diagnosis and animal health, Romania
- Institute of Food safety "BIOR", Animal Health and Environment, Latvia
- IZSLER, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Italy
- Laboratorio Central Veterinario-Sanidad Animal, Spain
- Laboratory vesicular diseases, Departement of Virology, The Netherlands
- National veterinary laboratory Technical University, Division of Virology Lindholm, Denmark
- National Veterinary Research Institute, Department of Foot and Mouth Disease, Poland
- State Veterinary Institute Prague, Czech Republic
- SVFI, State Veterinary institute in Zvolen, Slovakia

2.2 OPERATION OF PROFICIENCY TESTING ROUND AND INSTRUCTION TO PARTICIPANTS

➢ On July 18th, 23 Europeans laboratories received invitation mail to participate to VSV-PT. On September 26th, Laboratories which were interested in participating in this PT received invitation letter (Annexe MQE VII.01), information on the interlaboratory test plan (F MQE VII.01) and instructions for carrying out the test (F MQE VII.07). All participants completed and returned participation form (F MQE VII.03) before October 6th.

➢ Samples were sent to participants on October 27th under dry ice. All participants completed and returned acknowledgment of receipt (F MQE VII.04) before November 5th.

➢ Participants were given three weeks to return their results (by November 17th). 14 participants returned their results on time and one returned the results on December 8th. This participant did not receive the purchased RT-PCR reagents on time.

➢ All participants received individual report on their results on December 9th.

➢ The final report and a satisfaction questionnaire, to be completed and returned, were sent to all participants on December 16th (F MQE X.01).

3. PROFICIENCY TEST ITEMS

3.1 PREPARATION OF THE PROFICIENCY TEST ITEMS

➢ PT is based on a panel of 6 RNA samples (A to F).

➢ General preparation:

Two VSV strains were used to establish the PT panel: New Jersey Hazlehurst ($10^{6.54}$ $TCID_{50}$/25µl) (accession number M20166) and Indiana 1 Mudd-Summers ($10^{5.65}$ $TCID_{50}$/25µl) (accession number EU849003). RNA extractions were performed from 140µl of VSV infected or non infected Vero cells (African green monkey kidney) by using the automatic QIAcube (QIAGEN) and the QIAamp viral extraction kit, according to manufacturer's recommendations. RNA elution was performed in 100µl. PT samples were prepared by making dilutions of the RNA in RNA Safe Buffer (50ng/µl Carrier polyA-RNA, 0.05% Tween 20, 0.05% sodium acid).

➢ Preparation of samples A to D:

Pure RNA and serial dilutions from 10^{-1} to 10^{-6} were analysed by real-time RT-PCR. Based on the results obtained, RNA from VSV-NJ was diluted at 10^{-1} and 10^{-3} (respectively samples A and B), and RNA from VSV-IND1 was diluted at 10^{-2} and 10^{-4} (respectively samples C and D).

➢ Preparation of samples E and F:

RNA was extracted from non infected cells and diluted at 10^{-3} (samples E and F).

3.2 IDENTIFICATION OF THE PROFICIENCY TEST ITEMS

A panel of 6 samples representing 5 levels of contamination was sent to each laboratory:

Laboratory code	Sample A VSV NJ 10^{-1}	Sample B VSV NJ 10^{-3}	Sample C VSV IND1 10^{-2}	Sample D VSV IND1 10^{-4}	Sample E Negative	Sample F Negative
	Sample code	Sample code	Sample code	Sample code	Sample code	Sample code
1	178	49	158	33	120	157
2	21	66	26	147	162	20
3	94	9	89	141	72	73
4	83	19	93	52	129	6
5	75	125	128	37	136	45
6	70	105	174	131	88	104
7	40	57	43	154	143	76
8	56	109	29	172	16	27
9	167	140	170	155	117	39
10	77	134	22	150	11	164
11	64	79	151	7	90	149
12	146	102	84	108	62	51
13	121	74	148	124	61	2
14	142	85	168	82	166	44
15	137	161	25	179	38	4

3.3 HOMOGENEITY

Three panels were analyzed separately on October 21st, by real-time RT-PCR. Samples A and B from the three panels were tested positives for New Jersey serotype. Samples C and D from the three panels were tested positives for Indiana serotype. Samples E and F from the three panels were tested negatives. Standard deviation lower than 1 Ct value was retained for homogeneity conformity acceptation. Results of the three panels were satisfactory. Panels were considered as homogeneous.

3.4 STABILITY

From October 27th to November 17th, one complete panel was stored at -80°C, then analysed on November 17th, by real-time RT-PCR. Samples A and B were tested positives for New Jersey serotype. Samples C and D were tested positives for Indiana serotype. Samples E and F were tested negatives. Stability of the samples was accepted if the difference between the average Ct values of homogeneity and the Ct value obtained with stability panel samples is lower than 2 Ct. Results were satisfactory. Panels were considered stable at -80°C for 3 weeks.

4. RESULTS FROM ALL PARTICIPANTS

Results were considered valid if:

- Negatives samples (E and F) were tested negatives
- Positives samples (A to D) were detected as VSV and/or specifically typed.

4.1 METHODS AND QUALITATIVE RESULTS

• **Lab1**

Method 1: classical RT-PCR for typing describes by Hole, K. *et al.* (2010)

Primers: NJ forward 5'-TGATTCAATATAATTATTTTGGGAC-3' (7230-7254)
NJ reverse 5'-AGGCTCAGAGGCATGTTCAT-3' (7476-7495)
IND forward 5'-TGATACAGTACAATTATTTTGGGAC-3' (7230-7254)
IND reverse 5'-GAGACTTTCTGTTACGGGATCTGG-3' (7433-7456)

Kit: Multisccribe RT (Life technologies) + Go Taq hot start green master mix (Promega)

Amplification cycles: 48°C-30min, 95°C-2min, 40x (95°C-30sec, 54°C-30sec, 72°C-30sec)

Method 2: classical RT-PCR for detection describes by Fernandez, J. *et al.* (2008)

Primers: VSV forward 5'-AATGACGATGAGACYATGCAATC-3' (7019–7041)
VSV reverse 5'-CAAGTCACYCGTGACCATCT-3' (7128–7109)

Kit: Multisccribe RT (Life technologies) + Go Taq hot start green master mix (Promega)

Amplification cycles: 48°C-30min, 95°C-2min, 40x (94°C-30sec, 60°C-30sec, 72°C-30sec)

• **Lab1 results**

		VSV NJ 10^{-1}	VSV NJ 10^{-3}	VSV IND1 10^{-2}	VSV IND1 10^{-4}	Negative	Negative
Sample code		178	49	158	33	120	157
VSV detection	Classical RT-PCR (method 2)	**Detected**	**Detected**	**Detected**	**Detected**	**Und**	**Und**
	Real time RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
VSV typing	Classical RT-PCR (method 1)	**New Jersey**	**New Jersey**	**Indiana**	**Indiana**	**Und**	**Und**
	Real time RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
Overall		**Detection and typing**	**Detection and typing**	**Detection and typing**	**Detection and typing**	**Und**	**Und**

ND= not done / Und= undetected

• **Lab2**

Method: real time RT-PCR for typing describes by Hole, J. *et al.* (2010)

Primers: VSV Universal (Indiana) forward 5'-TGATACAGTACAATTATTTTGGGAC-3' (7230-7254)

VSV Universal (Indiana) reverse 5'-GAGACTTTCTGTTACGGGATCTGG-3' (7433-7456)

New Jersey forward 5'-TGATTCAATATAATTATTTTGGGAC-3' (7230-7254)

New Jersey Rev 5'-AGGCTCAGAGGCATGTTCAT-3' (7476-7495)

Probes: NJ probe 5'-FAM- TTGCACACCAGAACATTCAA-BHQ-3' (7334-7353)

IND probe 5'-FAM-ATGATGCATGATCCAGC-MGB-NFQ-3' (7274-7290)

Kit: RNA Ultrasense OneStep qRT-PCR System (Invitrogen)

Amplification cycles: 55°C-15min, 95°C-2min, 45x (95°C-15sec, 54°C-30sec, 72°C-1min), 40°C-2min

• **Lab2 results**

		VSV NJ 10^{-1}	VSV NJ 10^{-3}	VSV IND1 10^{-2}	VSV IND1 10^{-4}	Negative	Negative
Sample code		21	66	26	147	162	20
VSV detection	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
VSV typing	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	**New Jersey**	**New Jersey**	**Indiana**	**Indiana**	**Und**	**Und**
Overall		**Detection and typing**	**Detection and typing**	**Detection and typing**	**Detection and typing**	**Und**	**Und**

ND= not done / Und= undetected

• **Lab3**

Method: real time RT-PCR for typing adapted from Wilson, WC. *et al.* (2009)

Primers: VSV-NJ forward N746F-NA 5'- CTCACAACATGGGTCCTGAA-3'

VSV-NJ reverse N814R-92CRB 5'- TTCTTGACCTGGATACATCAT-3'

VSV-IND forward N1082F 5'- CGGAGGATTGACGACTAATGC-3'

VSV-IND reverse N1148R 5'- TCAAACCATCCGAGCCATTC-3'

Probes: NJ probe N791-Degen 5'- FAM-AGGGAAGTYGCAGACGARCTATGCC-BHQ1-3'

IND probe N1105-INCR 5'- FAM-CCACCTCAAGGCAGAGATGTGGT-BHQ1-3'

Kit: Quantitect Probe RT-PCR Kit (Qiagen)

Amplification cycles: 55°C-30min, 95°C-15min, 45 x (95°C-15sec, 55°C-60sec, 72°C-10sec)

• **Lab3 results**

		VSV NJ 10^{-1}	VSV NJ 10^{-3}	VSV IND1 10^{-2}	VSV IND1 10^{-4}	Negative	Negative
Sample code		94	9	89	141	72	73
VSV detection	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
VSV typing	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	**New Jersey**	**New Jersey**	**Indiana**	**Indiana**	**Und**	**Und**
Overall		**Detection and typing**	**Detection and typing**	**Detection and typing**	**Detection and typing**	**Und**	**Und**

ND= not done / Und= undetected

• **Lab4**

Method: real time RT-PCR for typing adapted from Wilson, WC. *et al.* (2009)

Primers: NJ forward N746F-92CRB 5'-CTCACAACATGGGTCCTCAA-3'

NJ forward N746F-92CLB 5'-CTAACAACATGGGTCCTTAA-3'

NJ forward N746F-1184HDB 5'-CTAACAACATGGGTTCTGAA-3'

NJ forward N746F-NA 5'-CTCACAACATGGGTCCTGAA-3'

NJ forward N746F-PAN35 5'-CTAACCACATGGGTTCTAAA-3'

NJ reverse N814R-92CRB 5'-TTCTTGACCTGGATACATCAT-3'

NJ reverse N814R-92CLB 5'-TTCTTGCCCCGGATACATCAT-3'

NJ reverse N814R-0185PNB 5'-TTCTTGACCTGGGTACATCAT-3'

IND forward N1082F 5'-CGGAGGATTGACGACTAATGC-3'

IND forward N1082F-97CRB 5'-CGGGGGATTGACAACCAATGC-3'

IND forward N1082F-85CLB 5'-CGGAGGATTAACAACCAATGC-3'

IND reverse N1148R 5'-TCAAACCATCCGAGCCATTC-3'

IND reverse N1148R-97CRB5'-TCAAACCACCCAAGCCATTC-3'

IND reverse N1148R-98COE 5'-TCAAACCATCCTAGCCATTC-3'

Probes: NJ probe N791-Degen 5'-FAM-AGGGAAGTYGCAGACGARCTATGCC-BHQ1-3'

IND probe N1105-INCR 5'-FAM-CCACCTCAAGGCAGAGATGTGGT-BHQ1-3'

Kit: One Step RT PCR kit (Qiagen)

Amplification cycles: 55°C-30min, 95°C-15min, 45x (95°C-15sec, 55°C-60sec, 72°C-10sec)

• **Lab4 results**

		VSV NJ 10^{-1}	VSV NJ 10^{-3}	VSV IND1 10^{-2}	VSV IND1 10^{-4}	Negative	Negative
Sample code		83	19	93	52	129	6
VSV detection	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
VSV typing	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	**New Jersey**	**New Jersey**	**Indiana**	**Indiana**	**Und**	**Und**
Overall		**Detection and typing**	**Detection and typing**	**Detection and typing**	**Detection and typing**	**Und**	**Und**

ND= not done / Und= undetected

• **Lab5** Method: (i) classical RT-PCR for detection and (ii) real time RT-PCR for typing adapted from Hole,K. *et al.* (2006)

Primers: VSV forward 5'- TGATACAGTACAATTATTTTGGGAC-3'

VSV reverse 5'- GAGACTTTCTGTTACGGGATCTGG-3'

Probes: NJ probe 5'-FAM-TTTATGCATGACCCWGCAATAAG-MGB-3'

IND probe 5'-VIC-ATGATGCATGATCCAGC-MGB-3'

Kit: (i) OneStep RT-PCR (Qiagen) for classical RT-PCR

(ii) Ag-Path (Applied) for real-time RT-PCR

Amplification cycles: (i) 50°C-30min, 95°C-10min, 35x (95°C-60sec, 56°C-60sec, 72°C-60sec), 72°C-5min

(ii) 45°C-10min, 95°C-10min, 45x (95°C-15sec, 60°C-60sec)

• **Lab5 results**

		VSV NJ 10^{-1}	VSV NJ 10^{-3}	VSV IND1 10^{-2}	VSV IND1 10^{-4}	Negative	Negative
Sample code		75	125	128	37	136	45
VSV detection	Classical RT-PCR	**Detected**	**Detected**	**Detected**	**Detected**	**Und**	**Und**
	Real time RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
VSV typing	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	**New Jersey**	**New Jersey**	**Indiana**	**Indiana**	**Und**	**Und**
Overall		**Detection and typing**	**Detection and typing**	**Detection and typing**	**Detection and typing**	**Und**	**Und**

ND= not done / Und= undetected

• **Lab6** Method: real-time RT-PCR for detection and typing from EURL-SOP (Ansès) adapted from Hole, K. *et al.* (2006)

Primers: VSV forward 5'-TGATACAGTACAATTATTTTGGGAC-3'

VSV reverse 5'-GAGACTTTCTGTTACGGGATCTGG-3'

β-actin forward 5'-CAGCACAATGAAGATCAAGATCATC-3'

β-actin reverse 5'-CGGACTCATCGTACTCCTGCTT-3'

Probes: NJ probe 5'-FAM-CATGACCCWGCAATAA-MGB-3'

IND probe 5'-FAM-ATGATGCATGATCCAGC-MGB-3'

β-actin probe 5'-VIC-TCGCTGTCCACCTTCCAGCAGATGT-TAMRA-3'

Kit: AgPath-ID One-step RT-PCR Reagents (Applied)

Amplification cycles: 45°C-10min, 95°C-10min, 45x (95°C-15sec, 60°C-60sec)

• **Lab6 results**

		VSV NJ 10^{-1}	VSV NJ 10^{-3}	VSV IND1 10^{-2}	VSV IND1 10^{-4}	Negative	Negative
Sample code		70	105	174	131	88	104
VSV detection	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
VSV typing	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	**New Jersey**	**New Jersey**	**Indiana**	**Indiana**	**Und**	**Und**
β-actin detection Real-time RT-PCR		**Detected**	**Detected**	**Detected**	**Detected**	**Detected**	**Detected**
Overall		**Detection and typing**	**Detection and typing**	**Detection and typing**	**Detection and typing**	**Und**	**Und**

ND= not done / Und= undetected

- **Lab7** Method: unpublished method

  Primers: VSV forward 5'-TAAATGAYGATGAGACYATGCAATC-3'

  VSV reverse 5'-ACAWGTCACTCGYGACCATCT-3'

  Probes: NJ probe 5'-FAM-CAGACTATTTGAACTATGGGAAAATYCC-BHQ1-3'

  IND probe 5'-HEX-CCGATTTTCCGTGGAGTGATTAGAGG-BHQ1-3'

  Kit: Quantifast RT PCR kit (Qiagen)

  Amplification cycles: 45°C-10min, 95°C-10min, 42x (95°C-15sec, 56°C-32sec, 72°C-30sec)

- **Lab7 results**

		VSV NJ 10^{-1}	VSV NJ 10^{-3}	VSV IND1 10^{-2}	VSV IND1 10^{-4}	Negative	Negative
Sample code		40	57	43	154	143	76
VSV detection	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
VSV typing	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	**New Jersey**	**New Jersey**	**Indiana**	**Indiana**	**Und**	**Und**
Overall		**Detection and typing**	**Detection and typing**	**Detection and typing**	**Detection and typing**	**Und**	**Und**

ND= not done / Und= undetected

- **Lab8** Method 1: classical RT-PCR for typing describes by Hole, K. *et al.* (2010)

  Primers: NJ forward 5'-TGATTCAATATAATTATTTTGGGAC-3' (7230-7254)

  NJ reverse 5'-AGGCTCAGAGGCATGTTCAT-3' (7476-7495)

  IND forward 5'-TGATACAGTACAATTATTTTGGGAC-3' (7230-7254)

  IND reverse 5'-GAGACTTTCTGTTACGGGATCTGG-3' (7433-7456)

  Method 2: classical RT-PCR for detection describes by Fernandez, J. *et al.* (2008)

  Primers: VSV forward 5'-AATGACGATGAGACYATGCAATC-3' (7019–7041)

  VSV reverse 5'-CAAGTCACYCGTGACCATCT-3' (7128–7109)

  Kit: OneStep RT-PCR (Qiagen)

  Amplification cycles: 50°C-30min, 95°C-15min, 40x (95°C-15sec, 55°C-30sec, 72°C-30sec)

- **Lab8 results**

		VSV NJ 10^{-1}	VSV NJ 10^{-3}	VSV IND1 10^{-2}	VSV IND1 10^{-4}	Negative	Negative
Sample code		56	109	29	172	16	27
VSV detection	Classical RT-PCR	**Detected**	**Detected**	**Detected**	**Detected**	**Und**	**Und**
	Real time RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
VSV typing	Classical RT-PCR	**New Jersey**	**New Jersey**	**Indiana**	**Indiana**	**Und**	**Und**
	Real time RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
Overall		**Detection and typing**	**Detection and typing**	**Detection and typing**	**Detection and typing**	**Und**	**Und**

ND= not done / Und= undetected

• **Lab9** Method: real time RT-PCR for typing, describes by Hole,K. *et al.* (2010)

Primers: VSV-IND forward 5'-TGATACAGTACAATTATTTTGGGAC-3'

VSV-IND reverse 5'-GAGACTTTCTGTTACGGGATCTGG-3'

VSV-NJ forward 5'-TGATTCAATATAATTATTTTGGGAC-3'

VSV-NJ reverse 5'-AGGCTCAGAGGCATGTTCAT-3'

Probes: NJ-1 probe 5'-FAM-TTTATGCATGACCCWGCAATAAG-MGB-3'

NJ-2 probe 5'-FAM-TTGCACACCAGAACATTCAA-BHQ1-3'

IND probe 5'-VIC-ATGATGCATGATCCAGC-MGB-3'

Kit: Quantitech Probe (Qiagen)

Amplification cycles: 50°C-30min, 95°C-15min, 45x (95°C-15sec, 54°C-30sec, 72°C-60sec)

• **Lab9 results**

		VSV NJ 10^{-1}	VSV NJ 10^{-3}	VSV IND1 10^{-2}	VSV IND1 10^{-4}	Negative	Negative
Sample code		167	140	170	155	117	39
VSV detection	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
VSV typing	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	**New Jersey**	**New Jersey**	**Indiana**	**Indiana**	**Und**	**Und**
Overall		**Detection and typing**	**Detection and typing**	**Detection and typing**	**Detection and typing**	**Und**	**Und**

ND= not done / Und= undetected

• **Lab10** Method: real-time RT-PCR for detection and typing from EURL-SOP (Ansès) adapted from Hole, K. *et al.* (2006)

Primers: VSV forward 5'-TGATACAGTACAATTATTTTGGGAC-3'

VSV reverse 5'-GAGACTTTCTGTTACGGGATCTGG-3'

β-actin forward 5'-CAGCACAATGAAGATCAAGATCATC-3'

β-actin reverse 5'-CGGACTCATCGTACTCCTGCTT-3'

Probes: NJ probe 5'-FAM-CATGACCCWGCAATAA-MGB-3'

IND probe 5'-FAM-ATGATGCATGATCCAGC-MGB-3'

β-actin probe 5'-VIC-TCGCTGTCCACCTTCCAGCAGATGT-TAMRA-3'

Kit: PCR Superscript III Platinum One Step Quantitative RT-PCR System (Life Technologies)

Amplification cycles: 45°C-10min, 95°C-10min, 45x (95°C-15sec, 60°C-60sec)

• Lab10 results		VSV NJ 10^{-1}	VSV NJ 10^{-3}	VSV IND1 10^{-2}	VSV IND1 10^{-4}	Negative	Negative
Sample code		77	134	22	150	11	164
VSV detection	Classical RT-PCR	*Detected*	*Weakly detected*	*Detected*	*Detected*	*Und*	*Und*
	Real time RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
VSV typing	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	New Jersey	New Jersey	Indiana	Indiana	Und	Und
β-actin detection Real-time RT-PCR		Detected	Detected	Detected	Detected	Detected	Detected
Overall		Detection and typing	Detection and typing	Detection and typing	Detection and typing	Und	Und

ND= not done / Und= undetected

• **Lab11**

Method: real-time RT-PCR for detection and typing from EURL-SOP (Ansès) adapted from Hole, K. *et al.* (2006)

Primers: VSV forward 5'-TGATACAGTACAATTATTTTGGGAC-3'

VSV reverse 5'-GAGACTTTCTGTTACGGGATCTGG-3'

β-actin forward 5'-CAGCACAATGAAGATCAAGATCATC-3'

β-actin reverse 5'-CGGACTCATCGTACTCCTGCTT-3'

Probes: NJ probe 5'-FAM-CATGACCCWGCAATAA-MGB-3'

IND probe 5'-FAM-ATGATGCATGATCCAGC-MGB-3'

β-actin probe 5'-VIC-TCGCTGTCCACCTTCCAGCAGATGT-TAMRA-3'

Kit: AgPath-ID One-step RT-PCR Reagents (Applied)

Amplification cycles: 45°C-10min, 95°C-10min, 45x (95°C-15sec, 60°C-60sec)

• Lab11 results		VSV NJ 10^{-1}	VSV NJ 10^{-3}	VSV IND1 10^{-2}	VSV IND1 10^{-4}	Negative	Negative
Sample code		64	79	151	7	90	149
VSV detection	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
VSV typing	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	New Jersey	New Jersey	Indiana	Indiana	Und	Und
β-actin detection Real-time RT-PCR		Detected	Detected	Detected	Detected	Detected	Detected
Overall		Detection and typing	Detection and typing	Detection and typing	Detection and typing	Und	Und

ND= not done / Und= undetected

• **Lab12**

Method: real-time RT-PCR for detection and typing from EURL-SOP (Ansès) adapted from Hole, K. *et al.* (2006)

Primers: VSV forward 5'-TGATACAGTACAATTATTTTGGGAC-3'

VSV reverse 5'-GAGACTTTCTGTTACGGGATCTGG-3'

β-actin forward 5'-CAGCACAATGAAGATCAAGATCATC-3'

β-actin reverse 5'-CGGACTCATCGTACTCCTGCTT-3'

Probes: NJ probe 5'-FAM-CATGACCCWGCAATAA-MGB-3'

IND probe 5'-FAM-ATGATGCATGATCCAGC-MGB-3'

β-actin probe 5'-VIC-TCGCTGTCCACCTTCCAGCAGATGT-TAMRA-3'

Kit: SuperScript™ III One-Step RT-PCR System with Platinum® Taq High Fidelity (Life Technologies)

Amplification cycles: 45°C-10min, 95°C-10min, 45x (95°C-15sec, 60°C-60sec)

• **Lab12 results**

		VSV NJ 10^{-1}	VSV NJ 10^{-3}	VSV IND1 10^{-2}	VSV IND1 10^{-4}	Negative	Negative
Sample code		146	102	84	108	62	51
VSV detection	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
VSV typing	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	**New Jersey**	**New Jersey**	**Indiana**	**Indiana**	**Und**	**Und**
β-actin detection Real-time RT-PCR		**Detected**	**Detected**	**Detected**	**Detected**	**Detected**	**Detected**
Overall		**Detection and typing**	**Detection and typing**	**Detection and typing**	**Detection and typing**	**Und**	**Und**

ND= not done / Und= undetected

• **Lab13**

Method: Primers from Rasmussen *et al.* (2006) modified and probes from Hoffmann, unpublished

Primers: VSV forward 5'-TAAATGAYGATGAGACYATGCAATC-3' (7017)

VSV reverse 5'-ACAWGTCACTCGYGACCATCT-3' (7129)

β-actin primers sequences non communicated

Probes: NJ probe 5'-FAM-CAGACTATTTGAACTATGGGAAAATYCC-3' (7145)

IND probe 5'-FAM-CCGATTTTCCGTGGAGTGATTAGAGG-3' (7180)

β-actin probe sequence non communicated

Kit: AgPath-ID (Ambion)

Amplification cycles: 42 cycles of amplification

• **Lab13 results**

		VSV NJ 10^{-1}	VSV NJ 10^{-3}	VSV IND1 10^{-2}	VSV IND1 10^{-4}	Negative	Negative
Sample code		121	74	148	124	61	2
VSV detection	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
VSV typing	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	**New Jersey**	**New Jersey**	**Indiana**	**Indiana**	**Und**	**Und**
β-actin detection Real-time RT-PCR		**Detected**	**Detected**	**Detected**	**Detected**	**Detected**	**Detected**
Overall		**Detection and typing**	**Detection and typing**	**Detection and typing**	**Detection and typing**	**Und**	**Und**

ND= not done / Und= undetected

• **Lab14**

Method: real time RT-PCR for typing, adapted from Hole,K. *et al.* (2010)

Primers: VSV-Indiana forward 5'-TGATACAGTACAATTATTTTGGGAC-3'

VSV-Indiana reverse 5'-GAGACTTTCTGTTACGGGATCTGG-3'

VSV-New Jersey forward 5'-TGATTCAATATAATTATTTTGGGGAC-3'

VSV- New Jersey reverse 5'-AGGCTCAGAGGCATGTTCAT-3'

Probes: NJ probe (essay 1) 5'-FAM-TTTATGCATGACCCWGCAATAAG-MGB-3'

NJ probe (essay 2) 5'-FAM-TTGCACACCAGAACATTCAA-BHQ1-3'

IND probe 5'-FAM-ATGATGCATGATCCAGC-MGB-3'

Kit: Superscript III platinum (Invitrogen)

Amplification cycles: 50°C-15min, 95°C-2min, 50x (95°C-15sec, 54°C-30sec)

• **Lab14 results**

		VSV NJ 10^{-1}	VSV NJ 10^{-3}	VSV IND1 10^{-2}	VSV IND1 10^{-4}	Negative	Negative
Sample code		142	85	168	82	166	44
VSV detection	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
VSV typing	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	**New Jersey**	**New Jersey**	Indiana	**Indiana**	**Und**	Und
Overall		**Detection and typing**	**Detection and typing**	**Detection and typing**	**Detection and typing**	**Und**	Und

ND= not done / Und= undetected

• **Lab15**

Method 1: real time RT-PCR for typing adapted from Wilson, WC. *et al.* (2009)

Primers: VSV NJ N746F-92CRB forward 5'-CTCACAACATGGGTCCTCAA-3'

VSV NJ N746F-92CLB forward 5'-CTAACAACATGGGTCCTTAA-3'

VSV NJ N746F-1184HDB forward 5'-CTAACAACATGGGTTCTGAA-3'

VSV NJ N746F-NA forward 5'-CTCACAACATGGGTCCTGAA-3'

VSV NJ N746F-UNA82 forward 5'-CTTACTACATGGGTCCTCAA-3'

VSV NJ N746F-PAN35 forward 5'-CTAACCACATGGGTTCTAAA-3'

VSV NJ N814R-92CRB reverse 5'-TTCTTGACCTGGATACATCAT-3'

VSV NJ N814R-92CLB reverse 5'-TTCTTGCCCCGGATACATCAT-3'

VSV NJ N814R-0185PNB reverse 5'-TTCTTGACCTGGGTACATCAT-3'

VSV NJ N814R-UNA82 reverse 5'-TTCCTGACCCGGGTACATCAT-3'

VSV IND N1082F forward 5'-CGGAGGATTGACGACTAATGC-3'

VSV IND N1082F-97CRB forward 5'-CGGGGGATTGACAACCAATGC-3'

VSV IND N1082F-85CLB forward 5'-CGGAGGATTAACAACCAATGC-3'

VSV IND N1148R reverse 5'-TCAAACCATCCGAGCCATTC-3'

VSV IND N1148R-97CRB reverse 5'-TCAAACCACCCAAGCCATTC-3'

VSV IND N1148R-98COE reverse 5'-TCAAACCATCCTAGCCATTC-3'

Probes: NJ probe N791-Degen 5'-TET-AGGGAAGTYGCAGACGARCTATGCC-MGB-3'

NJ probe N791-T4 5'-TET-AGAGAGGTYGCAGATGARCTGTGCC-MGB-3'

NJ probe N791-0804COE2 5'-TET-AGGGAAGTCGCAGATGAGCTGTGCC-MGB-3'

IND probe N1105-INCR 5'-FAM-CCACCTCAAGGCAGAGATGTGGT-MGB-3'

Kit: ABI EZ RT-PCR rTth Kit Reagents (Applied ref.N808-0236)

Amplification cycles: 55°C-25min, 95°C-2min, 40x (95°C-10sec, 55°C-60sec)

Method 2: in-house VSV RT LAMP New Jersey, in development, unpublished

• **Lab15 results**		VSV NJ 10^{-1}	VSV NJ 10^{-3}	VSV IND1 10^{-2}	VSV IND1 10^{-4}	Negative	Negative
Sample code		137	161	25	179	38	4
VSV detection	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
VSV typing	Classical RT-PCR	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Real time RT-PCR (method 1)	**New Jersey and Indiana**	**New Jersey and Indiana**	**Indiana**	**Und**	Und	**Und**
Alternative VSV RT LAMP NJ (method 2)		**Time of positive 16 min**	**Time of positive 8 min**	**Und**	**Und**	Und	**Und**
Overall		**Cross reaction**	**Cross reaction**	**Detection and typing**	**Und**	Und	**Und**

ND= not done / Und= undetected

4.2 QUANTITATIVE RESULTS

Ct values obtained by each participant who used real-time RT-PCR methods are reported below.

Lab 1 and 8 performed only conventional RT-PCR methods.

Lab	Method	Sample A VSV NJ 10^{-1}	Sample B VSV NJ 10^{-3}	Sample C VSV IND 10^{-2}	Sample D VSV IND 10^{-4}	Sample E Negative	Sample F Negative	Note
2	Hole *et al.* (2010)	18,52	25,76	23,17	30,33	Und	Und	
3	Wilson *et al.* (2009) adapted	15,49	21,16	19,16	25,64	Und	Und	
4	Wilson *et al.* (2009) adapted	17,88	25,75	21,42	29,23	Und	Und	
5	Hole *et al.* (2006) adapted	25,17	31,93	28,84	35,06	Und	Und	
6	EURL-SOP adapted from Hole *et al.* (2006)	24,5	31,42	26,45	32,56	Und	Und	with detection of β-actin as internal control
7	Unpublished	18,21	25,23	21,74	28,61	Und	Und	
9	Hole *et al.* (2010)	19,5	26,1	22,5	29,6	Und	Und	
10	EURL-SOP adapted from Hole *et al.* (2006)	25,23	32,09	23,32	30,52	Und	Und	with detection of β-actin as internal control
11	EURL-SOP adapted from Hole *et al.* (2006)	23,45	30,08	23,94	31,87	Und	Und	with detection of β-actin as internal control
12	EURL-SOP adapted from Hole *et al.* (2006)	26,37	32,14	24,89	32,88	Und	Und	with detection of β-actin as internal control
13	Rasmussen *et al.* (2006) adapted	20,39	27,16	22,57	29,06	Und	Und	with detection of β-actin as internal control
14	Hole *et al.* (2010) adapted	16,45	22,41	20,85	28,45	Und	Und	
15	Wilson *et al.* (2009) adapted	25,1	33	30,6	Und	Und	Und	(i)cross reaction between Ind and NJ for typing VSV NJ samples (ii)weak positive VSV Ind sample not detected

Und= undetected

5. CONCLUSIONS

➢ All negatives samples (E and F) were tested negatives.

➢ Positive samples A, B and C were detected as VSV by all participants. Sample D was detected as VSV positive sample by only 14 participants. A and B (NJ samples) were typed by 15 participants. C (IND sample) was typed by all participants. D (IND sample) was typed by 14 participants.

➢ Five participants have included the detection of the internal β-actin quality control. These 5 participants detected β-actin target in all samples of the panel.

➢ According to the Ct values obtained with samples B and D (weak samples), it appears that the method used by Lab 3 adapted from Wilson et al. (2009) gave the best Ct values on this panel (see table below).

Lab	Method	Sample B VSV NJ 10^{-3}	Sample D VSV IND 10^{-4}
3	Wilson *et al.* (2009) adapted	21,16	25,64
14	Hole *et al.* (2010) adapted	22,41	28,45
7	Unpublished	25,23	28,61
13	Rasmussen *et al.* (2006) adapted	27,16	29,06
4	Wilson *et al.* (2009) adapted	25,75	29,23
9	Hole *et al.* (2010)	26,1	29,6
2	Hole *et al.* (2010)	25,76	30,33
10	EURL-SOP adapted from Hole *et al.* (2006)	32,09	30,52
11	EURL-SOP adapted from Hole *et al.* (2006)	30,08	31,87
6	EURL-SOP adapted from Hole *et al.* (2006)	31,42	32,56
12	EURL-SOP adapted from Hole *et al.* (2006)	32,14	32,88
5	Hole *et al.* (2006) adapted	31,93	35,06
15	Wilson *et al.* (2009) adapted	33	Und

➢ Simplex real-time RT-PCR methods are known to be more sensitive than duplex real-time RT-PCR methods. This fact is confirmed by results obtained from different methods used by participants on this PT panel (see table below).

Lab	Method	Simplex / duplex	Sample B VSV NJ 10^{-3}	Sample D VSV IND 10^{-4}
3	Wilson *et al.* (2009) adapted	Simplex	21,16	25,64
14	Hole *et al.* (2010) adapted	Simplex	22,41	28,45
7	Unpublished	Duplex NJ/IND	25,23	28,61
4	Wilson *et al.* (2009) adapted	Simplex	25,75	29,23
2	Hole *et al.* (2010)	Simplex	25,76	30,33
9	Hole *et al.* (2010)	Duplex NJ/IND	26,1	29,6
13	Rasmussen *et al.* (2006) adapted	Duplex NJ/β-actin or IND/β-actin	27,16	29,06
11	EURL-SOP adapted from Hole *et al.* (2006)	Duplex NJ/β-actin or IND/β-actin	30,08	31,87
6	EURL-SOP adapted from Hole *et al.* (2006)	Duplex NJ/β-actin or IND/β-actin	31,42	32,56
5	Hole *et al.* (2006) adapted	Duplex NJ/IND	31,93	35,06
10	EURL-SOP adapted from Hole *et al.* (2006)	Duplex NJ/β-actin or IND/β-actin	32,09	30,52
12	EURL-SOP adapted from Hole *et al.* (2006)	Duplex NJ/β-actin or IND/β-actin	32,14	32,88
15	Wilson *et al.* (2009) adapted	Duplex NJ/IND	33	Und

However duplex real-time RT-PCR methods allow (i) target (VSV or IND or NJ) and internal control (β-actin) detection at the same time, (ii) or New Jersey and Indiana typing at the same time. In addition, one duplex real-time RT-PCR essay is low cost than two simplex real-time RT-PCR essays.

6. ACTIONS

➢ It is recommended that real time RT-PCR for VSV detection and/or typing be implemented in all labs. This method is known to be more sensitive than conventional RT-PCR.

➢ It is recommended to include detection of the internal β-actin quality control to ensure good extraction of total RNA.

➢ The EU-RL will evaluate all methods used by participants and more particularly the method published by Wilson *et al.* (2009) and share information on this evaluation with the Europeans NRL.

7. OTHER INFORMATION

➢ Next PT- VSV will be organized in 2016 and will include samples with more different contamination levels from more VSV strains.

➢ For any further information regarding the organization and the report of this PT, please contact the coordinator who will treat your request as soon as possible.

➢ Coordinator contact: Aurore Romey, aurore.romey@anses.fr, +33 1 49 77 13 15

8. REFERENCES

➢ Hole, K. *et al.* J vet Diagn Invest, 2006 18:139-146

➢ Hole, K. *et al.* J vet Diagn Invest, 2010, 22:428-433

➢ Wilson William, C *et al.* J vet Diagn Invest, 2009 21:179-186

➢ Fernandez, J. *et al.* Journal of Virological Methods, 2008 147:301-311

➢ Rasmussen Thomas, B. *et al.* Journal of Clinical Microbiology, Jan. 2006, 356-362

➢ https://eurl-equinedisease.anses.fr

9. ANNEXES

- Invitation lettre (Annexe MQE VII.01)
- Information on the interlaboratory test plan (F MQE VII.01) (page 1/2 and page 2/2)
- Participation form (F MQE VII.03)
- Instructions for carrying out the interlaboratory test (F MQE VII.07) (page 1/2 and page 2/2)
- Acknowledgment of receipt (F MQE VII.04)
- Results form (VSV2014 results) (page ½ and page 2/2)
- Satisfaction questionnaire for laboratories participating in an ILPT (F MQE X.01)

anses
alimentation, environnement, travail

Maisons-Alfort Laboratory for Animal Health

Unit Virology

Maisons-Alfort, 22/09/2014

Dossier followed by:
Aurore Romey

Direct line:
+33 1 49 77 13 15

Direct fax:

Email:
Aurore.romey@anses.fr

Our Ref.: PT/VSV/2014

Subject: Organisation of VSV proficiency test

Dear Colleague,

We hereby announce that we are organising an inter-laboratory proficiency test concerning molecular diagnosis of Vesicular Stomatitis Virus.

You will find enclosed with this letter a guide entitled "Information for participants on the inter-laboratory test plan" containing the necessary information on the inter-laboratory test procedure together with a participation form to be returned no later than 6th October 2014.

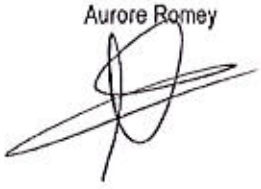
The results will be handled in confidence and returned without identifying participants. The final report will be distributed to all participants and to the organiser of the test.

Please do not hesitate to contact us if you have any queries or require further information.

Yours faithfully,

Name of coordinator of the inter-laboratory proficiency test

Aurore Romey



anses
alimentation, environnement, travail

INFORMATION FOR PARTICIPANTS ON THE INTERLABORATORY TEST PLAN

ILPT code	PT-VSV/2014/RT-PCR
Version	01

Test	
Name of test	Detection and characterisation of vesicular stomatitis virus by RT-PCR
Type	Proficiency Test
Purpose	Consolidate European Laboratory Network for the diagnosis of vesicular stomatitis, increase inventory of RT-PCR methods available and their performance, and validate the real-time RT-PCR method available on the website for VSV detection and typing
Participation fee	No

Identification of the Organiser	
Name	European Union Reference Laboratory for equine diseases
Address	23, avenue du Général de Gaulle 94706 Maisons-Alfort Cedex

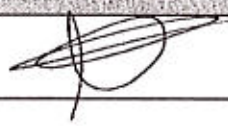
Identification of coordinator					
Name	Romey Aurore			**Unit/team**	Virology/BioPic
☎	+33149771315	**Fax**	+33143689762	**Email**	aurore.romey@anses.fr

Identification of the national external contact person *(to be completed by the organiser)*			
Name	No	☎	

Identification of subcontractors	
No subcontractor	

Information	
Analyte/matrix pair or method	VSV genome/RNA
Number of samples sent by the laboratory	6
Method of analysis	Conventionnal or real-time RT-PCR
Number of tests to be carried out on each sample	no requirement
Condition of samples and mode of dispatch	Samples sent frozen in dry ice
Time when samples will be shipped	Between 27 and 31 of October 2014
Analysis period	Between 3 and 14 of November 2014
Deadline for returning results	17 November 2014
Criteria for refusing results (results not used when processing data)	Results not clear

Decision rules for accepting results (acceptable range of values for results)	For real time RT-PCR: CT values or undet. For classical RT-PCR: gel photo. If more than one test are performed only one final result is given
Deadline for sending individual report	9 December 2014
Deadline for sending final report	16 December 2014
Disemination mode/Recipients of results and final report	By email and regular mail
Miscellaneous information	The samples must be processed using your normal procedures.

Signed at	22nd September 2014	on	Maisons-Alfort	Coordinator's signature	

anses
alimentation, environnement, travail

PARTICIPATION FORM

ILPT code	PT-VSV/2014/RT-PCR
Version	01

Name of test	Detection and characterisation of vesicular stomatitis virus by RT-PCR

Identification of participating laboratory

Name of Laboratory	

Address of the laboratory	Delivery address for samples (if different)

	List of tests organised (analyte/matrix pair or method)	Tariff code	Participation fee	Participation (Yes/No)	Method used (when a choice is available)
1	VSV genome/ RNA	No	No		
2					
3					
4					

Participation *(to be completed by the participating laboratory)*

Contact person during the inter-laboratory test					
☎		Fax		Email	

Comments/Explanations in the event of non-participation

Signed at	Name and signature of person responsible:
Date	

By signing this participation form the signatory agrees that his/her laboratory accepts the ILPT conditions set out in the attached participant information sheet and will pay the participation fee and postal costs.

This form must be signed and returned by email or by post no later than 06/10/2014 to:

Name	Aurore Romey				
Address	ANSES – Laboratoire de Santé Animale – Unité Virologie 23 Avenue du general De Gaulle				
Postcode	94706	City	Maisons-Alfort - France		
☎	+33149771315	Fax	+33143689762	Email	Aurore.romey@anses.fr

INSTRUCTIONS TO PARTICIPANTS FOR CARRYING OUT THE INTERLABORATORY TEST

ILPT code	PT-VSV/2014/RT-PCR
Version	01

Name of the test	Detection and characterisation of vesicular stomatitis virus by RT-PCR
Type	Proficiency Test
Purpose	Consolidate European Laboratory Network for the diagnosis of vesicular stomatitis, increase inventory of RT-PCR methods available and their performance, and validate the real-time RT-PCR method available on the website for VSV detection and typing

Identification of the Organiser

Name	European Union Reference Laboratory for equine diseases
Address	23, avenue du Général de Gaulle 94706 Maisons-Alfort Cedex

Identification of the coordinator

Name	Romey Aurore			**Unit/team**	Virology/BioPic
☎	+33149771315	**Fax**	+33143689762	**Email**	aurore.romey@anses.fr

Identification of the national external correspondant *(to be completed by the organiser)*

Name	No	☎	

Information

Analyte/matrix pair or method	VSV genome/RNA
Number of samples sent	6
How samples must be stored after receipt	Store at -80°C until use
Dispatch date of samples	Between 27 and 31 October 2014
Method of analysis	Conventionnal or Real-time RT-PCR
Number of tests to be performed on each sample	no requirement
Deadline for analysis	Between 03 and 14 November 2014
Deadline for returning results	17 November 2014
Conditions for presenting results (unit of measurement, number of significant figures ...)	Results should be presented in an Excel file sent by the organiser. For conventionnal RT-PCR, gel photo should be included For real-time RT-PCR, print screen of amplification curves should be included
Criteria for refusing results (results not used when processing data)	Results not clear
Decision rules for accepting results (acceptable range of values for results)	For real time RT-PCR: CT values or undet. For classical RT-PCR: gel photo. If more than one test are performed only one final result is given

How the analysis is to be carried out	Samples will be tested by real-time RT-PCR for detection of VS and characterization of NJ and IND strains. Samples can also be tested by conventional RT-PCR.
Miscellaneous information	The samples must be processed using your normal procedures.

Completed at	22nd September 2014	on	Maisons-Alfort	Coordinator's signature	

anses
French agency for food, environmental and occupational health & safety

ACKNOWLEDGMENT OF RECEIPT

ILPT code	PT-VSV/2014/RT-PCR
Version	01

Name of test	Real time or classical RT-PCR

Laboratory identification	
Laboratory code	

Contents of the consignment

- ☒ A cover letter concerning the samples
- ☒ Form « Instructions to participants on the implementation of the PT »
- ☒ Result forms to be filled out: number of forms : 1
- ☒ Samples: number of samples = 6
- ☐ Form « Deviation report - actions suggested by the participating laboratory»
- ☐ Other:

Receipt of the consignment		
Date and time of receipt at the laboratory		
Number of samples received		
All items received	☐ Yes	☐ No
Documents / samples missing		

Condition of samples when received

Samples should be received frozen

Condition of samples complying with above criteria: ☐ Satisfactory ☐ Unsatisfactory
If unsatisfactory, state why:

Number of unsatisfactory samples:

Samples damaged: ☐ Yes ☐ No

Number of damaged samples:

☐ Please send a new consignment of the following samples:

Observations

Name and signature of person responsible:

Acknowledgment of receipt should be sent to:

Name	Aurore ROMEY				
Address	23 avenue du Général de Gaulle				
Postcode	94706	City	Maisons-Alfort Cedex		
☎	+33149771317 or 1315	Fax	+33143689762	Email	aurore.romey@anses.fr

anses
alimentation, environnement, travail

VSV/2014 Results

ILPT code	PT-VSV/2014/RT-PCR

Lab name	
Lab code	
Date samples were analysed	
Date results were sent	

Ref. Method used	
RT-PCR kit used	
Amplification cycle	
Primers	
Probes	

Sample number	Ct value for Real time RT-PCR (1)				Positive/Negative (P/N) for Classical RT-PCR (2)			
	VSV	New Jersey	Indiana	β-actine	VSV	New Jersey	Indiana	β-actine

(1) join image copy of amplification curves (print screen) on page 2
(2) join photo of agarose gel on page 2

insert print screen of amplification curves

insert photo of agarose gel

Signature of person responsible for the ILPT at the participating laboratory			
Date		**Name and signature of person responsible for the ILPT**	

Results must be sent no later than 17th November 2014

Name	Aurore ROMEY		
Address	Unité Virologie 23 avenue du Général de Gaulle		
Postcode	94706	**City**	Maisons-Alfort Cedex
Tel.+33149771315	Fax.+33143689762		Email: aurore.romey@anses.fr

anses
French agency for food, environmental and occupational health safety

SATISFACTION QUESTIONNAIRE FOR LABORATORIES PARTICIPATING IN AN ILPT

ILPT	
Name	PT-VSV/2014/RT-PCR
Period	2014

Participating laboratory identification	
Last name, first name	
Establishment	

What is your overall level of satisfaction regarding implementation/organisation of the ILPT?

Highly satisfied ☐ Satisfied ☐ Moderately satisfied ☐ Unsatisfied ☐

Comments:

Are you satisfied with your interactions with the ILPT organiser (answers to your questions, feedback, etc.)?

Highly satisfied ☐ Satisfied ☐ Moderately satisfied ☐ Unsatisfied ☐

Comments:

Are you satisfied with the various messages/documents sent to you by the organiser (form and content)?

Highly satisfied ☐ Satisfied ☐ Moderately satisfied ☐ Unsatisfied ☐

Comments:

Are you satisfied with the way the ILPG results were rendered?

Highly satisfied ☐ Satisfied ☐ Moderately satisfied ☐ Unsatisfied ☐

Comments:

Are you satisfied with the return date of the final report?

Highly satisfied ☐ Satisfied ☐ Moderately satisfied ☐ Unsatisfied ☐

Comments:

Other remarks / points needing improvement