



Lyssavirus Unit

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APPENDICES

INTER-LABORATORY ASSAY FOR PERFORMANCE CHARACTERISTICS EVALUATION OF LYSSAVIRUS REAL TIME RT-PCR

Year 2022

Evelyne Picard-Meyer and Emmanuelle Robardet

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

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1. S Table 1: Results of SYBR Green RT-PCR by strain tested.

| Samples | Class | C _T values | SYBR Green pan-lyssavirus RT-PCR assay | | | | | | | | | | | | | | |
|----------|-------------------|---|--|----------------|--------------|----------------|----------------|---------------------|-------|----------------|---------------|-------|----------------|--------------|-------|----------------|--------------|
| | | | L1 | | | L2 | | | L4 | | | L6 | | | L7 | | |
| | | | CT | T _M | Conclusion | CT | T _M | Conclusion | CT | T _M | Conclusion | CT | T _M | Conclusion | CT | T _M | Conclusion |
| Negative | / | CT ₁ CT ₂ Mean C _T | No Ct | | | 32.71 39.37 | | | No Ct | | | No Ct | | | No Ct | | |
| | | | No Ct | | Not detected | 36.04 | 79.67 | Detected | No Ct | | Not detected | No Ct | | Not detected | No Ct | | Not detected |
| CVS (1) | strongly positive | CT ₁ CT ₂ Mean C _T | 24.11 | | | 36.72 35.52 | | | 24.87 | | | 27.7 | | | 23.41 | | |
| | | | 24.11 | 76.56 | Detected | 36.12 | 77.65 | Detected | 24.87 | 80.0 | Detected | 27.7 | 78.0 | Detected | 23.41 | 79.00 | Detected |
| CVS (1) | moderate positive | CT ₁ CT ₂ Mean C _T | 28.89 | | | 44.46 37.17 | | | 29.54 | | | 33.8 | | | 31.09 | | |
| | | | 28.89 | 76.42 | Detected | 40.82 | 72.38 | Not detected | 29.54 | 80.0 | Detected | 33.8 | 78.0 | Detected | 31.09 | 79.00 | Detected |
| CVS (1) | weak positive | CT ₁ CT ₂ Mean C _T | 33.53 | | | 40.20 No Ct | | | 34.9 | | | 35.2 | | | 26.25 | | |
| | | | 33.53 | 76.27 | Detected | 40.20 | | Not detected | 34.9 | 80.8 | Detected | 35.2 | 78.0 | Detected | 26.25 | 78.50 | Detected |
| CVS (2) | strongly positive | CT ₁ CT ₂ Mean C _T | 23.54 | | | 38.21 37.82 | | | 24.2 | | | 28 | | | 23.23 | | |
| | | | 23.54 | | Detected | 38.02 | 78.2 | Detected | 24.2 | 80.3 | Detected | 28 | 78.0 | Detected | 23.23 | 79.00 | Detected |
| CVS (2) | moderate positive | CT ₁ CT ₂ Mean C _T | 29.4 | | | 44.88 43.31 | | | 28.7 | | | 32.4 | | | 28.21 | | |
| | | | 29.4 | | Detected | 44.10 | 71.88 | Not detected | 28.7 | 80.2 | Detected | 32.4 | 78.0 | Detected | 28.21 | 79.00 | Detected |
| CVS (2) | weak positive | CT ₁ CT ₂ Mean C _T | 35.48 | | | 39.47 No Ct | | | 33.61 | | | 36.6 | | | 35.65 | | |
| | | | 35.48 | | Detected | 39.47 | 71.96 | Not detected | 33.61 | 80.3 | Detected | 36.6 | 78.0 | Detected | 35.65 | 79.50 | Detected |
| Greece | strongly positive | CT ₁ CT ₂ Mean C _T | 23.1 | | | 37.48 37.19 | | | 24.48 | | | 27.1 | | | 22.85 | | |
| | | | 23.1 | 75.99 | Detected | 37.34 | 77.25 | Detected | 24.48 | 79.7 | Detected | 27.1 | 77.5 | Detected | 22.85 | 78.50 | Detected |
| Greece | moderate positive | CT ₁ CT ₂ Mean C _T | 28.17 | | | 44.01 No Ct | | | 29.35 | | | 32.6 | | | 27.98 | | |
| | | | 28.17 | 76.06 | Detected | 44.01 | | Not detected | 29.35 | 80.0 | Detected | 32.6 | 77.5 | Detected | 27.98 | 78.50 | Detected |
| Greece | weak positive | CT ₁ CT ₂ Mean C _T | 35.51 | | | 41.61 No Ct | | | 36.83 | | | 36.3 | | | 32.85 | | |
| | | | 35.51 | 75.98 | Detected | 41.61 | 72.45 | Not detected | 36.83 | 80.0 | Detected (LD) | 36.3 | 77.5 | Detected | 32.85 | 78.50 | Detected |
| EBLV-1b | strongly positive | CT ₁ CT ₂ Mean C _T | 22.46 | | | 33.89 32.29 | | | 23.14 | | | 24.9 | | | 20.47 | | |
| | | | 22.46 | 75.70 | Detected | 33.09 | 77.74 | Detected | 23.14 | 79.3 | Detected | 24.9 | 77.5 | Detected | 20.47 | 78.00 | Detected |
| EBLV-1b | moderate positive | CT ₁ CT ₂ Mean C _T | 27.63 | | | 43.90 42.54 | | | 29.49 | | | 29.3 | | | 27.05 | | |
| | | | 27.63 | 75.70 | Detected | 43.22 | | Not detected | 29.49 | 79.7 | Detected | 29.3 | 77.5 | Detected | 27.05 | 78.00 | Detected |
| EBLV-1b | weak positive | CT ₁ CT ₂ Mean C _T | 34.84 | | | 40.01 40.38 | | | 32.97 | | | 35.1 | | | 32.15 | | |
| | | | 34.84 | 75.70 | Detected | 40.20 | 71.99 | Not detected | 32.97 | 79.8 | Detected | 35.1 | 77.5 | Detected | 32.15 | 78.00 | Detected |
| EBLV-2 | strongly positive | CT ₁ CT ₂ | 20.18 | | | 31.30 33.56 | | | 21.65 | | | 24.3 | | | 19.8 | | |

| | | | | | | | | | | | | | | | | | |
|--------|-------------------|---------------------|-------|----------|----------|--------------|----------|--------------|-------|----------|----------|------|----------|----------|-------|----------|----------|
| | | Mean C _T | 20.18 | Detected | 32.43 | 76.7 | Detected | 21.65 | 79.5 | Detected | 24.3 | 77.0 | Detected | 19.8 | 78.00 | Detected | |
| EBLV-2 | moderate positive | CT ₁ | 24.94 | | 36.12 | | | 27.38 | | | 29.8 | | | 24.65 | | | |
| | | CT ₂ | | | 40.01 | | | | | | | | | | | | |
| | | Mean C _T | 24.94 | Detected | 38.07 | 77.35 | Detected | 27.38 | 79.0 | Detected | 29.8 | 77.0 | Detected | 24.65 | 78.00 | Detected | |
| EBLV-2 | weak positive | CT ₁ | 31.72 | | No Ct | | | 31.91 | | | 35.6 | | | 29.66 | | | |
| | | CT ₂ | | | No Ct | | | | | | | | | | | | |
| | | Mean C _T | 31.72 | Detected | No Ct | Not detected | | 31.91 | 79.5 | Detected | 35.6 | 77.0 | Detected | 29.66 | 78.00 | Detected | |
| BBLV | strongly positive | CT ₁ | 22.74 | | 35.42 | | | 24.99 | | | 26.5 | | | 20.9 | | | |
| | | CT ₂ | | | 34.03 | | | | | | | | | | | | |
| | | Mean C _T | 22.74 | 75.34 | Detected | 34.73 | 76.76 | Not detected | 24.99 | 79.0 | Detected | 26.5 | 77.0 | Detected | 20.9 | 78.00 | Detected |
| BBLV | moderate positive | CT ₁ | 27.62 | | 41.84 | | | 31 | | | 33.1 | | | 32.77 | | | |
| | | CT ₂ | | | 42.95 | | | | | | | | | | | | |
| | | Mean C _T | 27.62 | Detected | 42.40 | 77.63 | Detected | 31 | 79.0 | Detected | 33.1 | 77.0 | Detected | 32.77 | 77.50 | Detected | |
| BBLV | weak positive | CT ₁ | 33.24 | | No Ct | | | 35.05 | | | 36 | | | 31.36 | | | |
| | | CT ₂ | | | No Ct | | | | | | | | | | | | |
| | | Mean C _T | 33.24 | Detected | No Ct | Not detected | | 35.05 | 79.2 | Detected | 36 | 77.0 | Detected | 31.36 | 77.50 | Detected | |

| Samples | Class | C _T values | SYBR Green pan-lyssavirus RT-PCR assay | | | | | | | | | | | | | | |
|----------|-------------------|---|--|----------------|---------------|-----------------|----------------|---------------|----------------|----------------|--------------|-----------------|----------------|--------------|----------------|----------------|---------------|
| | | | L8 | | | L11 | | | L12 | | | L15 | | | L16 | | |
| | | | CT | T _M | Conclusion | CT | T _M | Conclusion | CT | T _M | Conclusion | CT | T _M | Conclusion | CT | T _M | Conclusion |
| Negative | / | CT ₁ CT ₂ Mean C _r | No Ct | | | No Ct | | | No Ct | | | No Ct | | | No Ct | | |
| | | | No Ct | | Not detected | No Ct | | Not detected | No Ct | | Not detected | No Ct | | Not detected | No Ct | | Not detected |
| CVS (1) | strongly positive | CT ₁ CT ₂ Mean C _r | 23.91 | | | 23.33 30.46 | | | 27.71 27.88 | | | 24.68 24.71 | | | 32.81 33.23 | | |
| | | | 23.91 | 76.4 | Detected | 26.895 76.77 | | Detected | 27.80 79.8 | | Detected | 24.695 77.50 | | Detected | 33.02 77.3 | | Detected |
| CVS (1) | moderate positive | CT ₁ CT ₂ Mean C _r | 29.63 | | | 29.73 29.87 | | | 32.45 32.70 | | | 29.9 30.09 | | | 37.80 33.24 | | |
| | | | 29.63 | 76.5 | Detected | 29.8 76.57 | | Detected | 32.58 80 | | Detected | 29.995 77.40 | | Detected | 35.52 77.5 | | Detected (LD) |
| CVS (1) | weak positive | CT ₁ CT ₂ Mean C _r | 34.34 | | | 33.81 | | | | | | No Ct No Ct | | | No Ct No Ct | | |
| | | | 34.34 | 76.5 | Detected | 33.81 76.73 | | Detected (LD) | No Ct | | Not detected | No Ct | | Not detected | No Ct | | Inconclusive |
| CVS (2) | strongly positive | CT ₁ CT ₂ Mean C _r | 24 | | | 23.46 30.9 | | | 27.00 27.35 | | | 25.22 25.21 | | | 33.97 33.82 | | |
| | | | 24 | 76.5 | Detected | 27.18 76.84 | | Detected | 27.18 79.8 | | Detected | 25.215 77.15 | | Detected | 33.90 77.5 | | Detected |
| CVS (2) | moderate positive | CT ₁ CT ₂ Mean C _r | 29.7 | | | 29.12 29.22 | | | 32.79 32.69 | | | 30.2 30.14 | | | 40.59 39.60 | | |
| | | | 29.7 | 76.4 | Detected | 29.17 76.58 | | Detected | 32.74 80 | | Detected | 30.17 77.75 | | Detected | 40.10 | | Detected (LD) |
| CVS (2) | weak positive | CT ₁ CT ₂ Mean C _r | 35.88 | | | 34.66 34.45 | | | | | | No Ct No Ct | | | No Ct | | |
| | | | 35.88 | 76.6 | Detected (LD) | | 76.55 | Detected (LD) | No Ct | | Not detected | No Ct | | Not detected | No Ct | | Not detected |
| Greece | strongly positive | CT ₁ CT ₂ Mean C _r | 23.65 | | | 23.79 23.88 | | | 28.43 28.44 | | | 24.66 24.49 | | | 34.43 33.29 | | |
| | | | 23.65 | 76 | Detected | 23.835 76.14 | | Detected | 28.44 79.5 | | Detected | 24.575 77.85 | | Detected | 33.86 77.0 | | Detected |
| Greece | moderate positive | CT ₁ CT ₂ Mean C _r | 29.54 | | | 29.55 31.99 | | | 33.49 33.33 | | | 29.17 28.95 | | | 39.66 33.30 | | |
| | | | 29.54 | 76 | Detected | 30.77 76.22 | | Detected | 33.41 79.5 | | Detected | 29.06 77.85 | | Detected | 36.48 77 | | Detected (LD) |
| Greece | weak positive | CT ₁ CT ₂ Mean C _r | 33.61 | | | 34.56 32.27 | | | | | | No Ct No Ct | | | 33.31 | | |
| | | | 33.61 | 76 | Detected | 33.415 76.05 | | Detected (LD) | No Ct | | Not detected | No Ct | | Not detected | 33.31 | | Inconclusive |
| EBLV-1b | strongly positive | CT ₁ CT ₂ Mean C _r | 23.46 | | | 23.18 23.07 | | | 27.33 28.12 | | | 29.05 29.21 | | | 29.77 33.26 | | |
| | | | 23.46 | 75.75 | Detected | | 75.9 | Detected | 28.12 79.2 | | Detected | 29.13 77.50 | | Detected | 31.52 76.5 | | Detected |
| EBLV-1b | moderate positive | CT ₁ CT ₂ Mean C _r | 29.27 | | | 28.11 28.34 | | | 33.14 32.90 | | | No Ct No Ct | | | 34.46 33.27 | | |
| | | | 29.27 | 75.75 | Detected | 28.225 75.9 | | Detected | 33.02 79.5 | | Detected | No Ct | | Not detected | 33.87 76.7 | | Inconclusive |
| EBLV-1b | weak positive | CT ₁ CT ₂ Mean C _r | 35.69 | | | 34.31 35.06 | | | | | | No Ct No Ct | | | No Ct No Ct | | |
| | | | 35.69 | 75.9 | Detected (LD) | 34.685 75.92 | | Detected (LD) | No Ct | | Not detected | No Ct | | Not detected | No Ct | | Inconclusive |
| EBLV-2 | strongly positive | CT ₁ CT ₂ Mean C _r | 20.49 | | | 20.44 | | | 25.43 25.82 | | | 29.39 29.57 | | | 28.33 27.89 | | |
| | | | 20.49 | 75.75 | Detected | 20.44 75.92 | | Detected | 25.63 79 | | Detected | 29.48 77.10 | | Detected | 28.11 76.3 | | Detected |
| EBLV-2 | moderate positive | CT ₁ CT ₂ Mean C _r | 26.75 | | | 26.35 | | | 31.40 31.20 | | | No Ct No Ct | | | 35.02 34.16 | | |
| | | | 26.75 | 75.75 | Detected | 26.35 75.87 | | Detected | 31.30 79 | | Detected | No Ct | | Not detected | 34.59 76.7 | | Detected |
| EBLV-2 | weak positive | CT ₁ CT ₂ Mean C _r | 32.06 | | | 31.69 31.84 | | | 39.14 37.28 | | | No Ct No Ct | | | 42.81 42.78 | | |
| | | | 32.06 | 75.75 | Detected | 31.765 75.8 | | Detected | 38.21 79.2 | | Inconclusive | No Ct | | Not detected | 42.80 | | Inconclusive |
| BBLV | strongly positive | CT ₁ CT ₂ Mean C _r | 22.64 | | | 23.22 | | | 29.86 29.96 | | | 31.89 32.17 | | | 31.72 33.32 | | |
| | | | 22.64 | 75.5 | Detected | 23.22 75.6 | | Detected | 29.91 78.8 | | Detected | 32.03 77.90 | | Detected | 32.52 76.3 | | Detected |

| | | | | | | | | | | | | | | | |
|------|-------------------|---------------------|-------|------|---------------|-------|-------|---------------|-------|--------------|--------------|-------|--------------|--------------|--------------|
| BBLV | moderate positive | CT ₁ | 28.13 | | | 29.07 | | | 36.47 | | | 28.87 | | | 40.52 |
| | | CT ₂ | | | | | | | 35.37 | | | 28.81 | | | 41.15 |
| | | Mean C _T | 28.13 | 75.5 | Detected | 29.07 | 75.58 | Detected | 35.92 | 79 | Detected | 28.84 | 77.40 | Detected | 40.84 |
| BBLV | weak positive | CT ₁ | 35.13 | | | 34.69 | | | No Ct | | | No Ct | | | No Ct |
| | | CT ₂ | | | | 34.09 | | | No Ct | | | No Ct | | | No Ct |
| | | Mean C _T | 35.13 | 75.5 | Detected (LD) | 34.39 | 75.48 | Detected (LD) | No Ct | Not detected | Not detected | No Ct | Not detected | Not detected | No Ct |
| | | | | | | | | | | | | | | | Inconclusive |

| | | | SYBR Green pan-lyssavirus RT-PCR assay | | | | | | | | |
|----------|-------------------|-----------------------|--|----------------|--------------|-------|----------------|------------|-------|----------------|--------------|
| Samples | Class | C _T values | L17 | | | L21 | | | L25 | | |
| | | | CT | T _M | Conclusion | CT | T _M | Conclusion | CT | T _M | Conclusion |
| Negative | / | CT ₁ | no data | | | nd. | nd. | nd. | No Ct | | |
| | | CT ₂ | | | | nd. | nd. | nd. | No Ct | | |
| | | CT ₃ | | | | nd. | nd. | nd. | No Ct | | |
| | | Mean C _T | no data | | Not detected | nd. | nd. | nd. | No Ct | - | Not detected |
| CVS (1) | strongly positive | CT ₁ | 37.84 | | | 27.63 | | | 21.15 | | |
| | | CT ₂ | | | | | | | 21.37 | | |
| | | CT ₃ | | | | | | | 21.08 | | |
| | | Mean C _T | 37.84 | 83.6 | Detected | 27.63 | 75 | Detected | 21.20 | 77.7 | Detected |
| CVS (1) | moderate positive | CT ₁ | 44.96 | | | 32.85 | | | 25.72 | | |
| | | CT ₂ | | | | | | | 25.74 | | |
| | | CT ₃ | | | | | | | 25.75 | | |
| | | Mean C _T | 44.96 | 83.4 | Detected | 32.85 | 75 | Detected | 25.74 | 77.7 | Detected |
| CVS (1) | weak positive | CT ₁ | no data | | | 37.75 | | | 30.52 | | |
| | | CT ₂ | | | | | | | 29.92 | | |
| | | CT ₃ | | | | | | | 30.69 | | |
| | | Mean C _T | no data | | Not detected | 37.75 | 75 | Detected | 30.38 | 77.7 | Detected |
| CVS (2) | strongly positive | CT ₁ | 37.83 | | | 27.91 | | | 20.40 | | |
| | | CT ₂ | | | | | | | 20.53 | | |
| | | CT ₃ | | | | | | | 20.33 | | |
| | | Mean C _T | 37.83 | 83.6 | Detected | 27.91 | 75 | Detected | 20.42 | 78.0 | Detected |
| CVS (2) | moderate positive | CT ₁ | | | | 33.53 | | | 24.96 | | |
| | | CT ₂ | | | | | | | 25.21 | | |
| | | CT ₃ | | | | | | | 25.05 | | |
| | | Mean C _T | | 83.4 | Inconclusive | 33.53 | 75 | Detected | 25.07 | 77.9 | Detected |
| CVS (2) | weak positive | CT ₁ | | | | 37.75 | | | 30.48 | | |
| | | CT ₂ | | | | | | | 32.30 | | |
| | | CT ₃ | | | | | | | 30.31 | | |
| | | Mean C _T | | | Not detected | 37.75 | | Detected | 31.03 | 78.0 | Detected |
| Greece | strongly positive | CT ₁ | 38.06 | | | nd. | nd. | nd. | 20.47 | | |
| | | CT ₂ | | | | nd. | nd. | nd. | 20.41 | | |
| | | CT ₃ | | | | nd. | nd. | nd. | 20.33 | | |
| | | Mean C _T | 38.06 | 82.8 | Detected | nd. | nd. | nd. | 20.40 | 77.2 | Detected |
| Greece | moderate positive | CT ₁ | 44.28 | | | nd. | nd. | nd. | 25.17 | | |
| | | CT ₂ | | | | nd. | nd. | nd. | 25.20 | | |
| | | CT ₃ | | | | nd. | nd. | nd. | 25.50 | | |
| | | Mean C _T | 44.28 | 82.6 | Detected | nd. | nd. | nd. | 25.29 | 77.2 | Detected |
| Greece | weak positive | CT ₁ | | | | nd. | nd. | nd. | 31.69 | | |
| | | CT ₂ | | | | nd. | nd. | nd. | 30.98 | | |
| | | CT ₃ | | | | nd. | nd. | nd. | 31.56 | | |
| | | Mean C _T | | | Not detected | nd. | nd. | nd. | 31.41 | 77.1 | Detected |
| EBLV-1b | strongly positive | CT ₁ | 35.42 | | | nd. | nd. | nd. | 19.98 | | |
| | | CT ₂ | | | | nd. | nd. | nd. | 20.02 | | |
| | | CT ₃ | | | | nd. | nd. | nd. | 19.82 | | |
| | | Mean C _T | 35.42 | 81.1 | Detected | nd. | nd. | nd. | 19.94 | 77.0 | Detected |
| EBLV-1b | moderate positive | CT ₁ | 42.03 | | | nd. | nd. | nd. | 25.25 | | |
| | | CT ₂ | | | | nd. | nd. | nd. | 25.41 | | |
| | | CT ₃ | | | | nd. | nd. | nd. | 26.31 | | |
| | | Mean C _T | 42.03 | 81.4 | Detected | nd. | nd. | nd. | 25.66 | 77.0 | Detected |
| EBLV-1b | weak positive | CT ₁ | | | | nd. | nd. | nd. | 29.68 | | |
| | | CT ₂ | | | | nd. | nd. | nd. | 30.03 | | |
| | | CT ₃ | | | | nd. | nd. | nd. | 31.21 | | |
| | | Mean C _T | | 82.0 | Not detected | nd. | nd. | nd. | 30.31 | 77.0 | Detected |
| EBLV-2 | strongly positive | CT ₁ | | | | nd. | nd. | nd. | 17.24 | | |
| | | CT ₂ | | | | nd. | nd. | nd. | 17.12 | | |
| | | CT ₃ | | | | nd. | nd. | nd. | 17.25 | | |
| | | Mean C _T | | | Not detected | nd. | nd. | nd. | 17.20 | 77.2 | Detected |
| EBLV-2 | moderate positive | CT ₁ | | | | nd. | nd. | nd. | 22.59 | | |
| | | CT ₂ | | | | nd. | nd. | nd. | 22.86 | | |
| | | CT ₃ | | | | nd. | nd. | nd. | 22.90 | | |
| | | Mean C _T | | | Not detected | nd. | nd. | nd. | 22.78 | 77.2 | Detected |
| EBLV-2 | weak positive | CT ₁ | | | | nd. | nd. | nd. | 27.85 | | |
| | | CT ₂ | | | | nd. | nd. | nd. | 27.54 | | |
| | | CT ₃ | | | | nd. | nd. | nd. | 27.67 | | |
| | | Mean C _T | | | Not detected | nd. | nd. | nd. | 27.69 | 77.1 | Detected |
| BBLV | strongly positive | CT ₁ | 41.5 | | | nd. | nd. | nd. | 19.55 | | |
| | | CT ₂ | | | | nd. | nd. | nd. | 19.57 | | |
| | | CT ₃ | | | | nd. | nd. | nd. | 19.66 | | |
| | | Mean C _T | 41.5 | 80.7 | Detected | nd. | nd. | nd. | 19.59 | 76.7 | Detected |
| BBLV | moderate positive | CT ₁ | | | | nd. | nd. | nd. | 24.83 | | |
| | | CT ₂ | | | | nd. | nd. | nd. | 24.65 | | |

| | | | | | | | | | | |
|------|---------------|---------------------|------|---------------------|-----|-----|-----|-------|------|----------|
| | | CT ₃ | | | nd. | nd. | nd. | 24.99 | | |
| | | Mean C _T | 81.0 | Inconclusive | nd. | nd. | nd. | 24.82 | 76.7 | Detected |
| BBLV | weak positive | CT ₁ | | | nd. | nd. | nd. | 30.64 | | |
| | | CT ₂ | | | nd. | nd. | nd. | 31.11 | | |
| | | CT ₃ | | | nd. | nd. | nd. | 30.74 | | |
| | | Mean C _T | | Not detected | nd. | nd. | nd. | 30.83 | 76.8 | Detected |

2. S Table 2: Results of TaqMan specific probe RT-PCR by strain tested

| Lyssavirus specific probe RT-PCR assay | | | | | | | | | | | | | | | | | | | | |
|--|-------------------|---|----------------|----------------|----------------|--------------------|--------------------|---------------|----------------|----------------|----------------|--------------|----------------|--------------------|-------------------------|---------------|----------------|--------------------|----------------|--------------|
| Samples | Class | CT Value | RABV | EBLV-1 | EBLV-2 | RABV EBLV-1 EBLV-2 | RABV EBLV-1 EBLV-2 | RABV | EBLV-1/BBL V | EBLV-2 | EBLV-2 | EBLV-2 | EBLV-1 | RABV EBLV-1 EBLV-2 | RABV | EBLV-1 | EBLV-2 | RABV EBLV-1 EBLV-2 | | |
| | | | L1 | | | | L3 | | L10 | | | | L11 | | | | L17 | | | |
| | | | C _T | C _T | C _T | Conclusion | C _T | Conclusion | C _T | C _T | C _T | Conclusion | C _T | C _T | C _T | Conclusion | C _T | C _T | C _T | Conclusion |
| Negative | / | CT ₁ CT ₂ CT ₃ | No Ct | No Ct | No Ct | | No Ct | | No Ct | No Ct | No Ct | | No Ct | No Ct | No Ct | | No Ct | No Ct | No Ct | |
| | | Mean C _T | | | | Not detected | No Ct | Not detected | No Ct | No Ct | No Ct | Not detected | No Ct | No Ct | No Ct | Not detected | No Ct | No Ct | No Ct | Not detected |
| CVS (1) | strongly positive | CT ₁ CT ₂ CT ₃ | 27.91 | No Ct | No Ct | | 27.76 27.52 | | 25.18 | No Ct | No Ct | | No Ct | No Ct | 29.74 30.46 29.81 | | 25.44 | No Ct | No Ct | |
| | | Mean C _T | 27.91 | No Ct | No Ct | Detected | 27.64 | Detected | 25.18 | No Ct | No Ct | Detected | No Ct | No Ct | 30 | Detected | 25.44 | No Ct | No Ct | Detected |
| CVS (1) | moderate positive | CT ₁ CT ₂ CT ₃ | 32 | No Ct | No Ct | | 31.02 34.97 | | 29.7 | No Ct | No Ct | | No Ct | No Ct | 35.87 35.51 35.42 | | 30.9 | No Ct | No Ct | |
| | | Mean C _T | 32 | No Ct | No Ct | Detected | 32.995 | Detected | 29.7 | No Ct | No Ct | Detected | No Ct | No Ct | 35.6 | Detected (LD) | 30.9 | No Ct | No Ct | Detected |
| CVS (1) | weak positive | CT ₁ CT ₂ CT ₃ | 37.84 | No Ct | No Ct | | 44.94 42.92 | | 38.29 | No Ct | No Ct | | No Ct | No Ct | 36.07 No Ct | | No Ct | No Ct | No Ct | |
| | | Mean C _T | 37.84 | No Ct | No Ct | Detected | 43.93 | Detected (LD) | 38.29 | No Ct | No Ct | Detected | No Ct | No Ct | 36.07 | Inconclusive | No Ct | No Ct | No Ct | Not detected |
| CVS (2) | strongly positive | CT ₁ CT ₂ CT ₃ | 27.19 | No Ct | No Ct | | 28.72 28.09 | | 24.21 | No Ct | No Ct | | No Ct | No Ct | 30.19 30.9 29.87 | | 25.22 | No Ct | No Ct | |
| | | Mean C _T | 27.19 | No Ct | No Ct | Detected | 28.405 | Detected | 24.21 | No Ct | No Ct | Detected | No Ct | No Ct | 30.32 | Detected | 25.22 | No Ct | No Ct | Detected |
| CVS (2) | moderate positive | CT ₁ CT ₂ CT ₃ | 32.39 | No Ct | No Ct | | 27.01 27.65 | | 29.44 | No Ct | No Ct | | No Ct | No Ct | 34.94 33.94 34.2 | | 30.45 | No Ct | No Ct | |
| | | Mean C _T | 32.39 | No Ct | No Ct | Detected | 27.33 | Detected | 29.44 | No Ct | No Ct | Detected | No Ct | No Ct | 34.36 | Detected (LD) | 30.45 | No Ct | No Ct | Detected |
| CVS (2) | weak positive | CT ₁ CT ₂ CT ₃ | 36.72 | No Ct | No Ct | | No Ct No Ct | | 36.29 | No Ct | No Ct | | No Ct | No Ct | 40.03 39.45 38.62 | | No Ct | No Ct | No Ct | |
| | | Mean C _T | 36.72 | No Ct | No Ct | Detected | No Ct | Not detected | 36.29 | No Ct | No Ct | Detected | No Ct | No Ct | 39.36 | Inconclusive | No Ct | No Ct | No Ct | Not detected |
| Greece | strongly positive | CT ₁ CT ₂ CT ₃ | 24.68 | No Ct | No Ct | | 26.04 30.43 | | 22.95 | No Ct | No Ct | | No Ct | No Ct | 26.38 25.5 26.19 | | 26.71 | No Ct | No Ct | |
| | | Mean C _T | 24.68 | No Ct | No Ct | Detected | 28.235 | Detected | 22.95 | No Ct | No Ct | Detected | No Ct | No Ct | 26.02 | Detected | 26.71 | No Ct | No Ct | Detected |
| Greece | moderate positive | CT ₁ CT ₂ CT ₃ | 29.99 | No Ct | No Ct | | 29.39 29.68 | | 27.78 | No Ct | No Ct | | No Ct | No Ct | 31.78 31.99 32.42 | | 31.17 | No Ct | No Ct | |
| | | Mean C _T | 29.99 | No Ct | No Ct | Detected | 29.535 | Detected | 27.78 | No Ct | No Ct | Detected | No Ct | No Ct | 32.06 | Detected | 31.17 | No Ct | No Ct | Detected |
| Greece | weak positive | CT ₁ CT ₂ CT ₃ | 34.84 | No Ct | No Ct | | 40.45 40.34 | | 32.14 | No Ct | No Ct | | No Ct | No Ct | 36.02 38.14 36.48 | | No Ct | No Ct | No Ct | |
| | | Mean C _T | 34.84 | No Ct | No Ct | Detected | 40.395 | Detected (LD) | 32.14 | No Ct | No Ct | Detected | No Ct | No Ct | 36.88 | Inconclusive | No Ct | No Ct | No Ct | Not detected |
| EBLV-1b | strongly positive | CT ₁ CT ₂ CT ₃ | No Ct | 25.69 | No Ct | | 28.8 29.01 | | No Ct | 24.92 | No Ct | | No Ct | 24.65 23.98 | No Ct | | No Ct | 21.23 | No Ct | |

| | | | | | | | | | | | | | | | | | | | | |
|---------|-------------------|---------------------|-------|-------|-------|--------------|--------|--------------|-------|-------|-------|----------|-------|-------|-------|--------------|-------|-------|-------|--------------|
| EBLV-1b | moderate positive | Mean C _T | No Ct | 25.69 | No Ct | Detected | 28.905 | Detected | No Ct | 24.92 | No Ct | Detected | No Ct | 24.32 | No Ct | Detected | No Ct | 21.23 | No Ct | Detected |
| | | CT ₁ | No Ct | 30.71 | No Ct | | 33.45 | | No Ct | 29.98 | No Ct | | No Ct | 29.4 | No Ct | | No Ct | 26.1 | No Ct | |
| | | CT ₂ | | | | | 34.43 | | | | | | No Ct | 29.72 | No Ct | | | | | |
| | | CT ₃ | | | | | | | | | | | No Ct | | No Ct | | | | | |
| | | Mean C _T | No Ct | 30.71 | No Ct | Detected | 33.94 | Detected | No Ct | 29.98 | No Ct | Detected | No Ct | 29.56 | No Ct | Detected | No Ct | 26.1 | No Ct | Detected |
| EBLV-1b | weak positive | CT ₁ | No Ct | 35.94 | No Ct | | No Ct | | No Ct | 36.12 | No Ct | | No Ct | 36.49 | No Ct | | No Ct | 30.84 | No Ct | |
| | | CT ₂ | | | | | No Ct | | | | | | No Ct | 36.1 | No Ct | | | | | |
| | | CT ₃ | | | | | No Ct | | | | | | No Ct | | No Ct | | | | | |
| | | Mean C _T | No Ct | 35.94 | No Ct | Detected | No Ct | Not detected | No Ct | 36.12 | No Ct | Detected | No Ct | 36.29 | No Ct | Inconclusive | No Ct | 30.84 | No Ct | Detected |
| EBLV-2 | strongly positive | CT ₁ | No Ct | No Ct | 24.9 | | 24.89 | | No Ct | No Ct | 20.5 | | 23.17 | No Ct | No Ct | | No Ct | No Ct | 21.9 | |
| | | CT ₂ | | | | | 25.31 | | | | | | 22.91 | No Ct | No Ct | | | | | |
| | | CT ₃ | | | | | | | | | | | 22.75 | No Ct | No Ct | | | | | |
| | | Mean C _T | No Ct | No Ct | 24.9 | Detected | 25.1 | Detected | No Ct | No Ct | 20.5 | Detected | 22.94 | No Ct | No Ct | Detected | No Ct | No Ct | 21.9 | Detected |
| EBLV-2 | moderate positive | CT ₁ | No Ct | No Ct | 29.83 | | 29.09 | | No Ct | No Ct | 25.56 | | 28.77 | No Ct | No Ct | | No Ct | No Ct | 26.38 | |
| | | CT ₂ | | | | | 29.21 | | | | | | 28.77 | No Ct | No Ct | | | | | |
| | | CT ₃ | | | | | | | | | | | 28.70 | No Ct | No Ct | | | | | |
| | | Mean C _T | No Ct | No Ct | 29.83 | Detected | 29.15 | Detected | No Ct | No Ct | 25.56 | Detected | 28.75 | No Ct | No Ct | Detected | No Ct | No Ct | 26.38 | Detected |
| EBLV-2 | weak positive | CT ₁ | No Ct | No Ct | 35.13 | | 33.08 | | No Ct | No Ct | 31.01 | | 34.50 | No Ct | No Ct | | No Ct | No Ct | 30.86 | |
| | | CT ₂ | | | | | 33.43 | | | | | | 34.14 | No Ct | No Ct | | | | | |
| | | CT ₃ | | | | | | | | | | | 33.45 | No Ct | No Ct | | | | | |
| | | Mean C _T | No Ct | No Ct | 35.13 | Detected | 33.255 | Detected | No Ct | No Ct | 31.01 | Detected | 34.03 | No Ct | No Ct | Detected | No Ct | No Ct | 30.86 | Detected |
| BBLV | strongly positive | CT ₁ | No Ct | No Ct | No Ct | | No Ct | | No Ct | 23.76 | | | No Ct | No Ct | No Ct | | No Ct | No Ct | No Ct | |
| | | CT ₂ | | | | | No Ct | | | | | | No Ct | No Ct | No Ct | | | | | |
| | | CT ₃ | | | | | No Ct | | | | | | No Ct | No Ct | No Ct | | | | | |
| | | Mean C _T | No Ct | No Ct | No Ct | Not detected | No Ct | Not detected | No Ct | 23.76 | No Ct | Detected | No Ct | No Ct | No Ct | Not detected | No Ct | No Ct | No Ct | Not detected |
| BBLV | moderate positive | CT ₁ | No Ct | No Ct | No Ct | | No Ct | | No Ct | 28.7 | No Ct | | No Ct | No Ct | No Ct | | No Ct | No Ct | No Ct | |
| | | CT ₂ | | | | | No Ct | | | | | | No Ct | No Ct | No Ct | | | | | |
| | | CT ₃ | | | | | No Ct | | | | | | No Ct | No Ct | No Ct | | | | | |
| | | Mean C _T | No Ct | No Ct | No Ct | Not detected | No Ct | Not detected | No Ct | 28.7 | No Ct | Detected | No Ct | No Ct | No Ct | Not detected | No Ct | No Ct | No Ct | Not detected |
| BBLV | weak positive | CT ₁ | No Ct | No Ct | No Ct | | No Ct | | No Ct | 34.55 | No Ct | | No Ct | No Ct | No Ct | | No Ct | No Ct | No Ct | |
| | | CT ₂ | | | | | No Ct | | | | | | No Ct | No Ct | No Ct | | | | | |
| | | CT ₃ | | | | | No Ct | | | | | | No Ct | No Ct | No Ct | | | | | |
| | | Mean C _T | No Ct | No Ct | No Ct | Not detected | No Ct | Not detected | No Ct | 34.55 | No Ct | Detected | No Ct | No Ct | No Ct | Not detected | No Ct | No Ct | No Ct | Not detected |

| Lyssavirus specific probe RT-PCR assay | | | | | | | | | | | | | |
|--|-------------------|---|-------------------------|---------------------|----------------|--------------|--------------------|--------------|-----------------|------------|--------------------|--------------|---------------------|
| Samples | Class | CT Value | RABV EBLV-1 EBLV-2 BBLV | | RABV | | RABV EBLV-1 EBLV-2 | | RABV EBLV-1 | | RABV EBLV-1 EBLV-2 | | |
| | | | L18 | | L19 | | L20 | | L23 | | L24 | | |
| | | | C _T | Conclusion | C _T | Conclusion | C _T | Conclusion | C _T | Conclusion | C _T | Conclusion | |
| Negative | / | CT ₁ CT ₂ CT ₃ | No Ct No Ct | | No Ct | | | 39.88 | | No Ct | | No Ct | |
| | | Mean C _T | No Ct | Not detected | No Ct | Not detected | | 39.88 | Detected | No Ct | Not detected | No Ct | Not detected |
| CVS (1) | strongly positive | CT ₁ CT ₂ CT ₃ | 26.25 25.50 | | 26.91 | | | 25.76 | | 27.84 | | 28.63 | |
| | | Mean C _T | 25.88 | Detected | | Detected | | 25.76 | Detected | 27.84 | Detected | 28.63 | Detected |
| CVS (1) | moderate positive | CT ₁ CT ₂ CT ₃ | 31.75 29.79 | | 31.73 | | | 31.07 | | 31.96 | | 33.76 | |
| | | Mean C _T | 30.77 | Detected | 31.73 | Detected | | 31.07 | Detected | 31.96 | Detected | 33.76 | Detected |
| CVS (1) | weak positive | CT ₁ CT ₂ CT ₃ | No Ct No Ct | | 36.47 | | | 36.71 | | 35.87 | | 35.67 | |
| | | Mean C _T | No Ct | Not detected | 36.47 | Detected | | 36.71 | Detected | 35.87 | Detected | 35.67 | Detected |
| CVS (2) | strongly positive | CT ₁ CT ₂ CT ₃ | 26.20 24.80 | | 26.78 | | | 26.03 | | 27.58 | | 28.67 | |
| | | Mean C _T | 25.50 | Detected | 26.78 | Detected | | 26.03 | Detected | 27.58 | Detected | 28.67 | Detected |
| CVS (2) | moderate positive | CT ₁ CT ₂ CT ₃ | 31.12 29.90 | | 31.87 | | | 31.4 | | 31.22 | | 33.42 | |
| | | Mean C _T | | Detected | | Detected | | 31.4 | Detected | 31.22 | Detected | 33.42 | Detected |
| CVS (2) | weak positive | CT ₁ CT ₂ CT ₃ | No Ct | | 36.12 | | | 35.7 | | 36.29 | | No Ct | |
| | | Mean C _T | No Ct | Not detected | 36.12 | Detected | | 35.7 | Detected | 36.29 | Detected | No Ct | Not detected |
| Greece | strongly positive | CT ₁ CT ₂ CT ₃ | 23.95 24.30 | | 24.32 | | | 24.39 | | 25.67 | | 24.4 | |
| | | Mean C _T | 24.13 | Detected | 24.32 | Detected | | 24.39 | Detected | 25.67 | Detected | 24.4 | Detected |
| Greece | moderate positive | CT ₁ CT ₂ CT ₃ | 28.23 29.50 | | 29.13 | | | 29.15 | | 30.05 | | 28.81 | |
| | | Mean C _T | 28.87 | Detected | 29.13 | Detected | | 29.15 | Detected | 30.05 | Detected | 28.81 | Detected |
| Greece | weak positive | CT ₁ CT ₂ CT ₃ | 33.21 34.76 | | 33.44 | | | 33.35 | | 33.46 | | 36.71 | |
| | | Mean C _T | 33.99 | Detected | 33.44 | Detected | | 33.35 | Detected | 33.46 | Detected | 36.71 | Detected |
| EBLV-1b | strongly positive | CT ₁ CT ₂ CT ₃ | 27.20 26.40 | | No Ct | | | 23.66 | | 26.61 | | 24.14 | |
| | | Mean C _T | 26.80 | Detected | No Ct | Not detected | | 23.66 | Detected | 26.61 | Detected | 24.14 | Detected |
| EBLV-1b | moderate positive | CT ₁ CT ₂ CT ₃ | 31.80 32.50 | | No Ct | | | 28.83 | | 31.75 | | 29.17 | |

| | | | | | | | | | | | | |
|---------|-------------------|---------------------|--------------|---------------------|-------|--------------|--------------|-----------------|--------------|-----------------|-------|--------------|
| | | Mean C _T | 32.15 | Detected | No Ct | Not detected | 28.83 | Detected | 31.75 | Detected | 29.17 | Inconclusive |
| EBLV-1b | weak positive | CT ₁ | No Ct | | No Ct | | 35.23 | | 34.76 | | 34.25 | |
| | | CT ₂ | No Ct | | | | | | | | | |
| | | CT ₃ | | | | | | | | | | |
| | | Mean C _T | No Ct | Not detected | No Ct | Not detected | 35.23 | Detected | 34.76 | Detected | 34.25 | Inconclusive |
| EBLV-2 | strongly positive | CT ₁ | 22.27 | | No Ct | | 23.06 | | No Ct | Detected | 23.22 | |
| | | CT ₂ | 23.50 | | | | | | | | | |
| | | CT ₃ | | | | | | | | | | |
| | | Mean C _T | 23.14 | Detected | No Ct | Not detected | 23.06 | Detected | No Ct | Not detected | 23.22 | Detected |
| EBLV-2 | moderate positive | CT ₁ | 26.89 | | No Ct | | 28.03 | | No Ct | | 28.4 | |
| | | CT ₂ | 27.30 | | | | | | | | | |
| | | CT ₃ | | | | | | | | | | |
| | | Mean C _T | 27.10 | Detected | No Ct | Not detected | 28.03 | Detected | No Ct | Not detected | 28.4 | Detected |
| EBLV-2 | weak positive | CT ₁ | 31.66 | | No Ct | | 34.92 | | No Ct | | 34.25 | |
| | | CT ₂ | 31.80 | | | | | | | | | |
| | | CT ₃ | | | | | | | | | | |
| | | Mean C _T | 31.73 | Detected | No Ct | Not detected | 34.92 | Detected | No Ct | Not detected | 34.25 | Detected |
| BBLV | strongly positive | CT ₁ | 24.54 | | No Ct | | 29.94 | | 31.78 | | No Ct | |
| | | CT ₂ | 22.50 | | | | | | | | | |
| | | CT ₃ | | | | | | | | | | |
| | | Mean C _T | 23.52 | Detected | No Ct | Not detected | 29.94 | Detected | 31.78 | Detected | No Ct | Not detected |
| BBLV | moderate positive | CT ₁ | 29.99 | | No Ct | | 33.21 | | 33 | | No Ct | |
| | | CT ₂ | 27.61 | | | | | | | | | |
| | | CT ₃ | | | | | | | | | | |
| | | Mean C _T | 28.80 | Detected | No Ct | Not detected | 33.21 | Detected | 33 | Detected | No Ct | Not detected |
| BBLV | weak positive | CT ₁ | 34.67 | | No Ct | | 40.55 | | 38.89 | | No Ct | |
| | | CT ₂ | 32.60 | | | | | | | | | |
| | | CT ₃ | | | | | | | | | | |
| | | Mean C _T | 33.64 | Detected | No Ct | Not detected | 40.55 | Detected | 38.89 | Detected | No Ct | Not detected |

3. S Table 3: Results of *pan-Lyssavirus* TaqMan RT-PCR by strain tested

| Samples | Class | C _T values | Pan-lyssavirus probe based RT-PCR assay | | | | | | | | | | | | | | | | | |
|----------|-------------------|--|---|------------|----------------|---------------|-----|----------------|----------|------------|----------------|--------------|-----|------------|----------|----------------|----------|--|------------------|---------------|
| | | | L5 | | L9 | | L13 | | L14 | | L19' | | L21 | | L22 | | | | | |
| | | | CT | Conclusion | CT | Conclusion | CT | Conclusion | CT | Conclusion | Ct | Conclusion | Ct | Conclusion | Ct | Conclusion | | | | |
| Negative | / | CT ₁ CT ₂ CT ₃ Mean C _T | 35.66 | | No Ct No Ct | | | No Ct No Ct | | | No Ct No Ct | | | No Ct | | No Ct No Ct | | | | |
| CVS (1) | strongly positive | CT ₁ CT ₂ CT ₃ Mean C _T | 30.39 | | 26.41 26.27 | | | 20.57 20.24 | | | 28.1 27.6 | | | 25.50 | | 27.63 | | | 27.939 27.993 | |
| CVS (1) | moderate positive | CT ₁ CT ₂ CT ₃ Mean C _T | 30.39 | Detected | 26.34 | Detected | | 20.405 | Detected | | 27.85 | Detected | | 25.50 | Detected | 27.63 | Detected | | 27.966 | Detected |
| CVS (1) | weak positive | CT ₁ CT ₂ CT ₃ Mean C _T | 31.1 | | 31.34 31.42 | | | 26.09 25.75 | | | 32.7 33.3 | | | 30.91 | | 32.85 | | | 33.717 33.818 | |
| CVS (1) | strongly positive | CT ₁ CT ₂ CT ₃ Mean C _T | 31.1 | Detected | 31.38 | Detected | | 25.92 | Detected | | 33 | Detected | | 30.91 | Detected | 32.85 | Detected | | 33.7675 | Detected |
| CVS (1) | weak positive | CT ₁ CT ₂ CT ₃ Mean C _T | 34.74 | | 36.33 37.71 | | | 25.08 25.15 | | | 38.6 | | | 35.70 | | 37.75 | | | 38.871 38.424 | |
| CVS (1) | weak positive | CT ₁ CT ₂ CT ₃ Mean C _T | 34.74 | Detected | 37.02 | Detected (LD) | | 29.11206 | Detected | | 38.6 | Inconclusive | | 35.70 | Detected | 37.75 | Detected | | 38.6475 | Detected (LD) |
| CVS (2) | strongly positive | CT ₁ CT ₂ CT ₃ Mean C _T | 27.16 | | 25.99 25.89 | | | 20.53 20.19 | | | 28.3 28.4 | | | 25.43 | | 27.91 | | | 27.656 27.585 | |
| CVS (2) | moderate positive | CT ₁ CT ₂ CT ₃ Mean C _T | 27.16 | Detected | 25.94 | Detected | | 20.36 | Detected | | 28.35 | Detected | | 25.43 | Detected | 27.91 | Detected | | 27.6205 | Detected |
| CVS (2) | moderate positive | CT ₁ CT ₂ CT ₃ Mean C _T | 33.5 | | 30.95 31.52 | | | 25.82 25.93 | | | 33.1 33.2 | | | 30.59 | | 33.53 | | | 33.501 33.672 | |
| CVS (2) | weak positive | CT ₁ CT ₂ CT ₃ Mean C _T | 33.5 | Detected | 31.235 | Detected | | 25.875 | Detected | | 33.15 | Detected | | 30.59 | Detected | 33.53 | Detected | | 33.5865 | Detected |
| CVS (2) | weak positive | CT ₁ CT ₂ CT ₃ Mean C _T | 35.55 | | 36.49 37.39 | | | 29.92 29.98 | | | 38.9 | | | 35.68 | | 36.64 | | | 38.121 37.944 | |
| CVS (2) | weak positive | CT ₁ CT ₂ CT ₃ Mean C _T | 35.55 | Detected | 36.94 | Detected (LD) | | 29.95 | Detected | | 38.9 | Inconclusive | | 35.68 | Detected | 36.64 | Detected | | 38.0325 | Detected (LD) |
| Greece | strongly positive | CT ₁ CT ₂ CT ₃ Mean C _T | 27.17 | | 25.48 25.06 | | | 20.79 20.8 | | | 27.7 27.7 | | | 24.55 | | 24.89 | | | 27.922 27.863 | |
| Greece | strongly positive | CT ₁ CT ₂ CT ₃ Mean C _T | 27.17 | Detected | 25.27 | Detected | | 20.795 | Detected | | 27.7 | Detected | | 24.55 | Detected | 24.89 | Detected | | 27.8925 | Detected |
| Greece | moderate positive | CT ₁ CT ₂ CT ₃ Mean C _T | 32.46 | | 30.15 29.76 | | | 25.72 24.96 | | | 32 32.2 | | | 30.36 | | 30.53 | | | 32.718 32.786 | |
| Greece | moderate positive | CT ₁ CT ₂ CT ₃ Mean C _T | 32.46 | Detected | 29.955 | Detected | | 25.34 | Detected | | 32.1 | Detected | | 30.36 | Detected | 30.53 | Detected | | 32.752 | Detected |
| Greece | weak positive | CT ₁ CT ₂ CT ₃ Mean C _T | 36.19 | | 38.02 37.54 | | | 30.01 29.39 | | | 37.8 | | | 34.35 | | 35.3 | | | 38.334 39.139 | |
| Greece | weak positive | CT ₁ CT ₂ CT ₃ Mean C _T | 36.19 | Detected | 37.78 | Detected (LD) | | 29.7 | Detected | | 37.8 | Inconclusive | | 34.35 | Detected | 35.3 | Detected | | 38.7365 | Detected (LD) |
| EBLV-1b | strongly positive | CT ₁ CT ₂ CT ₃ Mean C _T | 25.79 | | 25.42 23.86 | | | 20.47 20.44 | | | 27.6 27.4 | | | 26.63 | | 23.62 | | | 26.278 26.92 | |
| EBLV-1b | strongly positive | CT ₁ CT ₂ CT ₃ Mean C _T | 25.79 | Detected | 24.64 | Detected | | 20.455 | Detected | | 27.5 | Detected | | 26.63 | Detected | 23.62 | Detected | | 26.599 | Detected |
| EBLV-1b | moderate positive | CT ₁ CT ₂ CT ₃ | 31.27 | | 30.32 30.65 | | | 23.05 22.97 | | | 31.8 31.5 | | | 31.43 | | 28.16 | | | 32.937 32.492 | |

| | | | | | | | | | | | | | | | | |
|---------|-------------------|---------------------|-------|----------|--------|---------------|--------|----------|-------|---------------|-------|--------------|-------|----------|---------|---------------|
| | | Mean C _T | 31.27 | Detected | 30.485 | Detected | 23.01 | Detected | 31.65 | Detected | 31.43 | Detected | 28.16 | Detected | 32.7145 | Detected |
| EBLV-1b | weak positive | CT ₁ | 36.66 | | 34.58 | | 29.82 | | 36.1 | | 35.97 | | 34.36 | | 37.49 | |
| | | CT ₂ | | | 35.11 | | 29.54 | | 38.6 | | | | | | 37.204 | |
| | | CT ₃ | | | | | | | | | | | | | | |
| | | Mean C _T | 36.66 | Detected | 34.845 | Detected | 29.68 | Detected | 37.35 | Inconclusive | 35.97 | Detected | 34.36 | Detected | 37.347 | Detected (LD) |
| EBLV-2 | strongly positive | CT ₁ | 22.9 | | 25.25 | | 18.32 | | 28.5 | | 23.46 | | 18.81 | | 26.947 | |
| | | CT ₂ | | | 25.9 | | 18.17 | | 28.6 | | | | | | 26.715 | |
| | | CT ₃ | | | | | | | | | | | | | | |
| | | Mean C _T | 22.9 | Detected | 25.575 | Detected | 18.245 | Detected | 28.55 | Detected | 23.46 | Detected | 18.81 | Detected | 26.831 | Detected |
| EBLV-2 | moderate positive | CT ₁ | 28.22 | | 30.5 | | 25.92 | | 33.1 | | 28.32 | | 27.64 | | 32.525 | |
| | | CT ₂ | | | 30.14 | | 25.6 | | 33.4 | | | | | | 32.119 | |
| | | CT ₃ | | | | | | | | | | | | | | |
| | | Mean C _T | 28.22 | Detected | 30.32 | Detected | 25.76 | Detected | 33.25 | Detected | 28.32 | Detected | 27.64 | Detected | 32.322 | Detected |
| EBLV-2 | weak positive | CT ₁ | 32.72 | | 37.44 | | 28.77 | | 42.5 | | 34.55 | | 32.94 | | 37.531 | |
| | | CT ₂ | | | 36.29 | | 28.33 | | 39.3 | | | | | | 37.895 | |
| | | CT ₃ | | | | | | | | | | | | | | |
| | | Mean C _T | 32.72 | Detected | 36.865 | Detected (LD) | 28.55 | Detected | 40.9 | Inconclusive | 34.55 | Detected | 32.94 | Detected | 37.713 | Detected (LD) |
| BBLV | strongly positive | CT ₁ | 29.88 | | 30.99 | | 21.79 | | 33 | | No Ct | | 26.44 | | 30.965 | |
| | | CT ₂ | | | 30.73 | | 21.57 | | 32.7 | | | | | | 30.618 | |
| | | CT ₃ | | | | | | | | | | | | | | |
| | | Mean C _T | 29.88 | Detected | 30.86 | Detected | 21.68 | Detected | 32.85 | Detected | No Ct | Not Detected | 26.44 | Detected | 30.7915 | Detected |
| BBLV | moderate positive | CT ₁ | 31.26 | | 37.06 | | 26.8 | | 35.8 | | No Ct | | 31.26 | | 35.906 | |
| | | CT ₂ | | | 36.19 | | 27.07 | | 37.6 | | | | | | 36.38 | |
| | | CT ₃ | | | | | | | | | | | | | | |
| | | Mean C _T | 31.26 | Detected | 36.625 | Detected (LD) | 26.935 | Detected | 36.7 | Detected (LD) | No Ct | Not Detected | 31.26 | Detected | 36.143 | Detected |
| BBLV | weak positive | CT ₁ | 33.93 | | No Ct | | 30.14 | | | | No Ct | | 35.78 | | >45 | |
| | | CT ₂ | | | No Ct | | 30.55 | | 39.3 | | | | | | >45 | |
| | | CT ₃ | | | | | | | | | | | | | | |
| | | Mean C _T | 33.93 | Detected | No Ct | Not Detected | 30.345 | Detected | 39.3 | Inconclusive | No Ct | Not Detected | 35.78 | Detected | No Ct | Not detected |

ND: not detected; NP: not performed; res: result

4. S Table 4: SYBR Green RT-PCR test results for the determination of the PCR detection limit

| SYBR Green pan-lyssavirus RT-PCR assay | | | L1 | | L2 | | L4 | | L6 | | L7 | | L8 | | L11 | | L12 | | L15 | | L16 | | L17 | | L21 | | L25 | |
|--|-----------------|---------------------------|-------|----------------|-------|----------------|-------|----------------|------|----------------|-------|----------------|-------|----------------|-------|----------------|-------|----------------|-------|----------------|-------|----------------|-------|----------------|-------|----------------|-------|----------------|
| Dilutions | CT replicates | Nb of copies/ μ L RNA | CT | T _M | CT | T _M | CT | T _M | CT | T _M | CT | T _M | CT | T _M | CT | T _M | CT | T _M | CT | T _M | CT | T _M | CT | T _M | CT | T _M | CT | T _M |
| Log 1 | CT ₁ | 1. 10 ⁶ | 17.69 | 76.35 | 21.93 | 78.2 | 17.9 | 80.2 | 20.6 | 78 | 17.24 | 79 | 18.34 | 76.5 | 17.35 | 76.83 | 20.12 | 80 | 17.64 | 77.25 | 24.06 | 76.8 | 29.03 | 83.58 | 17.63 | 75 | 14.38 | 77.7 |
| | CT ₂ | | / | / | 21.86 | | 17.96 | | 20.5 | | / | / | / | / | 17.25 | | 20.09 | | 17.66 | | 24.88 | | / | / | / | / | 14.42 | |
| | CT ₃ | | / | / | / | / | NT | | 20.8 | | / | / | / | / | 17.2 | | / | / | 17.76 | | 24.54 | | / | / | / | / | / | / |
| Log 2 | CT ₁ | 1. 10 ⁵ | 20.96 | 76.27 | 27.64 | 78.25 | 20.54 | | 23.9 | 78 | 20.71 | 79 | 22.18 | 76.5 | 20.65 | 76.8 | 23.52 | 79.8 | 22.91 | 77.15 | 29.22 | 77 | 33.14 | 83.58 | 20.14 | 75 | 17.83 | 77.7 |
| | CT ₂ | | / | / | 26.7 | | 20.49 | | 23.8 | | / | / | / | / | 20.45 | | 23.48 | | 22.72 | | 29.01 | | / | / | / | / | 17.68 | |
| | CT ₃ | | / | / | / | / | 20.8 | | 24.1 | | / | / | / | / | 20.31 | | | | 23.01 | | 29.32 | | / | / | / | / | / | / |
| Log 3 | CT ₁ | 1. 10 ⁴ | 24.3 | 76.2 | 31.14 | 78.28 | 23.86 | | 27.6 | | 24.25 | 79 | 25.87 | 76.5 | 23.61 | 76.8 | 26.47 | 79.8 | 25.47 | 77.35 | 34.37 | 77.3 | 36.89 | 83.58 | 23.25 | 75 | 21.09 | 77.7 |
| | CT ₂ | | / | / | 31.34 | | 23.93 | | 27.4 | | / | / | / | / | 23.78 | | 26.78 | | 25.5 | | 34.14 | | / | / | / | / | 21.29 | |
| | CT ₃ | | / | / | / | / | 23.76 | | 27.6 | | / | / | / | / | 23.99 | | | | 25.21 | | 34.06 | | / | / | / | / | / | / |
| Log 4 | CT ₁ | 1. 10 ³ | 27.76 | 76.2 | 34.97 | 78.28 | 26.91 | | 31.4 | 78 | 27.8 | 79 | 29.51 | 76.5 | 27.41 | 76.75 | 30.25 | 79.8 | 30.64 | 77.35 | 37.61 | 77.3 | 41.32 | 83.71 | 27.65 | 75 | 24.48 | 77.6 |
| | CT ₂ | | / | / | 34.32 | | 27.24 | | 31.1 | | / | / | / | / | 27.26 | | 30.05 | | 30.25 | | 37.74 | | / | / | / | / | 24.6 | |
| | CT ₃ | | / | / | / | / | NT | | 31.4 | | / | / | / | / | 27.54 | | | | 30.28 | | 37.73 | | / | / | / | / | / | / |
| Log 5 | CT ₁ | 1. 10 ² | 31.12 | 76.27 | 36.24 | 78.3 | 30.42 | | 34.9 | 78 | 31.03 | 79 | 32.54 | 76.5 | 30.63 | 76.7 | 33.89 | 80 | 33.15 | 77.1 | 44.77 | | 45 | | 32.18 | 75 | 28.35 | |
| | CT ₂ | | / | / | 36.53 | | 30.78 | | 34.9 | | / | / | / | / | 31.51 | | 34.72 | | 33.28 | | 43.24 | | / | / | / | / | 27.72 | |
| | CT ₃ | | / | / | / | / | 30.28 | | 35.3 | | / | / | / | / | 31.22 | | | | 33.74 | | 43.66 | | / | / | / | / | / | / |
| Log 6 | CT ₁ | 1. 10 ¹ | 34.65 | 76.27 | 37.27 | 72.03 | 33.16 | | 38 | 78 | 34.58 | 79 | 36.03 | 76.6 | 32.6 | 76.7 | 36.74 | 80 | 38.62 | 77.5 | 0 | | 45 | | 34.47 | 74.5 | 31.86 | 77.6 |
| | CT ₂ | | / | / | 26.87 | | 33.67 | | 38.5 | | / | / | / | / | 34.16 | | 39.69 | | 38.55 | | 0 | | / | / | / | / | 31.3 | |
| | CT ₃ | | / | / | / | / | NT | | | | / | / | / | / | 32.51 | | | | 39.2 | | 0 | | / | / | / | / | / | / |
| Log 7 | CT ₁ | 1. | / | / | 36.43 | 72.01 | 36.1 | | 0 | | 40.77 | 72 | 0 | | 34.38 | 76.7 | 0 | | 0 | | 0 | | 45 | | 37.43 | 74.5 | 36.53 | 77.6 |
| | CT ₂ | | / | / | 36.18 | | 35.74 | | 0 | | / | / | / | / | | | 0 | | 0 | | 0 | | / | / | / | / | 33.06 | |
| | CT ₃ | | / | / | / | / | NT | | | | / | / | / | / | | | | | | | 0 | | / | / | / | / | / | / |

5. S Table 5: TaqMan specific probe RT-PCR test results for the determination of the PCR detection limit

| Lyssavirus specific probe RT-PCR assay | | | L1 | L3 | L10 | L11 | L17 | L19 | L20 | L21 | L23 | L24 |
|--|-----------------|---------------------------|-------|----|-------|-------|-------|-------|-------|-------|-------|-------|
| Dilutions | CT replicates | Nb of copies/ μ L RNA | CT | CT | CT | CT | CT | CT | CT | CT | CT | CT |
| Log 1 | CT ₁ | 1. 10 ⁶ | 20.61 | 20 | 18.31 | 22.74 | 18.8 | 19.56 | 25.72 | 17.63 | 21.16 | 22.93 |
| | CT ₂ | | | 21 | | 22.66 | | | | | | 22.86 |
| | CT ₃ | | | | | 22.29 | | | | | | 22.84 |
| Log 2 | CT ₁ | 1. 10 ⁵ | 24.08 | 24 | 21.97 | 26.52 | 22.18 | 23.46 | 27.45 | 20.14 | 24.97 | 26.16 |
| | CT ₂ | | | 24 | | 26.67 | | | | | | 25.97 |
| | CT ₃ | | | | | 26.34 | | | | | | 26.13 |
| Log 3 | CT ₁ | 1. 10 ⁴ | 28.12 | 27 | 25.17 | 29.48 | 25.22 | 26.92 | 32.34 | 23.25 | 27.89 | 29.39 |
| | CT ₂ | | | 26 | | 29.36 | | | | | | 29.48 |
| | CT ₃ | | | | | 29.19 | | | | | | 29.46 |
| Log 4 | CT ₁ | 1. 10 ³ | 31.74 | 29 | 28.41 | 33.51 | 28.66 | 30.42 | 35.34 | 27.65 | 30.38 | 32.83 |
| | CT ₂ | | | 27 | | 32.62 | | | | | | 32.74 |
| | CT ₃ | | | | | 32.69 | | | | | | 33.48 |
| Log 5 | CT ₁ | 1. 10 ² | 35.91 | 32 | 31.86 | 35.35 | 32.53 | 34.12 | 37.93 | 32.18 | 32.86 | 36.11 |
| | CT ₂ | | | 33 | | 34.99 | | | | | | 37.34 |
| | CT ₃ | | | | | 34.64 | | | | | | 34.64 |
| Log 6 | CT ₁ | 1. 10 ¹ | 37.42 | 35 | 40.05 | 0 | 45 | 36.86 | 0 | 34.47 | 36.58 | 0 |
| | CT ₂ | | | 35 | | 0 | | | | | | 0 |
| | CT ₃ | | | | | 0 | | | | | | 36.74 |
| Log 7 | CT ₁ | 1. | NT | 41 | 0 | 0 | 45 | 0 | 40.43 | 37.43 | 34.86 | 0 |
| | CT ₂ | | | 44 | | 0 | | | | | | 0 |
| | CT ₃ | | | | | 0 | | | | | | 44.43 |

6. S Table 6: *Pan-Lyssavirus* TaqMan RT-PCR test results for the determination of the PCR detection limit

| <i>Pan-lyssavirus</i> probe based RT-PCR assay | | | L5 | L9 | L13 | L14 | L19 | L21 | L22 |
|--|-----------------|---------------------------|-------|-------|-------|-------|-------|-------|-------|
| Dilutions | CT replicates | Nb of copies/ μ L RNA | CT | CT | CT | CT | CT | CT | CT |
| Log 1 | CT ₁ | 1. 10 ⁶ | 18.08 | 20.11 | 15.42 | 18.3 | 18.93 | 17.63 | 22.46 |
| | CT ₂ | | | 19.69 | 15.42 | 18.5 | | | |
| | CT ₃ | | | 19.99 | 18.5 | | | | |
| Log 2 | CT ₁ | 1. 10 ⁵ | 22.51 | 23.04 | 18.33 | 21.8 | 22.14 | 20.14 | 26.07 |
| | CT ₂ | | | 23.19 | 18.27 | 21.8 | | | |
| | CT ₃ | | | 24.17 | 22 | | | | |
| Log 3 | CT ₁ | 1. 10 ⁴ | 24.15 | 26.34 | 22.55 | 25.2 | 25.27 | 23.25 | 28.99 |
| | CT ₂ | | | 26.82 | 22.68 | 25.3 | | | |
| | CT ₃ | | | 26.75 | 25.3 | | | | |
| Log 4 | CT ₁ | 1. 10 ³ | 25.12 | 29.83 | 25.62 | 28.5 | 29.01 | 27.65 | 31.9 |
| | CT ₂ | | | 29.92 | 25.45 | 28.6 | | | |
| | CT ₃ | | | 30.17 | 28.7 | | | | |
| Log 5 | CT ₁ | 1. 10 ² | 27.71 | 33.69 | 28.87 | 31.8 | 33.08 | 32.18 | 35.02 |
| | CT ₂ | | | 32.89 | 28.59 | 31.09 | | | |
| | CT ₃ | | | 32.83 | 32.1 | | | | |
| Log 6 | CT ₁ | 1. 10 ¹ | 0 | 36.38 | 31.26 | 35.4 | 36.52 | 34.47 | 37.88 |
| | CT ₂ | | | 40.85 | 31.46 | 36 | | | |
| | CT ₃ | | | 38.12 | 35 | | | | |
| Log 7 | CT ₁ | 1. | 0 | 41.97 | 33.55 | 0 | 0 | 37.43 | 45 |
| | CT ₂ | | | 0 | 36.77 | 40.9 | | | |
| | CT ₃ | | | 0 | 37.4 | | | | |

7. S Table 7: Sample decoding

| Samples | CVS (1) SP | CVS (1) MP | CVS (1) WP | EBLV-1b SP | EBLV-1b MP | EBLV-1b WP | Greece SP | Greece MP | Greece WP | BBLV SP | BBLV MP | BBLV WP | EBLV-2 SP | EBLV-2 MP | EBLV-2 WP | CVS (2) SP | CVS (2) MP | CVS (2) WP | Decoy / | Negative / |
|---------|---------------|---------------|---------------|---------------|---------------|---------------|--------------|--------------|--------------|------------|------------|------------|--------------|--------------|--------------|---------------|---------------|---------------|------------|---------------|
| L1 | 6513 | 2668 | 7822 | 132 | 5287 | 1441 | 4580 | 7345 | 3499 | 4809 | 3354 | 8508 | 7428 | 3582 | 6053 | 8425 | 8654 | 2273 | 3517 | 7363 |
| L2 | 3917 | 6453 | 2379 | 3231 | 5767 | 1693 | 8594 | 4520 | 7056 | 5518 | 3834 | 6370 | 4832 | 758 | 610 | 6059 | 2982 | 2296 | 3998 | 8072 |
| L3 | 2704 | 1706 | 7318 | 5323 | 4325 | 3328 | 771 | 6383 | 5386 | 1000 | 2392 | 8004 | 6009 | 2621 | 7940 | 1769 | 4388 | 7007 | 2556 | 3554 |
| L4 | 3954 | 337 | 1501 | 3268 | 8651 | 5035 | 2021 | 7404 | 3788 | 1335 | 6718 | 3102 | 7259 | 3642 | 6342 | 5638 | 2561 | 8485 | 6882 | 8109 |
| L5 | 205 | 8664 | 3904 | 2824 | 4673 | 4133 | 7272 | 6731 | 6191 | 891 | 350 | 2200 | 1119 | 2969 | 8745 | 5422 | 8041 | 1660 | 514 | 1054 |
| L6 | 2452 | 1683 | 3304 | 1766 | 997 | 228 | 519 | 8750 | 7981 | 8833 | 8064 | 7295 | 5757 | 4988 | 1535 | 1288 | 7212 | 6526 | 8228 | 8997 |
| L7 | 2320 | 4627 | 324 | 4939 | 7246 | 2943 | 387 | 2694 | 5001 | 3006 | 5313 | 7620 | 5625 | 7932 | 7555 | 7080 | 7308 | 927 | 5477 | 3169 |
| L8 | 5107 | 7186 | 2655 | 4421 | 6500 | 6188 | 3175 | 5253 | 4941 | 2489 | 2177 | 4255 | 8412 | 8101 | 7495 | 1096 | 7020 | 6334 | 2341 | 2652 |
| L9 | 5744 | 1899 | 7053 | 8363 | 4518 | 672 | 3811 | 6576 | 2730 | 6430 | 2585 | 7739 | 6659 | 2813 | 5284 | 7656 | 7885 | 1504 | 2748 | 6594 |
| L10 | 6225 | 8760 | 7077 | 5539 | 8074 | 4001 | 4292 | 6828 | 2754 | 1216 | 6142 | 8677 | 7139 | 3065 | 2917 | 8366 | 5289 | 4603 | 6305 | 1379 |
| L11 | 4783 | 1395 | 2787 | 7402 | 6404 | 3016 | 2850 | 8462 | 7464 | 5469 | 2081 | 1083 | 5697 | 4700 | 7628 | 3848 | 4076 | 85 | 2244 | 5632 |
| L12 | 109 | 5492 | 4266 | 8423 | 4806 | 1189 | 7176 | 3559 | 8942 | 6490 | 2873 | 8256 | 1023 | 8797 | 2496 | 8402 | 5326 | 4640 | 3037 | 4263 |
| L13 | 2741 | 4590 | 6440 | 7750 | 7209 | 6669 | 808 | 2658 | 2117 | 5817 | 5276 | 4736 | 6045 | 2821 | 4671 | 1348 | 1577 | 6586 | 5440 | 3590 |
| L14 | 1454 | 685 | 2307 | 768 | 8999 | 8230 | 8522 | 7753 | 6983 | 7836 | 7066 | 6297 | 2369 | 7916 | 7147 | 291 | 6214 | 5528 | 4840 | 5609 |
| L15 | 7703 | 1010 | 5708 | 3712 | 6019 | 8326 | 5770 | 8077 | 1384 | 8389 | 1696 | 6394 | 2008 | 1631 | 3938 | 3463 | 3692 | 8701 | 1860 | 8553 |
| L16 | 4567 | 6646 | 2114 | 3881 | 5960 | 8038 | 2634 | 4713 | 6791 | 1948 | 4027 | 6105 | 7872 | 7267 | 6955 | 2946 | 6479 | 5793 | 4190 | 2112 |
| L17 | 4975 | 1130 | 6284 | 7594 | 3749 | 8903 | 3042 | 5807 | 1961 | 5661 | 1816 | 6970 | 5890 | 8360 | 4515 | 6887 | 7116 | 735 | 8589 | 5825 |
| L18 | 8532 | 2068 | 384 | 7846 | 1382 | 6308 | 6599 | 135 | 5061 | 5913 | 8449 | 1985 | 446 | 2689 | 5225 | 1673 | 7597 | 6911 | 8612 | 3686 |
| L19 | 4471 | 3473 | 4866 | 480 | 6092 | 5094 | 2538 | 1540 | 7152 | 7547 | 4159 | 3161 | 7776 | 4094 | 706 | 5926 | 6155 | 8774 | 4323 | 5321 |
| L20 | 5263 | 1647 | 420 | 4577 | 961 | 6344 | 3330 | 8714 | 5097 | 254 | 8028 | 4411 | 6178 | 2268 | 7651 | 4557 | 1481 | 795 | 5801 | 418 |
| L21 | 7667 | 7126 | 2366 | 1285 | 3135 | 2595 | 5734 | 5193 | 4653 | 8353 | 7812 | 662 | 8581 | 7747 | 7207 | 3884 | 6503 | 122 | 7976 | 8516 |
| L22 | 457 | 8688 | 1309 | 6380 | 5611 | 4842 | 5134 | 4365 | 3596 | 4448 | 3679 | 2910 | 1371 | 6919 | 6149 | 5903 | 2826 | 2140 | 3842 | 4611 |
| L23 | 4086 | 8784 | 4481 | 96 | 2403 | 4710 | 2154 | 4461 | 6768 | 7163 | 470 | 2777 | 7391 | 7015 | 322 | 8846 | 75 | 5084 | 7243 | 4936 |
| L24 | 6417 | 8495 | 1574 | 5731 | 5419 | 7498 | 4484 | 4172 | 6251 | 1408 | 3486 | 5565 | 7332 | 6726 | 8805 | 2406 | 8329 | 7643 | 3650 | 1572 |
| L25 | 4206 | 361 | 5515 | 6825 | 2979 | 8134 | 8883 | 5037 | 1192 | 4892 | 1047 | 6201 | 5121 | 7591 | 3746 | 6118 | 6347 | 8966 | 7820 | 5056 |

Abbreviations: Strongly positive: SP ; Weak positive: WP ; Moderate positive: MP