Table S1: Screening chronic wasting disease (CWD) in 104 white-tailed deer by RT-QuIC based on cycle threshold1 and ELISA

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | RT-QuIC | | | |  |
|  | Mann-Whitney U-test | | Probability test | |  |
| ID2 | P-value | Pos/Neg | #Pos/#tested3 | Pos/Neg | ELISA |
| Obex-01 | 0.227 | Neg | 1/8 | Neg | Neg |
| Obex-07 | 0.011 | Pos | 4/4 | Pos | Pos |
| Obex-08 | 0.227 | Neg | 2/8 | Neg | Neg |
| Obex-18 | 0.227 | Neg | 2/8 | Neg | Neg |
| Obex-19 | 0.011 | Pos | 4/4 | Pos | Pos |
| Obex-20 | 0.011 | Pos | 4/4 | Pos | Pos |
| Obex-25 | 0.227 | Neg | 1/8 | Neg | Neg |
| Obex-29 | 0.227 | Neg | 1/8 | Neg | Neg |
| Obex-34 | 0.227 | Neg | 1/8 | Neg | Neg |
| Obex-45 | 0.011 | Pos | 4/4 | Pos | Pos |
| Obex-55 | 0.011 | Pos | 4/4 | Pos | Pos |
| Obex-56 | 0.227 | Neg | 2/8 | Neg | Neg |
| Obex-75 | 0.500 | Neg | 1/8 | Neg | Neg |
| Obex-80 | 0.796 | Neg | 1/8 | Neg | Neg |
| Obex-97 | 0.500 | Neg | 2/8 | Neg | Neg |
| Obex-100 | 0.500 | Neg | 1/8 | Neg | Neg |
| RLN-07 | 0.013 | Pos | 4/4 | Pos | Pos |
| RLN-08 | 0.062 | Neg | 3/8 | Neg | Neg |
| RLN-09 | 0.500 | Neg | 1/8 | Neg | Neg |
| RLN-18 | 0.647 | Neg | 1/8 | Neg | Neg |
| RLN-19 | 0.013 | Pos | 4/4 | Pos | Pos |
| RLN-20 | 0.013 | Pos | 4/4 | Pos | Pos |
| RLN-21 | 0.500 | Neg | 1/8 | Neg | Neg |
| RLN-28 | 0.500 | Neg | 1/8 | Neg | Neg |
| RLN-38 | 0.500 | Neg | 1/8 | Neg | Neg |
| RLN-39 | 0.500 | Neg | 1/8 | Neg | Neg |
| RLN-40 | 0.500 | Neg | 1/8 | Neg | Neg |
| RLN-45 | 0.011 | Pos | 4/4 | Pos | Pos |
| RLN-55 | 0.011 | Pos | 4/4 | Pos | Pos |
| RLN-61 | 0.093 | Neg | 1/8 | Neg | Neg |
| RLN-62 | 1.000 | Neg | 0/8 | Neg | Neg |
| RLN-77 | 0.227 | Neg | 1/8 | Neg | Neg |
| RLN-84 | 0.227 | Neg | 1/8 | Neg | Neg |
| RLN-88 | 1.000 | Neg | 0/8 | Neg | Neg |
| RLN-92 | 0.227 | Neg | 1/8 | Neg | Neg |
| RLN-100 | 0.227 | Neg | 1/8 | Neg | Neg |

1. Obex and retropharyngeal lymph node (RLN) tissue specimens from 104 white-tailed deer were tested by RT-QuIC in quadruplicate with an assay duration of 27 and 28 hours, respectively. CWD positivity was determined using the Mann-Whitney U-test or the probability test based on cycle threshold (Tstdev). When specimens had one or two replicate(s) crossed Tstdev, the specimens were re-tested in quadruplicate.
2. This table listed tissue specimens that were determined CWD positive using the Mann-Whitney U-test or had at least one replicate crossed Tstdev.
3. A total of 8 replicate were tested when specimens were subjected to re-testing.

Table S2: Screening chronic wasting disease (CWD) in 104 white-tailed deer by RT-QuIC based on max-point ratio (MPR)1 and ELISA

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | RT-QuIC | | | |  |
|  | Welch’s t-test | | Probability test | |  |
| ID2 | P-value | Pos/Neg | #Pos/#tested3 | Pos/Neg | ELISA |
| Obex-01 | 0.191 | Neg | 1/8 | Neg | Neg |
| Obex-07 | 0.000 | Pos | 4/4 | Pos | Pos |
| Obex-08 | 0.204 | Neg | 3/8 | Neg | Neg |
| **Obex-10** | 0.029 | Pos | 0/4 | Neg | Neg |
| **Obex-12** | 0.009 | Pos | 0/4 | Neg | Neg |
| Obex-18 | 0.195 | Neg | 1/8 | Neg | Neg |
| Obex-19 | 0.007 | Pos | 4/4 | Pos | Pos |
| Obex-20 | 0.000 | Pos | 4/4 | Pos | Pos |
| Obex-25 | 0.238 | Neg | 1/8 | Neg | Neg |
| Obex-29 | 0.234 | Neg | 1/8 | Neg | Neg |
| Obex-34 | 0.266 | Neg | 1/8 | Neg | Neg |
| Obex-45 | 0.009 | Pos | 4/4 | Pos | Pos |
| Obex-55 | 0.008 | Pos | 4/4 | Pos | Pos |
| Obex-56 | 0.208 | Neg | 2/8 | Neg | Neg |
| Obex-75 | 0.450 | Neg | 2/8 | Neg | Neg |
| Obex-80 | 0.301 | Neg | 2/8 | Neg | Neg |
| Obex-97 | 0.526 | Neg | 2/8 | Neg | Neg |
| Obex-100 | 0.598 | Neg | 1/8 | Neg | Neg |
| RLN-07 | 0.003 | Pos | 4/4 | Pos | Pos |
| RLN-08 | 0.090 | Neg | 3/8 | Neg | Neg |
| RLN-09 | 0.105 | Neg | 1/8 | Neg | Neg |
| RLN-19 | 0.000 | Pos | 4/4 | Pos | Pos |
| RLN-20 | 0.000 | Pos | 4/4 | Pos | Pos |
| RLN-21 | 0.318 | Neg | 1/8 | Neg | Neg |
| RLN-38 | 0.392 | Neg | 1/8 | Neg | Neg |
| RLN-40 | 0.452 | Neg | 1/8 | Neg | Neg |
| **RLN-43** | 0.005 | Pos | 0/4 | Neg | Neg |
| RLN-45 | 0.000 | Pos | 4/4 | Pos | Pos |
| **RLN-49** | 0.010 | Pos | 0/4 | Neg | Neg |
| **RLN-54** | 0.005 | Pos | 0/4 | Neg | Neg |
| RLN-55 | 0.002 | Pos | 4/4 | Pos | Pos |
| **RLN-57** | 0.012 | Pos | 0/4 | Neg | Neg |
| **RLN-58** | 0.002 | Pos | 0/4 | Neg | Neg |
| **RLN-60** | 0.016 | Pos | 0/4 | Neg | Neg |
| RLN-61 | 0.105 | Neg | 2/8 | Neg | Neg |
| **RLN-62** | 0.032 | Pos | 0/4 | Neg | Neg |
| **RLN-63** | 0.031 | Pos | 0/4 | Neg | Neg |
| RLN-77 | 0.210 | Neg | 1/8 | Neg | Neg |
| RLN-92 | 0.240 | Neg | 1/8 | Neg | Neg |
| RLN-100 | 0.338 | Neg | 1/8 | Neg | Neg |

1. Obex and retropharyngeal lymph node (RLN) tissue specimens from 104 white-tailed deer were tested by RT-QuIC in quadruplicate with an assay duration of 27 and 28 hours for obex and RLN, respectively. CWD positivity was determined using the Welch’s t-test or the probability test based on MPR threshold (TMPR) of 2.3 and 2.6 for obex and RLN, respectively. When specimens had one or two replicate(s) crossed TMPR, the specimens were re-tested in quadruplicate.
2. This table listed tissue specimens that were determined CWD positive using the Welch’s t-test or had at least one replicate crossed TMPR.
3. A total of 8 replicates were tested when specimens were subjected to re-testing.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Threshold | Tissue | Pos Both Durations | Pos 40 hours, Neg Ideal | Neg 40 hours, Pos Ideal | Neg Both Durations | McNemar's Statistic | P2 |
| TSTDEV | Obex | 31 | 33 | 0 | 352 | 31.03 | 2.54E-08 |
| TSTDEV | RLN | 32 | 23 | 0 | 361 | 21.04 | 4.49E-06 |
| TMPR | Obex | 31 | 34 | 0 | 351 | 32.03 | 1.52E-08 |
| TMPR | RLN | 32 | 15 | 0 | 369 | 13.07 | 3.01E-04 |

Table S3: Contingency tables for a replicate-level McNemar test1 between ideal assay durations and a 40 h assay duration

1. Obex and retropharyngeal lymph node (RLN) tissue specimens from 104 white-tailed deer were tested by RT-QuIC in quadruplicate with an assay duration of 27 hours for obex and RLN with TSTDEV, and 27 hours and 28 hours for obex and RLN with TMPR, respectively. TSTDEV was defined per plate as the average of all tested wells plus 10 standard deviations. TMPR was defined per well as the maximum fluorescence over the fluorescence at ~52 min, and was set to 2.3 and 2.6 for obex and RLN at ideal durations, respectively, and 2.7 and 3.1 at 40 hours, for obex and RLN, respectively.
2. The probability value (P) was calculated using 1 degree of freedom.