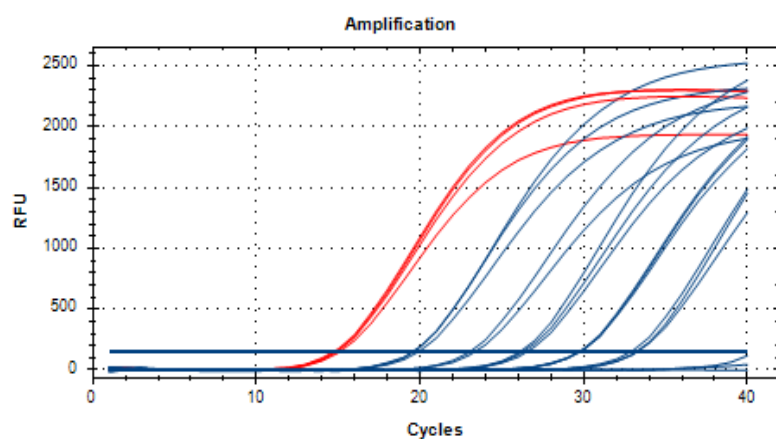


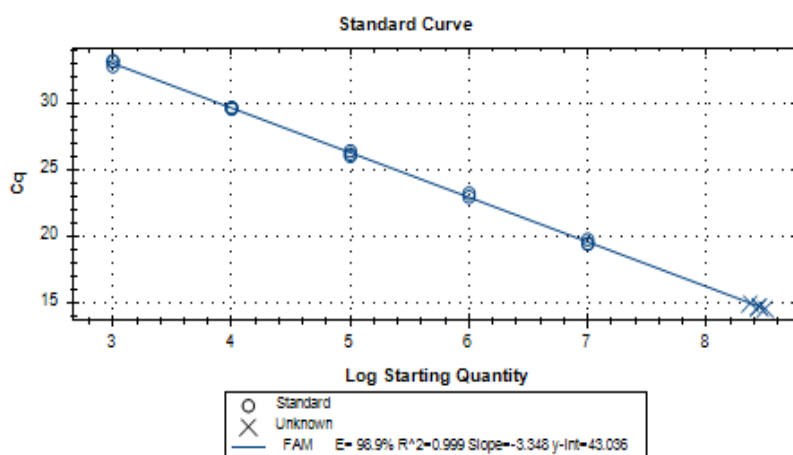
## SUPPLEMENTARY MATERIAL

### S1: Viral stock quantification (gc/μl reaction)

| Well | Target | Content | Sample    | Cq    | Starting Quantity (SQ) |
|------|--------|---------|-----------|-------|------------------------|
| A02  | BTV    | Unkn    | cDNA 3395 | 15,02 | 2,34E+08               |
| B02  | BTV    | Unkn    | cDNA 3395 | 14,60 | 3,12E+08               |
| C02  | BTV    | Unkn    | cDNA 3395 | 14,77 | 2,78E+08               |
| E02  | BTV    | Unkn    | cDNA 3395 | 14,77 | 2,78E+08               |
| H02  | BTV    | NTC     | NTC       |       |                        |
| G02  | BTV    | NTC     | NTC RT    |       |                        |
| E11  | BTV    | Std     | ST3       | 33,17 | 1,00E+03               |
| F11  | BTV    | Std     | ST3       | 32,77 | 1,00E+03               |
| G11  | BTV    | Std     | ST3       | 33,14 | 1,00E+03               |
| B11  | BTV    | Std     | ST4       | 29,60 | 1,00E+04               |
| C11  | BTV    | Std     | ST4       | 29,58 | 1,00E+04               |
| D11  | BTV    | Std     | ST4       | 29,68 | 1,00E+04               |
| A11  | BTV    | Std     | ST5       | 26,42 | 1,00E+05               |
| G09  | BTV    | Std     | ST5       | 26,03 | 1,00E+05               |
| H09  | BTV    | Std     | ST5       | 26,18 | 1,00E+05               |
| E09  | BTV    | Std     | ST6       | 23,25 | 1,00E+06               |
| F09  | BTV    | Std     | ST6       | 22,97 | 1,00E+06               |
| A09  | BTV    | Std     | ST7       | 19,78 | 1,00E+07               |
| B09  | BTV    | Std     | ST7       | 19,48 | 1,00E+07               |
| D09  | BTV    | Std     | ST7       | 19,45 | 1,00E+07               |

2,75E+08





| Fluor | Efficiency % | Slope  | Y-Intercept | R <sup>2</sup> |
|-------|--------------|--------|-------------|----------------|
| FAM   | 98,93        | -3,348 | 43,036      | 0,999          |

## S2: Standardization of the RNA elution from filter cards

A general linear model was fitted, and orthogonal contrasts were performed between treatments using Bonferroni correction.

| Condition | Card Hydration              | Post-Hydration Incubation Temp | Post-Hydration Incubation Time | Agitation |
|-----------|-----------------------------|--------------------------------|--------------------------------|-----------|
| 1         | TE Buffer                   | 37°C                           | 30 minutes                     | No        |
| 2         | TE Buffer                   | 60°C                           | 30 minutes                     | No        |
| 3         | LYSIS Buffer                | 37°C                           | 30 minutes                     | No        |
| 4         | LYSIS Buffer                | 60°C                           | 30 minutes                     | No        |
| 5         | CHELEX <sup>3</sup>         | 60°C                           | 25 minutes                     | 1000 rpm  |
| 6         | TED10 <sup>4</sup> + CHELEX | 26°C and then 60°C             | 15 min and then 25 min         | 1000 rpm  |

1, 2: Tris-EDTA Buffer. 3, 4: RL buffer solution + RNA Carrier (TIANGEN, 1 µg/µl)<sup>50</sup>. 5: Chelex 100 5% resin solution. 6: 90% TE Buffer + 10% Dimethyl sulfoxide

Cq values for each condition

| C1    | C2    | C3    | C4    | C5    | C6    |
|-------|-------|-------|-------|-------|-------|
| 24.45 | 26.08 | 27.98 | 28.45 | 25    | 27.32 |
| 24.3  | 26.17 | 26.02 | 26.07 | 26.76 | 26.87 |
| 26.61 | 25.92 | 28.95 | 26.2  | 25.2  | 27.23 |
| 24.7  | 26.46 | 26.68 | 26.56 | 27.1  | 27.5  |

### Summary statistics

| Condition | Variable | n | Mean  | SD   | Min   | Max   | Median |
|-----------|----------|---|-------|------|-------|-------|--------|
| C1        | Cq       | 4 | 25.02 | 1.08 | 24.3  | 26.61 | 24.58  |
| C2        | Cq       | 4 | 26.16 | 0.23 | 25.92 | 26.46 | 26.13  |
| C3        | Cq       | 4 | 27.41 | 1.31 | 26.02 | 28.95 | 27.33  |
| C4        | Cq       | 4 | 26.82 | 1.11 | 26.07 | 28.45 | 26.38  |

|    |    |   |       |      |       |      |       |
|----|----|---|-------|------|-------|------|-------|
| C5 | Cq | 4 | 26.02 | 1.07 | 25    | 27.1 | 25.98 |
| C6 | Cq | 4 | 27.23 | 0.26 | 26.87 | 27.5 | 27.28 |

| Contrasts | p-value |
|-----------|---------|
| C1-C2     | 0.0523  |
| C1-C3     | 0.0113  |
| C1-C4     | 0.0311  |
| C1-C5     | 0.2038  |
| C1-C6     | 0.0008  |

### S3: Analytical sensitivity of the FPC/RT-qPCR methodology

A general linear model was fitted, and orthogonal contrasts between treatments were performed with Bonferroni correction.

| Sample | Treatment | Dilution | Concentration (gc/μl) | Log  |
|--------|-----------|----------|-----------------------|------|
| 1      | S         | -1       | 1.33E+05              | 5.12 |
| 2      | S         | -1       | 1.33E+05              | 5.13 |
| 3      | S         | -1       | 2.80E+05              | 5.45 |
| 4      | S         | -2       | 4.56E+04              | 4.66 |
| 5      | S         | -2       | 5.46E+04              | 4.74 |
| 6      | S         | -2       | 3.91E+04              | 4.59 |
| 7      | S         | -2       | 2.47E+04              | 4.39 |
| 8      | S         | -2       | 4.64E+04              | 4.67 |
| 9      | S         | -3       | 8.76E+03              | 3.94 |
| 10     | S         | -3       | 5.27E+03              | 3.72 |
| 11     | S         | -3       | 7.08E+03              | 3.85 |
| 12     | S         | -3       | 5.07E+03              | 3.71 |
| 13     | S         | -3       | 6.30E+03              | 3.80 |
| 14     | S         | -3       | 8.06E+03              | 3.91 |
| 15     | S         | -3       | 7.54E+03              | 3.88 |
| 16     | S         | -4       | 7.59E+02              | 2.88 |
| 17     | S         | -4       | 9.59E+02              | 2.98 |
| 18     | S         | -4       | 1.86E+02              | 2.27 |
| 19     | S         | -4       | 1.80E+02              | 2.26 |
| 20     | S         | -5       | 2.34E+01              | 1.37 |
| 21     | S         | -5       | 6.47E+01              | 1.81 |
| 22     | S         | -5       | 5.98E+00              | 0.78 |
| 23     | S         | -5       | ND                    |      |
| 24     | S         | -6       | 8.20E+00              | 0.91 |
| 25     | S         | -6       | 2.07E+01              | 1.32 |
| 26     | S         | -6       | 7.16E+00              | 0.85 |
| 27     | S         | -6       | ND                    |      |

|    |   |    |          |      |
|----|---|----|----------|------|
| 28 | T | -1 | 7.69E+05 | 5.89 |
| 29 | T | -1 | 4.65E+05 | 5.67 |
| 30 | T | -1 | 4.51E+05 | 5.65 |
| 31 | T | -2 | 6.49E+04 | 4.81 |
| 32 | T | -2 | 3.55E+04 | 4.55 |
| 33 | T | -2 | 7.79E+04 | 4.89 |
| 34 | T | -2 | 1.98E+04 | 4.30 |
| 35 | T | -2 | 7.04E+04 | 4.85 |
| 36 | T | -2 | 6.23E+03 | 3.79 |
| 37 | T | -2 | 7.25E+03 | 3.86 |
| 38 | T | -3 | 2.61E+03 | 3.42 |
| 39 | T | -3 | 1.95E+03 | 3.29 |
| 40 | T | -3 | 2.88E+04 | 4.46 |
| 41 | T | -3 | 2.78E+03 | 3.44 |
| 42 | T | -3 | 3.60E+03 | 3.56 |
| 43 | T | -3 | 3.57E+03 | 3.55 |
| 44 | T | -3 | 4.22E+03 | 3.63 |
| 45 | T | -4 | 1.38E+02 | 2.14 |
| 46 | T | -4 | 1.12E+02 | 2.05 |
| 47 | T | -4 | 1.07E+02 | 2.03 |
| 48 | T | -4 | 1.37E+02 | 2.14 |
| 49 | T | -5 | 6.33E+01 | 1.80 |
| 50 | T | -5 | 4.26E+01 | 1.63 |
| 51 | T | -5 | 3.70E+01 | 1.57 |
| 52 | T | -5 | 3.79E+01 | 1.58 |
| 53 | T | -6 | ND       |      |
| 54 | T | -6 | ND       |      |
| 55 | T | -6 | 4.49E+00 | 0.65 |
| 56 | T | -6 | 7.67E+00 | 0.88 |

ND: non detectable

### Summary statistics

| Dilution | Treatment | Variable            | n | Mean | SD   | Min  | Max  | Median |
|----------|-----------|---------------------|---|------|------|------|------|--------|
| -1       | S         | LOG10_Concentration | 3 | 5.23 | 0.19 | 5.12 | 5.45 | 5.12   |
| -1       | T         | LOG10_Concentration | 3 | 5.74 | 0.13 | 5.65 | 5.89 | 5.67   |
| -2       | S         | LOG10_Concentration | 5 | 4.61 | 0.13 | 4.39 | 4.74 | 4.66   |
| -2       | T         | LOG10_Concentration | 7 | 4.44 | 0.46 | 3.79 | 4.89 | 4.55   |
| -3       | S         | LOG10_Concentration | 7 | 3.83 | 0.09 | 3.71 | 3.94 | 3.85   |
| -3       | T         | LOG10_Concentration | 7 | 3.62 | 0.39 | 3.29 | 4.46 | 3.55   |
| -4       | S         | LOG10_Concentration | 4 | 2.6  | 0.39 | 2.26 | 2.98 | 2.57   |
| -4       | T         | LOG10_Concentration | 4 | 2.09 | 0.06 | 2.03 | 2.14 | 2.09   |
| -5       | S         | LOG10_Concentration | 3 | 1.32 | 0.52 | 0.78 | 1.81 | 1.37   |
| -5       | T         | LOG10_Concentration | 4 | 1.64 | 0.11 | 1.57 | 1.8  | 1.6    |
| -6       | S         | LOG10_Concentration | 3 | 1.03 | 0.25 | 0.85 | 1.32 | 0.91   |
| -6       | T         | LOG10_Concentration | 2 | 0.77 | 0.16 | 0.65 | 0.88 | 0.77   |

Shapiro-Wilks (modified)

| Variable                 | n  | SD   | W*   | p(Unilateral D) |
|--------------------------|----|------|------|-----------------|
| RE 1 LOG10_Concentration | 52 | 0.75 | 0.96 | 0.273           |

| Dilution | T vs S  |
|----------|---------|
| -1       | 0.0185* |
| -2       | 0.3711  |
| -3       | 0.1899  |
| -4       | 0.0413* |
| -5       | 0.3339  |
| -6       | 0.2565  |

#### S4: Stability assay

Comparisons performed between treatments at different time points. Results are presented for two concentrations of virus diluted in blood. Analysis was conducted by fitting a general linear model and comparing treatments using orthogonal contrasts

#### Blood spiked with BTV 1/10

| SAMPLE | TIME (DAYS) | CONSERVATION | log gc/ul |
|--------|-------------|--------------|-----------|
| 1      | 1           | F            | 5.55      |
| 2      | 1           | F            | 5.84      |
| 3      | 1           | F            | 5.22      |
| 4      | 1           | F            | 5.55      |
| 5      | 1           | F            | 5.58      |
| 6      | 1           | UF           | 5.56      |
| 7      | 1           | UF           | 5.51      |
| 8      | 1           | UF           | 5.37      |
| 9      | 1           | UF           | 5.20      |
| 10     | 1           | UF           | 5.35      |
| 11     | 1           | R            | 5.03      |
| 12     | 1           | R            | 5.63      |
| 13     | 1           | R            | 5.55      |
| 14     | 1           | R            | 5.31      |
| 15     | 1           | R            | 5.11      |
| 16     | 1           | RT           | 5.54      |
| 17     | 1           | RT           | 5.73      |
| 18     | 1           | RT           | 5.58      |
| 19     | 1           | RT           | 5.64      |
| 20     | 1           | RT           | 5.56      |
| 21     | 7           | F            | 5.06      |

|    |    |    |      |
|----|----|----|------|
| 22 | 7  | F  | 5.64 |
| 23 | 7  | F  | 5.41 |
| 24 | 7  | F  | 5.45 |
| 25 | 7  | F  | 5.43 |
| 26 | 7  | UF | 4.91 |
| 27 | 7  | UF | 5.04 |
| 28 | 7  | UF | 5.18 |
| 29 | 7  | UF |      |
| 30 | 7  | UF |      |
| 31 | 7  | R  | 4.92 |
| 32 | 7  | R  | 5.40 |
| 33 | 7  | R  | 5.17 |
| 34 | 7  | R  | 5.13 |
| 35 | 7  | R  | 5.46 |
| 36 | 7  | RT | 5.23 |
| 37 | 7  | RT | 4.79 |
| 38 | 7  | RT | 5.60 |
| 39 | 7  | RT | 5.83 |
| 40 | 7  | RT | 5.73 |
| 41 | 30 | F  | 5.29 |
| 42 | 30 | F  | 5.06 |
| 43 | 30 | F  | 5.62 |
| 44 | 30 | F  | 5.51 |
| 45 | 30 | F  | 5.72 |
| 46 | 30 | UF | 5.27 |
| 47 | 30 | UF | 5.67 |
| 48 | 30 | UF | 5.42 |
| 49 | 30 | UF | 5.47 |
| 50 | 30 | UF | 4.99 |
| 51 | 30 | R  | 4.85 |
| 52 | 30 | R  | 5.81 |
| 53 | 30 | R  | 5.84 |
| 54 | 30 | R  | 5.79 |
| 55 | 30 | R  | 5.14 |
| 56 | 30 | RT | 5.10 |
| 57 | 30 | RT | 5.23 |
| 58 | 30 | RT | 5.16 |
| 59 | 30 | RT |      |
| 60 | 30 | RT | 4.97 |
| 61 | 60 | F  | 5.01 |
| 62 | 60 | F  | 5.41 |
| 63 | 60 | F  | 4.85 |
| 64 | 60 | F  | 5.08 |
| 65 | 60 | F  | 4.87 |
| 66 | 60 | UF |      |

|     |     |    |      |
|-----|-----|----|------|
| 67  | 60  | UF | 5.74 |
| 68  | 60  | UF | 5.16 |
| 69  | 60  | UF |      |
| 70  | 60  | UF | 5.29 |
| 71  | 60  | R  | 5.08 |
| 72  | 60  | R  | 5.47 |
| 73  | 60  | R  | 5.22 |
| 74  | 60  | R  | 5.11 |
| 75  | 60  | R  | 5.63 |
| 76  | 60  | RT | 5.52 |
| 77  | 60  | RT | 5.29 |
| 78  | 60  | RT | 4.45 |
| 79  | 60  | RT | 5.45 |
| 80  | 60  | RT | 5.65 |
| 81  | 120 | F  | 5.45 |
| 82  | 120 | F  | 5.47 |
| 83  | 120 | F  | 5.37 |
| 84  | 120 | F  | 5.55 |
| 85  | 120 | F  | 4.95 |
| 86  | 120 | UF | 5.07 |
| 87  | 120 | UF | 5.27 |
| 88  | 120 | UF | 4.81 |
| 89  | 120 | UF | 5.04 |
| 90  | 120 | UF | 5.09 |
| 91  | 120 | R  |      |
| 92  | 120 | R  | 5.18 |
| 93  | 120 | R  | 5.21 |
| 94  | 120 | R  | 4.94 |
| 95  | 120 | R  | 5.14 |
| 96  | 120 | RT | 4.62 |
| 97  | 120 | RT | 5.37 |
| 98  | 120 | RT | 4.93 |
| 99  | 120 | RT | 5.40 |
| 100 | 120 | RT | 5.31 |
| 101 | 360 | F  | 5.68 |
| 102 | 360 | F  | 5.51 |
| 103 | 360 | F  | 5.38 |
| 104 | 360 | F  | 5.60 |
| 105 | 360 | F  | 5.86 |
| 106 | 360 | UF | 5.54 |
| 107 | 360 | UF | 5.31 |
| 108 | 360 | UF | 5.68 |
| 109 | 360 | UF | 5.85 |
| 110 | 360 | UF | 5.69 |
| 111 | 360 | R  | 5.30 |

|     |     |    |      |
|-----|-----|----|------|
| 112 | 360 | R  | 5.15 |
| 113 | 360 | R  | 5.55 |
| 114 | 360 | R  | 5.24 |
| 115 | 360 | R  | 5.30 |
| 116 | 360 | RT | 4.65 |
| 117 | 360 | RT | 4.83 |
| 118 | 360 | RT | 4.83 |
| 119 | 360 | RT | 4.56 |
| 120 | 360 | RT | 4.41 |

#### Summary statistics

| Time (days) | Conservation | n | Mean | SD   | Min  | Max  | Median |
|-------------|--------------|---|------|------|------|------|--------|
| <b>1</b>    | F            | 5 | 5,55 | 0,22 | 5,22 | 5,84 | 5,55   |
| <b>1</b>    | R            | 5 | 5,33 | 0,26 | 5,03 | 5,63 | 5,31   |
| <b>1</b>    | RT           | 5 | 5,61 | 0,08 | 5,54 | 5,73 | 5,58   |
| <b>1</b>    | UF           | 5 | 5,40 | 0,14 | 5,20 | 5,56 | 5,37   |
| <b>7</b>    | F            | 5 | 5,40 | 0,21 | 5,06 | 5,64 | 5,43   |
| <b>7</b>    | R            | 5 | 5,22 | 0,22 | 4,92 | 5,46 | 5,17   |
| <b>7</b>    | RT           | 5 | 5,44 | 0,43 | 4,79 | 5,83 | 5,60   |
| <b>7</b>    | UF           | 3 | 5,04 | 0,14 | 4,91 | 5,18 | 5,04   |
| <b>30</b>   | F            | 5 | 5,44 | 0,27 | 5,06 | 5,72 | 5,51   |
| <b>30</b>   | R            | 5 | 5,49 | 0,46 | 4,85 | 5,84 | 5,79   |
| <b>30</b>   | RT           | 4 | 5,12 | 0,11 | 4,97 | 5,23 | 5,13   |
| <b>30</b>   | UF           | 5 | 5,36 | 0,25 | 4,99 | 5,67 | 5,42   |
| <b>60</b>   | F            | 5 | 5,04 | 0,23 | 4,85 | 5,41 | 5,01   |
| <b>60</b>   | R            | 5 | 5,30 | 0,24 | 5,08 | 5,63 | 5,22   |
| <b>60</b>   | RT           | 5 | 5,27 | 0,48 | 4,45 | 5,65 | 5,45   |
| <b>60</b>   | UF           | 3 | 5,40 | 0,30 | 5,16 | 5,74 | 5,29   |
| <b>120</b>  | F            | 5 | 5,36 | 0,24 | 4,95 | 5,55 | 5,45   |
| <b>120</b>  | R            | 4 | 5,12 | 0,12 | 4,94 | 5,21 | 5,16   |
| <b>120</b>  | RT           | 5 | 5,13 | 0,34 | 4,62 | 5,40 | 5,31   |
| <b>120</b>  | UF           | 5 | 5,06 | 0,16 | 4,81 | 5,27 | 5,07   |
| <b>360</b>  | F            | 5 | 5,61 | 0,18 | 5,38 | 5,86 | 5,60   |
| <b>360</b>  | R            | 5 | 5,31 | 0,15 | 5,15 | 5,55 | 5,30   |
| <b>360</b>  | RT           | 5 | 4,66 | 0,18 | 4,41 | 4,83 | 4,65   |
| <b>360</b>  | UF           | 5 | 5,61 | 0,20 | 5,31 | 5,85 | 5,68   |

#### Orthogonal contrasts

| Time (days) | Orthogonal contrast | Estimate | SE     | t       | p value | 95% CI low | 95% CI high | Residual df | Bonferroni p (x3) | Significant p<0.05 | Significant Bonf. |
|-------------|---------------------|----------|--------|---------|---------|------------|-------------|-------------|-------------------|--------------------|-------------------|
| 1           | C1: F vs UF+R+RT    | 0,3100   | 0,2939 | 1,0549  | 0,3071  | -0,3129    | 0,9329      | 16,0000     | 0,9214            | No                 | No                |
| 1           | C2: UF vs R+RT      | -0,1400  | 0,2078 | -0,6738 | 0,5101  | -0,5805    | 0,3005      | 16,0000     | 1,0000            | No                 | No                |
| 1           | C3: R vs RT         | -0,2840  | 0,1200 | -2,3673 | 0,0309  | -0,5383    | -0,0297     | 16,0000     | 0,0926            | Yes                | No                |
| 7           | C1: F vs UF+R+RT    | 0,4987   | 0,4526 | 1,1018  | 0,2891  | -0,4720    | 1,4694      | 14,0000     | 0,8674            | No                 | No                |
| 7           | C2: UF vs R+RT      | -0,5653  | 0,3744 | -1,5101 | 0,1533  | -1,3683    | 0,2376      | 14,0000     | 0,4598            | No                 | No                |
| 7           | C3: R vs RT         | -0,2200  | 0,1798 | -1,2233 | 0,2414  | -0,6057    | 0,1657      | 14,0000     | 0,7242            | No                 | No                |
| 30          | C1: F vs UF+R+RT    | 0,3550   | 0,4821 | 0,7364  | 0,4728  | -0,6725    | 1,3825      | 15,0000     | 1,0000            | No                 | No                |
| 30          | C2: UF vs R+RT      | 0,1270   | 0,3443 | 0,3688  | 0,7174  | -0,6069    | 0,8609      | 15,0000     | 1,0000            | No                 | No                |
| 30          | C3: R vs RT         | 0,3710   | 0,2066 | 1,7958  | 0,0927  | -0,0694    | 0,8114      | 15,0000     | 0,2781            | No                 | No                |
| 60          | C1: F vs UF+R+RT    | -0,8387  | 0,5262 | -1,5937 | 0,1333  | -1,9673    | 0,2900      | 14,0000     | 0,4000            | No                 | No                |
| 60          | C2: UF vs R+RT      | 0,2193   | 0,4353 | 0,5039  | 0,6222  | -0,7143    | 1,1529      | 14,0000     | 1,0000            | No                 | No                |
| 60          | C3: R vs RT         | 0,0300   | 0,2091 | 0,1435  | 0,8880  | -0,4185    | 0,4785      | 14,0000     | 1,0000            | No                 | No                |
| 120         | C1: F vs UF+R+RT    | 0,7745   | 0,3704 | 2,0912  | 0,0539  | -0,0149    | 1,5639      | 15,0000     | 0,1618            | No                 | No                |
| 120         | C2: UF vs R+RT      | -0,1315  | 0,2645 | -0,4971 | 0,6263  | -0,6954    | 0,4324      | 15,0000     | 1,0000            | No                 | No                |
| 120         | C3: R vs RT         | -0,0085  | 0,1587 | -0,0536 | 0,9580  | -0,3468    | 0,3298      | 15,0000     | 1,0000            | No                 | No                |
| 360         | C1: F vs UF+R+RT    | 1,2400   | 0,2773 | 4,4717  | 0,0004  | 0,6521     | 1,8279      | 16,0000     | 0,0012            | Yes                | Yes               |
| 360         | C2: UF vs R+RT      | 1,2640   | 0,1961 | 6,4463  | 0,0000  | 0,8483     | 1,6797      | 16,0000     | 0,0000            | Yes                | Yes               |
| 360         | C3: R vs RT         | 0,6520   | 0,1132 | 5,7593  | 0,0000  | 0,4120     | 0,8920      | 16,0000     | 0,0001            | Yes                | Yes               |

| TIME (DAYS) | p-valor (CONSERVATION) |
|-------------|------------------------|
| 1           | 0.1069                 |
| 7           | 0.2482                 |
| 30          | 0.3319                 |
| 60          | 0.4731                 |
| 120         | 0.2401                 |
| 360         | <0.0001                |

### Blood spiked with BTV 1/1000

| SAMPLE | TIME (DAYS) | CONSERVATION | log gg/ul |
|--------|-------------|--------------|-----------|
| 1      | 1           | F            | 3.87      |
| 2      | 1           | F            | 3.81      |
| 3      | 1           | F            | 3.62      |
| 4      | 1           | F            | 3.99      |
| 5      | 1           | F            | 3.82      |
| 6      | 1           | UF           | 3.93      |
| 7      | 1           | UF           | 3.81      |
| 8      | 1           | UF           | 3.98      |
| 9      | 1           | UF           | 3.15      |
| 10     | 1           | UF           | 3.57      |
| 11     | 1           | R            | 3.86      |
| 12     | 1           | R            | 3.69      |
| 13     | 1           | R            | 4.02      |
| 14     | 1           | R            | 3.97      |
| 15     | 1           | R            | 3.85      |
| 16     | 1           | RT           | 4.08      |
| 17     | 1           | RT           | 3.87      |
| 18     | 1           | RT           | 3.90      |

|    |    |    |      |
|----|----|----|------|
| 19 | 1  | RT | 4.11 |
| 20 | 1  | RT | 3.69 |
| 21 | 7  | F  | 3.53 |
| 22 | 7  | F  | 3.39 |
| 23 | 7  | F  | 2.94 |
| 24 | 7  | F  | 2.71 |
| 25 | 7  | F  | 3.57 |
| 26 | 7  | UF | 3.09 |
| 27 | 7  | UF | 2.92 |
| 28 | 7  | UF | 3.43 |
| 29 | 7  | UF | 3.03 |
| 30 | 7  | UF | 3.31 |
| 31 | 7  | R  | 2.72 |
| 32 | 7  | R  | 3.40 |
| 33 | 7  | R  | 3.51 |
| 34 | 7  | R  | 3.29 |
| 35 | 7  | R  | 3.25 |
| 36 | 7  | RT | 3.15 |
| 37 | 7  | RT | 3.10 |
| 38 | 7  | RT | 3.33 |
| 39 | 7  | RT | 3.26 |
| 40 | 7  | RT | 3.41 |
| 41 | 30 | F  | 3.03 |
| 42 | 30 | F  | 3.48 |
| 43 | 30 | F  | 3.42 |
| 44 | 30 | F  | 3.58 |
| 45 | 30 | F  | 3.13 |
| 46 | 30 | UF | 3.42 |
| 47 | 30 | UF | 3.47 |
| 48 | 30 | UF | 3.02 |
| 49 | 30 | UF | 3.11 |
| 50 | 30 | UF | 3.31 |
| 51 | 30 | R  | 3.42 |
| 52 | 30 | R  | 3.58 |
| 53 | 30 | R  | 3.33 |
| 54 | 30 | R  | 3.06 |
| 55 | 30 | R  | 3.01 |
| 56 | 30 | RT | 2.99 |
| 57 | 30 | RT | 3.03 |
| 58 | 30 | RT | 2.96 |
| 59 | 30 | RT | 3.03 |
| 60 | 30 | RT | 2.70 |
| 61 | 60 | F  | 2.87 |
| 62 | 60 | F  | 3.27 |
| 63 | 60 | F  | 3.40 |

|     |     |    |      |
|-----|-----|----|------|
| 64  | 60  | F  | 3.33 |
| 65  | 60  | F  | 2.46 |
| 66  | 60  | UF | 3.69 |
| 67  | 60  | UF | 3.01 |
| 68  | 60  | UF | 3.68 |
| 69  | 60  | UF | 3.34 |
| 70  | 60  | UF | 2.83 |
| 71  | 60  | R  | 2.46 |
| 72  | 60  | R  | 2.59 |
| 73  | 60  | R  | 3.01 |
| 74  | 60  | R  | 3.22 |
| 75  | 60  | R  | 3.01 |
| 76  | 60  | RT | 2.99 |
| 77  | 60  | RT | 3.03 |
| 78  | 60  | RT | 2.55 |
| 79  | 60  | RT | 3.03 |
| 80  | 60  | RT | 2.92 |
| 81  | 120 | F  | 3.10 |
| 82  | 120 | F  | 3.46 |
| 83  | 120 | F  | 3.18 |
| 84  | 120 | F  | 2.93 |
| 85  | 120 | F  | 3.06 |
| 86  | 120 | UF | 3.17 |
| 87  | 120 | UF | 2.90 |
| 88  | 120 | UF | 3.24 |
| 89  | 120 | UF | 3.09 |
| 90  | 120 | UF | 3.34 |
| 91  | 120 | R  | 3.27 |
| 92  | 120 | R  | 3.30 |
| 93  | 120 | R  | 3.24 |
| 94  | 120 | R  | 3.04 |
| 95  | 120 | R  | 3.04 |
| 96  | 120 | RT | 3.31 |
| 97  | 120 | RT | 2.87 |
| 98  | 120 | RT | 3.47 |
| 99  | 120 | RT | 3.30 |
| 100 | 120 | RT | 3.45 |
| 101 | 360 | F  | 3.47 |
| 102 | 360 | F  | 3.77 |
| 103 | 360 | F  | 3.53 |
| 104 | 360 | F  | 3.80 |
| 105 | 360 | F  | 3.45 |
| 106 | 360 | UF | 3.61 |
| 107 | 360 | UF | 3.48 |
| 108 | 360 | UF | 3.61 |

|     |     |    |      |
|-----|-----|----|------|
| 109 | 360 | UF | 3.68 |
| 110 | 360 | UF | 3.01 |
| 111 | 360 | R  | 2.95 |
| 112 | 360 | R  | 3.01 |
| 113 | 360 | R  | 2.88 |
| 114 | 360 | R  | 3.00 |
| 115 | 360 | R  | 2.43 |
| 116 | 360 | RT | 2.43 |
| 117 | 360 | RT | 2.22 |
| 118 | 360 | RT | 2.08 |
| 119 | 360 | RT | 2.35 |

#### Summary statistics

| Calibrator   | Time (days) | Conservation | n | Mean | SD   | Min  | Max  | Median |
|--------------|-------------|--------------|---|------|------|------|------|--------|
| <b>1/100</b> | 1           | F            | 5 | 3,82 | 0,13 | 3,62 | 3,99 | 3,82   |
| <b>1/100</b> | 1           | R            | 5 | 3,88 | 0,13 | 3,69 | 4,02 | 3,86   |
| <b>1/100</b> | 1           | RT           | 5 | 3,93 | 0,17 | 3,69 | 4,11 | 3,90   |
| <b>1/100</b> | 1           | UF           | 5 | 3,69 | 0,34 | 3,15 | 3,98 | 3,81   |
| <b>1/100</b> | 7           | F            | 5 | 3,23 | 0,38 | 2,71 | 3,57 | 3,39   |
| <b>1/100</b> | 7           | R            | 5 | 3,23 | 0,30 | 2,72 | 3,51 | 3,29   |
| <b>1/100</b> | 7           | RT           | 5 | 3,25 | 0,13 | 3,10 | 3,41 | 3,26   |
| <b>1/100</b> | 7           | UF           | 5 | 3,16 | 0,21 | 2,92 | 3,43 | 3,09   |
| <b>1/100</b> | 30          | F            | 5 | 3,33 | 0,24 | 3,03 | 3,58 | 3,42   |
| <b>1/100</b> | 30          | R            | 5 | 3,28 | 0,24 | 3,01 | 3,58 | 3,33   |
| <b>1/100</b> | 30          | RT           | 5 | 2,94 | 0,14 | 2,70 | 3,03 | 2,99   |
| <b>1/100</b> | 30          | UF           | 5 | 3,27 | 0,20 | 3,02 | 3,47 | 3,31   |
| <b>1/100</b> | 60          | F            | 5 | 3,07 | 0,40 | 2,46 | 3,40 | 3,27   |
| <b>1/100</b> | 60          | R            | 5 | 2,86 | 0,32 | 2,46 | 3,22 | 3,01   |
| <b>1/100</b> | 60          | RT           | 5 | 2,90 | 0,20 | 2,55 | 3,03 | 2,99   |
| <b>1/100</b> | 60          | UF           | 5 | 3,31 | 0,39 | 2,83 | 3,69 | 3,34   |
| <b>1/100</b> | 120         | F            | 5 | 3,15 | 0,20 | 2,93 | 3,46 | 3,10   |
| <b>1/100</b> | 120         | R            | 5 | 3,18 | 0,13 | 3,04 | 3,30 | 3,24   |
| <b>1/100</b> | 120         | RT           | 5 | 3,28 | 0,24 | 2,87 | 3,47 | 3,31   |
| <b>1/100</b> | 120         | UF           | 5 | 3,15 | 0,17 | 2,90 | 3,34 | 3,17   |
| <b>1/100</b> | 360         | F            | 5 | 3,60 | 0,17 | 3,45 | 3,80 | 3,53   |
| <b>1/100</b> | 360         | R            | 5 | 2,85 | 0,24 | 2,43 | 3,01 | 2,95   |
| <b>1/100</b> | 360         | RT           | 4 | 2,27 | 0,15 | 2,08 | 2,43 | 2,29   |
| <b>1/100</b> | 360         | UF           | 5 | 3,48 | 0,27 | 3,01 | 3,68 | 3,61   |

## Orthogonal contrasts

| Time (days) | Orthogonal contrast | Estimate | SE     | t       | p value | 95% CI low | 95% CI high | Residual df | Bonferroni p (x3) | Significant p<0.05 | Significant Bonf. |
|-------------|---------------------|----------|--------|---------|---------|------------|-------------|-------------|-------------------|--------------------|-------------------|
| 1           | C1: F vs UF+R+RT    | -0,0300  | 0,3276 | -0,0916 | 0,9282  | -0,7246    | 0,6646      | 16,0000     | 1,0000            | No                 | No                |
| 1           | C2: UF vs R+RT      | -0,4320  | 0,2317 | -1,8647 | 0,0807  | -0,9231    | 0,0591      | 16,0000     | 0,2420            | No                 | No                |
| 1           | C3: R vs RT         | -0,0520  | 0,1338 | -0,3888 | 0,7026  | -0,3356    | 0,2316      | 16,0000     | 1,0000            | No                 | No                |
| 7           | C1: F vs UF+R+RT    | 0,0440   | 0,4236 | 0,1039  | 0,9186  | -0,8540    | 0,9420      | 16,0000     | 1,0000            | No                 | No                |
| 7           | C2: UF vs R+RT      | -0,1720  | 0,2995 | -0,5742 | 0,5738  | -0,8070    | 0,4630      | 16,0000     | 1,0000            | No                 | No                |
| 7           | C3: R vs RT         | -0,0160  | 0,1729 | -0,0925 | 0,9274  | -0,3826    | 0,3506      | 16,0000     | 1,0000            | No                 | No                |
| 30          | C1: F vs UF+R+RT    | 0,4960   | 0,3206 | 1,5470  | 0,1414  | -0,1837    | 1,1757      | 16,0000     | 0,4242            | No                 | No                |
| 30          | C2: UF vs R+RT      | 0,3100   | 0,2267 | 1,3674  | 0,1904  | -0,1706    | 0,7906      | 16,0000     | 0,5712            | No                 | No                |
| 30          | C3: R vs RT         | 0,3380   | 0,1309 | 2,5823  | 0,0200  | 0,0605     | 0,6155      | 16,0000     | 0,0601            | Yes                | No                |
| 60          | C1: F vs UF+R+RT    | 0,1260   | 0,5201 | 0,2423  | 0,8117  | -0,9766    | 1,2286      | 16,0000     | 1,0000            | No                 | No                |
| 60          | C2: UF vs R+RT      | 0,8580   | 0,3678 | 2,3330  | 0,0330  | 0,0784     | 1,6376      | 16,0000     | 0,0991            | Yes                | No                |
| 60          | C3: R vs RT         | -0,0460  | 0,2123 | -0,2166 | 0,8312  | -0,4961    | 0,4041      | 16,0000     | 1,0000            | No                 | No                |
| 120         | C1: F vs UF+R+RT    | -0,1680  | 0,2914 | -0,5764 | 0,5723  | -0,7858    | 0,4498      | 16,0000     | 1,0000            | No                 | No                |
| 120         | C2: UF vs R+RT      | -0,1620  | 0,2061 | -0,7861 | 0,4433  | -0,5989    | 0,2749      | 16,0000     | 1,0000            | No                 | No                |
| 120         | C3: R vs RT         | -0,1020  | 0,1190 | -0,8573 | 0,4040  | -0,3542    | 0,1502      | 16,0000     | 1,0000            | No                 | No                |
| 360         | C1: F vs UF+R+RT    | 2,2100   | 0,3414 | 6,4728  | 0,0000  | 1,4823     | 2,9377      | 15,0000     | 0,0000            | Yes                | Yes               |
| 360         | C2: UF vs R+RT      | 1,8320   | 0,2439 | 7,5119  | 0,0000  | 1,3122     | 2,3518      | 15,0000     | 0,0000            | Yes                | Yes               |
| 360         | C3: R vs RT         | 0,5840   | 0,1463 | 3,9911  | 0,0012  | 0,2721     | 0,8959      | 15,0000     | 0,0035            | Yes                | Yes               |

| TIME (DAYS) | p-value (CONSERVATION) |
|-------------|------------------------|
| 1           | 0.3373                 |
| 7           | 0.9492                 |
| 30          | 0.0418                 |
| 60          | 0.2827                 |
| 120         | 0.5771                 |
| 360         | <0.0001                |

Since independent filter paper cards were processed at each sampling time, observations were considered independent and a repeated-measures design was not applicable. The effects of storage time and temperature on viral genome stability were therefore evaluated using a two-way factorial general linear model, including time, temperature, and the time × temperature interaction. Analyses were performed separately for each viral concentration, considering time as categoric factor.

$\log_{10} \text{ gc}/\mu\text{L} \sim \text{Time} + \text{Temperature} + \text{Time} \times \text{Temperature}$

| BTV dilution | time      | temperature | Time x temperature |
|--------------|-----------|-------------|--------------------|
| 1/10         | p= 0.0119 | p= 0.0474   | p= 0.000016        |
| 1/100        | p<0.0001  | p= 0.00045  | p>0.0000001        |

## S5. Agreement between BTV detection from blood and from FPCs

| Sample ID | Sample type | Specie | Cq value*    | qPCR result | Ab ELISA result |
|-----------|-------------|--------|--------------|-------------|-----------------|
| BOV LH1   | blood       | bovine | Undetermined | ND          | Positive        |
| BOV LH1   | FPC         | bovine | Undetermined | ND          | nd              |
| BOV LH2   | blood       | bovine | Undetermined | ND          | Positive        |
| BOV LH2   | FPC         | bovine | Undetermined | ND          | nd              |

|          |       |        |              |            |          |
|----------|-------|--------|--------------|------------|----------|
| BOV LH3  | blood | bovine | Undetermined | ND         | Negative |
| BOV LH3  | FPC   | bovine | Undetermined | ND         | nd       |
| BOV LH4  | blood | bovine | 33,60        | DETECTABLE | Positive |
| BOV LH4  | FPC   | bovine | 34,62        | DETECTABLE | nd       |
| BOV LH5  | blood | bovine | 34,38        | DETECTABLE | Positive |
| BOV LH5  | FPC   | bovine | 36,51        | DETECTABLE | nd       |
| BOV LH6  | blood | bovine | Undetermined | ND         | Positive |
| BOV LH6  | FPC   | bovine | Undetermined | ND         | nd       |
| BOV LH11 | blood | bovine | 34,19        | DETECTABLE | Positive |
| BOV LH11 | FPC   | bovine | 37,24        | DETECTABLE | nd       |
| BOV LH12 | blood | bovine | 31,42        | DETECTABLE | Positive |
| BOV LH12 | FPC   | bovine | 29,62        | DETECTABLE | nd       |
| BOV LH13 | blood | bovine | 33,96        | DETECTABLE | Positive |
| BOV LH13 | FPC   | bovine | 35,37        | DETECTABLE | nd       |
| BOV LH16 | blood | bovine | Undetermined | ND         | Positive |
| BOV LH16 | FPC   | bovine | Undetermined | ND         | nd       |
| BOV LH19 | blood | bovine | 37,66        | DETECTABLE | Positive |
| BOV LH19 | FPC   | bovine | 38           | DETECTABLE | nd       |
| BOV LH22 | blood | bovine | Undetermined | ND         | Positive |
| BOV LH22 | FPC   | bovine | Undetermined | ND         | nd       |
| BOV LH23 | blood | bovine | Undetermined | ND         | Positive |
| BOV LH23 | FPC   | bovine | Undetermined | ND         | nd       |
| BOV LH27 | blood | bovine | 38,65        | DETECTABLE | Positive |
| BOV LH27 | FPC   | bovine | 39,6         | DETECTABLE | nd       |
| BOV LH28 | blood | bovine | Undetermined | ND         | Negative |
| BOV LH28 | FPC   | bovine | Undetermined | ND         | nd       |
| BOV LH29 | blood | bovine | 39,91        | DETECTABLE | Negative |
| BOV LH29 | FPC   | bovine | Undetermined | ND         | nd       |
| BOV LH32 | blood | bovine | Undetermined | ND         | Positive |
| BOV LH32 | FPC   | bovine | Undetermined | ND         | nd       |
| BOV LH33 | blood | bovine | 33,52        | DETECTABLE | Positive |
| BOV LH33 | FPC   | bovine | 30,29        | DETECTABLE | nd       |
| BOV LH34 | blood | bovine | Undetermined | ND         | Positive |
| BOV LH34 | FPC   | bovine | Undetermined | ND         | nd       |
| BOV LH37 | blood | bovine | 37,19        | DETECTABLE | Positive |
| BOV LH37 | FPC   | bovine | 38,42        | DETECTABLE | nd       |
| BOV LH39 | blood | bovine | Undetermined | ND         | Positive |
| BOV LH39 | FPC   | bovine | Undetermined | ND         | nd       |
| BOV LH41 | blood | bovine | Undetermined | ND         | Positive |
| BOV LH41 | FPC   | bovine | Undetermined | ND         | nd       |
| BOV LH42 | blood | bovine | Undetermined | ND         | Negative |
| BOV LH42 | FPC   | bovine | Undetermined | ND         | nd       |
| BOV LH43 | blood | bovine | Undetermined | ND         | Positive |
| BOV LH43 | FPC   | bovine | 39,49        | DETECTABLE | nd       |
| BOV LH44 | blood | bovine | Undetermined | ND         | Positive |

|          |       |        |              |            |          |
|----------|-------|--------|--------------|------------|----------|
| BOV LH44 | FPC   | bovine | 38,45        | DETECTABLE | nd       |
| BOV LH9  | blood | bovine | 37,18        | DETECTABLE | Positive |
| BOV LH9  | FPC   | bovine | 38,43        | DETECTABLE | nd       |
| OV LH1   | blood | ovine  | Undetermined | ND         | Negative |
| OV LH1   | FPC   | ovine  | Undetermined | ND         | nd       |
| OV LH2   | blood | ovine  | Undetermined | ND         | Negative |
| OV LH2   | FPC   | ovine  | Undetermined | ND         | nd       |
| OV LH3   | blood | ovine  | Undetermined | ND         | Positive |
| OV LH3   | FPC   | ovine  | Undetermined | ND         | nd       |
| OV LH4   | blood | ovine  | Undetermined | ND         | Positive |
| OV LH4   | FPC   | ovine  | Undetermined | ND         | nd       |
| OV VC10  | blood | ovine  | 33,09        | DETECTABLE | Positive |
| OV VC10  | FPC   | ovine  | 37,9         | DETECTABLE | nd       |
| OV VC11  | blood | ovine  | 39,75        | DETECTABLE | Negative |
| OV VC11  | FPC   | ovine  | 38,06        | DETECTABLE | nd       |
| OV VC12  | blood | ovine  | 38,13        | DETECTABLE | Positive |
| OV VC12  | FPC   | ovine  | 37,9         | DETECTABLE | nd       |
| OV VC13  | blood | ovine  | 33           | DETECTABLE | Positive |
| OV VC13  | FPC   | ovine  | 31,39        | DETECTABLE | nd       |
| OV VC15  | blood | ovine  | 31,62        | DETECTABLE | Positive |
| OV VC15  | FPC   | ovine  | 33,03        | DETECTABLE | nd       |
| OV VC17  | blood | ovine  | Undetermined | ND         | Negative |
| OV VC17  | FPC   | ovine  | Undetermined | ND         | nd       |
| OV VC18  | blood | ovine  | 37,41        | DETECTABLE | Positive |
| OV VC18  | FPC   | ovine  | 36,73        | DETECTABLE | nd       |
| OV VC19  | blood | ovine  | 34,7         | DETECTABLE | Positive |
| OV VC19  | FPC   | ovine  | 35,09        | DETECTABLE | nd       |
| OV VC21  | blood | ovine  | 39,26        | DETECTABLE | Negative |
| OV VC21  | FPC   | ovine  | 38,9         | DETECTABLE | nd       |
| OV VC22  | blood | ovine  | 35,97        | DETECTABLE | Positive |
| OV VC22  | FPC   | ovine  | 35,05        | DETECTABLE | nd       |
| OV VC23  | blood | ovine  | 33,47        | DETECTABLE | Positive |
| OV VC23  | FPC   | ovine  | 37,24        | DETECTABLE | nd       |
| OV VC28  | blood | ovine  | 36,84        | DETECTABLE | Negative |
| OV VC28  | FPC   | ovine  | 39,55        | DETECTABLE | nd       |
| OV VC30  | blood | ovine  | 36,82        | DETECTABLE | Positive |
| OV VC30  | FPC   | ovine  | 35,2         | DETECTABLE | nd       |
| OV VC31  | blood | ovine  | 36,55        | DETECTABLE | Positive |
| OV VC31  | FPC   | ovine  | 36,17        | DETECTABLE | nd       |
| OV VC34  | blood | ovine  | Undetermined | ND         | Negative |
| OV VC34  | FPC   | ovine  | Undetermined | ND         | nd       |
| OV VC35  | blood | ovine  | Undetermined | ND         | Negative |
| OV VC35  | FPC   | ovine  | Undetermined | ND         | nd       |
| OV VC37  | blood | ovine  | 33,8         | DETECTABLE | Negative |
| OV VC37  | FPC   | ovine  | 36,1         | DETECTABLE | nd       |

|         |       |       |       |            |          |
|---------|-------|-------|-------|------------|----------|
| OV VC38 | blood | ovine | 32,17 | DETECTABLE | Positive |
| OV VC38 | FPC   | ovine | 36,77 | DETECTABLE | nd       |

(\*) Cq values reported represent the mean Cq of the three RT-qPCR replicates

The agreement between the two viral detection techniques was assessed using Cohen's Kappa index

|                | DECTABLE BLOOD | ND BLOOD  | TOTAL     |
|----------------|----------------|-----------|-----------|
| DETECTABLE FPC | 25             | 2         | <b>27</b> |
| ND FPC         | 1              | 20        | <b>21</b> |
| TOTAL          | <b>26</b>      | <b>22</b> | <b>48</b> |

Kappa de Cohen index= 0.874 (IC 95%: 0.735 – 1.000)