## Contrived Sample Shipping Stability Study

Boca Biolistics (5001 NW 13th Ave, Suite H, Pompano Beach, FL 33064 [www.bocabio.com](http://www.bocabio.com)) samples of known titer (Table 9) were used to determine the stability of viral particle on the swabs. Samples 5, 10, 17, and 24 were chosen as these were approximately 2x LoD, 2x LoD, 5x LoD, and negative, respectively. 1 µL of each sample (NP swab eluate in VTM) was pipetted onto the tip of a Rhinostic swab. The material was allowed to dry onto the swab for 30 minutes and then the swab and cap was tightened onto the collection tube. Sample were then subjected to the summer or winter cycling. After cycling, the samples were processed in accordance with the Quaeris assay, i.e. resuspended in 300µL of PBS with 2µL being used as input into the rRT-PCR reaction. For each sample, 10 replicate swabs were made for the summer cycling, 10 replicates were made for the winter cycling, and 13-16 replicates (depending on the amount of total sample) were made as “no cycling” controls for the seasonal temperature studies.

The no cycling control for all four samples used in the shipping stability study (Table 14A and 14B) were allowed to dry for two hours and then was resuspended and processed with the Quaeris protocol (**Appendix F**). In short, 300 µL of PBS was added to each Rhinostic collection tube with swab. The swab was gently shaken and then put onto a heat block for 30 minutes at 65°C to inactivate the virus. After viral inactivation, 2 µL of the PBS eluant were added directly to a rRT-PCR mastermix (NEB Luna E3007E) and run on a QuantStudio7.

**Table 23A: No Cycling Controls of Samples used for Summer and Winter Cycling**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **No Cycling** | | | | | | | |
|  | **Sample S000566036 - 2x LOD** | | | | **Sample S000566067 - 2x LOD** | | | |
| **Swab #** | **N1** | **RdRp** | **RNasP** | **CALL** | **N1** | **RdRp** | **RNasP** | **CALL** |
| 1 | 32.7 | 35.6 | 36.2 | POS | 33.6 | 36.9 | 36.2 | POS |
| 2 | 32.7 | 36.8 | 34.7 | POS | 33.7 | 37.3 | 35.4 | POS |
| 3 | 31.6 | 36.3 | 35.2 | POS | 32.9 | 36.4 | 35.0 | POS |
| 4 | 31.9 | 35.1 | 33.6 | POS | 34.3 | und | 37.4 | INC |
| 5 | 33.1 | 37.0 | 34.6 | POS | 33.3 | 37.6 | 35.9 | POS |
| 6 | 33.1 | 35.9 | 35.3 | POS | 32.5 | 36.5 | 37.5 | POS |
| 7 | 32.4 | 35.9 | 35.1 | POS | 32.9 | 35.7 | 36.2 | POS |
| 8 | 32.6 | 38.1 | 34.9 | INC | 33.7 | 36.7 | 38.8 | POS |
| 9 | 32.7 | 38.0 | 34.8 | POS | 34.1 | 36.9 | 34.9 | POS |
| 10 | 32.2 | 35.4 | 33.4 | POS | 33.0 | 37.5 | 37.3 | POS |
| **Mean CT** | **32.5** | **36.4** | **34.8** |  | **33.4** | **36.8** | **36.4** |  |
| std | 0.5 | 1.0 | 0.8 |  | 0.6 | 0.6 | 1.2 |  |

**Table 23B: No Cycling Controls of Samples used for Summer and Winter Cycling**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **No Cycling** | | | | | | | |
|  | **Sample S000566033 - 5x LOD** | | | | **Sample S000558382 – Negative** | | | |
| **Swab #** | **N1** | **RdRp** | **RNasP** | **CALL** | **N1** | **RdRp** | **RNasP** | **CALL** |
| 1 | 29.6 | 33.6 | 34.9 | POS | Und | und | 33.4 | NEG |
| 2 | 29.8 | 34.2 | 34.8 | POS | Und | und | 32.7 | NEG |
| 3 | 32.4 | 37.0 | Und | POS | Und | und | 34.0 | NEG |
| 4 | 29.6 | 33.9 | 36.0 | POS | Und | und | 34.0 | NEG |
| 5 | 29.8 | 34.3 | 34.0 | POS | Und | und | 32.7 | NEG |
| 6 | 30.1 | 34.5 | 35.0 | POS | Und | und | 32.7 | NEG |
| 7 | 33.3 | 37.3 | Und | POS | Und | und | 33.2 | NEG |
| 8 | 30.1 | 35.1 | 34.5 | POS | Und | und | 33.2 | NEG |
| 9 | 29.8 | 33.7 | 35.4 | POS | Und | und | 33.6 | NEG |
| 10 | 30.2 | 34.1 | 34.7 | POS | Und | und | 35.4 | INC |
| **Mean CT** | **30.5** | **34.8** | **34.9** |  | **N/A** | **N/A** | **33.5** |  |
| std | 3.1 | 1.3 | 0.6 |  | N/A | N/A | 0.8 |  |

All the replicates of all the samples yielded the expected call based on the criteria set out in Figure 1 with three exceptions. One replicate of each of the 2x LoD samples and the negative control were also inconclusive (Table 23A and B).

The summer cycling controls were kept at the following temperatures for the prescribed times shown in Table 24 by placing them into incubators at the appropriate temperatures.

**Table 24: Summer Profile**

|  |  |  |  |
| --- | --- | --- | --- |
| **Temperature** | **Cycle Period** | **Cycle Period Hours** | **Total Time Hours** |
| 40°C | 1 | 8 | 8 |
| 22°C | 2 | 4 | 12 |
| 40°C | 3 | 2 | 14 |
| 30°C | 4 | 36 | 50 |
| 40°C | 5 | 6 | 56 |

The winter cycling controls were kept at the following temperatures for the prescribed times shown in Table 25 by placing them into temperature-controlled environments at the appropriate temperatures.

**Table 25: Winter Profile**

|  |  |  |  |
| --- | --- | --- | --- |
| **Temperature** | **Cycle Period** | **Cycle Period Hours** | **Total Time Hours** |
| -10C | 1 | 8 | page2image3617928 |
| 18C | 2 | 4 | page2image35366412 |
| -10C | 3 | 2 | 14 |
| 10C | 4 | 36 | 50 |
| -10C | 5 | 6 | 56 |

After the full winter or summer cycle, samples were resuspended and processed in the Quaeris assay following the same SOP as used for processing of the no cycling controls (see SOP in **Appendix F**); i.e., all four samples used in the shipping stability study (Tables 17A-D) were allowed to dry for two hours and then was resuspended and processed with the Quaeris protocol (**Appendix F**). In short, 300 µL of PBS was added to each Rhinostic collection tube with swab. The swab was gently shaken and then put onto a heat block for 30 minutes at 65°C to inactivate the virus. After viral inactivation, 2 µL of the PBS eluant were added directly to a rRT-PCR mastermix (NEB Luna E3007E) and run on a QuantStudio7.

Results for all four contrived samples in the seasonal temperature studies are shown below in Table 26A for sample 5 (2x LoD), Table 26B for sample 10 (2x LoD), Table 2617C for sample 17 (5x LoD), and Table 26D for sample 25 (negative control).

**Table 26A: Summer and Winter Shipping Stability (Sample S000566036 - 2x LoD)**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Summer Cycle** | | | | **Winter Cycle** | | | |
| **Swab #** | **N1** | **RdRp** | **RNasP** | **Call** | **N1** | **RdRp** | **RNasP** | **Call** |
| 1 | 32.4 | 34.6 | 33.7 | POS | 31.6 | 33.2 | 31.8 | POS |
| 2 | 32.3 | 34.8 | 32.2 | POS | 32.1 | 33.7 | 33.4 | POS |
| 3 | 32.6 | 35.3 | 33.2 | POS | 31.6 | 33.4 | 32.6 | POS |
| 4 | 31.9 | 35.0 | 33.0 | POS | 33.0 | 33.9 | 33.9 | POS |
| 5 | 33.3 | 34.6 | 32.0 | POS | 33.5 | 35.2 | 32.2 | POS |
| 6 | 33.4 | 36.2 | 33.9 | POS | 31.8 | 33.3 | 33.7 | POS |
| 7 | 32.3 | 35.0 | 33.9 | POS | 33.1 | 34.9 | 33.6 | POS |
| 8 | 32.7 | 34.4 | 32.2 | POS | 32.8 | 34.9 | 31.5 | POS |
| 9 | 32.0 | 34.9 | 33.0 | POS | 32.9 | 34.4 | 32.2 | POS |
| 10 | 32.2 | 34.4 | 32.1 | POS | 35.3 | 36.7 | und | POS |
| mean | 32.5 | 34.9 | 32.9 |  | 32.8 | 34.4 | 32.8 |  |
| std | 0.5 | 0.5 | 0.8 |  | 1.1 | 1.1 | 0.9 |  |

**Table 26B: Summer and Winter Shipping Stability (Sample S000566067 - 2x LoD)**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Summer Cycle** | | | | **Winter Cycle** | | | |
| **Swab #** | **N1** | **RdRp** | **RNasP** | **Call** | **N1** | **RdRp** | **RNaseP** | **Call** |
| 1 | 32.4 | 34.9 | 35.4 | POS | 31.6 | 35.1 | und | POS |
| 2 | 32.3 | 34.3 | 32.8 | POS | 32.1 | 36.8 | 35.3 | POS |
| 3 | 32.6 | 34.4 | 35.1 | POS | 31.6 | 34.2 | 36.1 | POS |
| 4 | 31.9 | 33.8 | und | POS | 33.0 | 35.7 | 33.0 | POS |
| 5 | 33.3 | 35.2 | 34.2 | POS | 33.5 | 33.9 | 35.3 | POS |
| 6 | 33.4 | 34.2 | 35.4 | POS | 31.8 | 33.0 | 33.2 | POS |
| 7 | 32.3 | 36.3 | und | POS | 33.1 | 35.9 | 35.4 | POS |
| 8 | 32.7 | und | 34.3 | INC | 32.8 | 35.1 | 33.4 | POS |
| 9 | 32.0 | 35.4 | 35.8 | POS | 32.9 | 35.0 | 36.0 | POS |
| 10 | 32.2 | 34.5 | 35.5 | POS | 35.3 | 34.3 | und | POS |
| mean | 32.5 | 34.8 | 34.8 |  | 32.8 | 34.9 | 34.7 |  |
| std | 0.5 | 0.8 | 1.0 |  | 1.1 | 1.1 | 1.3 |  |

**Table 26C: Summer and Winter Shipping Stability (Sample S000566033 - 5x LoD)**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Summer Cycle** | | | | **Winter Cycle** | | | |
| **Swab #** | **N1** | **RdRp** | **RNasP** | **Call** | **N1** | **RdRp** | **RNaseP** | **Call** |
| 1 | 33.1 | 35.2 | und | POS | 33.6 | 34.4 | Und | POS |
| 2 | 30.1 | 32.4 | 32.9 | POS | 29.9 | 32.3 | 32.9 | POS |
| 3 | 30.5 | 33.1 | 34.0 | POS | 31.7 | 34.4 | 34.8 | POS |
| 4 | 30.8 | 33.0 | 33.2 | POS | 33.5 | 36.6 | 35.0 | POS |
|  | **Summer Cycle** | | | | **Winter Cycle** | | | |
| **Swab #** | **N1** | **RdRp** | **RNAseP** | **Call** | **N1** | **RdRp** | **RNAseP** | **Call** |
| 5 | 31.3 | 33.4 | 33.4 | POS | 33.2 | 33.7 | 34.6 | POS |
| 6 | 30.1 | 32.6 | 32.7 | POS | 30.2 | 31.9 | 34.8 | POS |
| 7 | 30.1 | 32.3 | 38.4 | POS | 31.0 | 32.3 | 38.5 | POS |
| 8 | 30.5 | 33.3 | 33.0 | POS | 30.8 | 32.7 | 35.4 | POS |
| 9 | 31.1 | 33.9 | 35.0 | POS | 30.9 | 32.9 | 33.9 | POS |
| 10 | 30.6 | 32.6 | 34.0 | POS | 30.1 | 31.5 | 35.7 | POS |
| mean | 30.8 | 33.2 | 34.1 |  | 31.5 | 33.3 | 35.1 |  |
| std | 0.9 | 0.9 | 1.8 |  | 1.4 | 1.5 | 1.5 |  |

**Table 26D: Summer and Winter Shipping Stability (Sample 25 – Negative)**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Sample S000558382 – Negative** | | | | | | | |
|  | **Summer Cycle** | | | | **Winter Cycle** | | | |
| **Swab #** | **N1** | **RdRp** | **RNasP** | **Call** | **N1** | **RdRp** | **RNaseP** | **Call** |
| 1 | und | und | 30.6 | NEG | Und | und | 35.1 | INC |
| 2 | und | und | 31.0 | NEG | Und | und | 32.8 | NEG |
| 3 | und | und | 31.9 | NEG | Und | und | 30.9 | NEG |
| 4 | und | und | 31.1 | NEG | Und | und | 30.8 | NEG |
| 5 | und | und | 32.2 | NEG | Und | und | 32.4 | NEG |
| 6 | und | und | 31.0 | NEG | Und | und | 31.0 | NEG |
| 7 | und | und | 31.5 | NEG | Und | und | 31.6 | NEG |
| 8 | und | und | 35.0 | INC | Und | und | 31.0 | NEG |
| 9 | und | und | 31.1 | NEG | Und | und | 31.3 | NEG |
| 10 | und | und | 31.6 | NEG | Und | und | 32.0 | NEG |
| mean | N/A | N/A | 31.7 |  | N/A | N/A | 31.9 |  |
| std | N/A | N/A | 1.3 |  | N/A | N/A | 1.3 |  |

There was no significant change in the Ct values for N1 or RdRp after summer or winter temperature cycling for any of the samples (Tables 26A-D). Based on the criteria set out in Figure 1, all 30 positive samples would be called as positive for the winter cycle and 29 of the 30 would be called as positive for the summer cycle (one inconclusive). Of the 10 negative samples one replicate for summer cycle (swab #8) and one replicate for the winter cycle (swab #1) were inconclusive.

Based on the results of the winter and summer cycling stability studies, the samples are stable for 56 hours after collection.