**SUPPLEMENT:**

**Table S1.** Source and details of human and animal serum samples tested in egg-derived virus and VLP HAI validation assays.

|  |  |  |
| --- | --- | --- |
| **No.** | **Sample #** | **Serum type/source** |
| 1 | Sample #1 | Human serum/Valley Biomedical, anti-HA antibodies depleted in Novavax |
| 2 | Sample #2 | Human serum/BioIVT, anti-HA antibodies depleted in Novavax |
| 3 | Sample #3 | Human serum/BioIVT, screened HAI negative for at least 1 influenza strain |
| 4 | Sample #4 | Human serum/BioIVT, anti-HA antibodies depleted in Novavax |
| 5 | Sample #5 | Human serum/BioIVT, anti-HA antibodies depleted in Novavax |
| 6 | Sample #6 | Human serum/BioIVT, anti-HA antibodies depleted in Novavax |
| 7 | Sample #7 | Human serum/BioIVT, screened HAI positive for at least 1 influenza strain |
| 8 | Sample #8 | Human serum/BioIVT, anti-HA antibodies depleted in Novavax |
| 9 | Sample #9 | Human serum/BioIVT, anti-HA antibodies depleted in Novavax |
| 10 | Sample #10 | Human serum/BioIVT, anti-HA antibodies depleted in Novavax |
| 11 | Sample #11 | Human serum/BioIVT, anti-HA antibodies depleted in Novavax |
| 12 | Sample #12 | Human serum/BioIVT, screened HAI positive for at least 1 influenza strain |
| 13 | Sample #13 | Human serum/BioIVT, screened HAI positive for at least 1 influenza strain |
| 14 | Sample #14 | Human serum/BioIVT, screened HAI positive for at least 1 influenza strain |
| 15 | Sample #15 | Human serum/BioIVT, screened HAI positive for at least 1 influenza strain |
| 16 | Sample #16 | Human serum/BioIVT, screened HAI positive for at least 1 influenza strain |
| 17 | Sample #17 | Human serum/BioIVT, reported receipt of influenza vaccine within the 2017-2018 Flu season |
| 18 | Sample #18 | Human serum/BioIVT, reported receipt of influenza vaccine within the 2017-2018 Flu season |
| 19 | Sample #19 | Human serum/BioIVT, reported receipt of influenza vaccine within the 2017-2018 Flu season |
| 20 | Sample #20 | Human serum/BioIVT, reported receipt of influenza vaccine within the 2017-2018 Flu season |
| 21 | Sample #21 | Human serum/BioIVT, reported receipt of influenza vaccine within the 2017-2018 Flu season |
| 22 | Sample #22 | Human serum/BioIVT, reported receipt of influenza vaccine within the 2017-2018 Flu season |
| 23 | Sample #23 | Human serum/BioIVT reported receipt of influenza vaccine within the 2017-2018 Flu season |
| 24 | Sample #24 | Human serum/BioIVT, reported receipt of influenza vaccine within the 2017-2018 Flu season |
| 25 | Sample #25 | Human serum/BioIVT, reported receipt of influenza vaccine within the 2017-2018 Flu season |
| 26 | Sample #26 | Human serum/BioIVT, reported receipt of influenza vaccine within the prior year |
| 27 | Sample #27 | Sheep serum/NIBSC |
| 28 | Sample #28 | Sheep serum/NIBSC |
| 29 | Sample #29 | Sheep serum/NIBSC |
| 30 | Sample #301 | Sheep serum/NIBSC |
| 31 | Sample #312 | Sheep serum/NIBSC |
| 32 | Sample #322 | Sheep serum/NIBSC |
| 33 | Sample #33 | Sheep serum/NIBSC |
| 34 | Sample #34 | Human serum/Biological Specialty Co./BioIVT, reported receipt of influenza vaccine within the 2017-2018 Flu season |
| 35 | Sample #35 | Human serum/Biological Specialty Co./BioIVT, reported receipt of influenza vaccine within the 2017-2018 Flu season |
| 36 | Sample #36 | Human serum/Biological Specialty Co./BioIVT, reported receipt of influenza vaccine within the 2017-2018 Flu season |
| 37 | Sample #37 | Human serum/Biological Specialty Co./BioIVT, reported receipt of influenza vaccine within the 2017-2018 Flu season |
| 38 | Sample #38 | Human serum/Biological Specialty Co./BioIVT, reported receipt of influenza vaccine within the 2017-2018 Flu season |
| 39 | Sample #39 | Human serum/Biological Specialty Co./BioIVT, reported receipt of influenza vaccine within the 2017-2018 Flu season |
| 40 | Sample #40 | Human serum/Biological Specialty Co./BioIVT, reported receipt of influenza vaccine within the 2017-2018 Flu season |
| 41 | Sample #41 | Sheep serum/Novavax |
| 42 | Sample #421 | Sheep serum/Novavax |
| 43 | Sample #43 | Sheep serum/Novavax |
| 44 | Sample #44 | Sheep serum /Novavax (Study NVX 733-025, Day 63 serum) |
| 45 | Sample #45 | Sheep serum /Novavax (Study NVX 733-025, Day 63 serum) |
| 46 | Sample #463 | Sheep serum /Novavax (Study NVX 733-027, Day 44 serum) |
| 47 | Sample #47 | Sheep serum /Novavax (Study NVX 733-027, Day 44 serum) |
| 48 | Sample #48 | Sheep serum /Novavax (Study NVX 733-027, Day 45 serum) |
| 49 | Sample #49 | Sheep serum /Novavax (Study NVX 733-027, Day 44 serum) |
| 50 | Sample #503 | Sheep serum /Novavax (Study NVX 733-031, Day 45 serum) |
| 51 | Sample #513 | Sheep serum /Novavax (Study NVX 733-031, Day 45 serum) |
| 52 | Sample #523 | Sheep serum /Novavax (Study NVX 733-034, Day 35 serum) |
| 53 | Sample #533 | Sheep serum /Novavax (Study NVX 733-034, Day 35 serum) |

1Samples used only in the egg-derived virus HAI assay. 2Samples used in the VLP HAI assay: NIBSC Influenza anti-B/Brisbane/60/2008-HA Serum, Code 15/312; NIBSC Influenza Anti-B/Colorado/06/2017-Like HA Serum, Code 18/170. 3Samples used only in the VLP HAI assay.

HA, hemagglutinin; HAI, hemagglutination inhibition; NIBSC, National Institute for Biological Standards and Control; VLP, virus-like particle.

**Table S2.** Results of linearity regression parameters of egg-derived virus HAI assay for four homologous seasonal influenza strains (A/Kansas, A/Brisbane, B/Maryland, and B/Phuket).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Strain** | **Sample** | **Parameter** | **Estimate** | **95% LCL** | **95% UCL** |
| A/Kansas/14/2017 | Sample #42 | Slope | 1.004 | 0.883 | 1.125 |
| Intercept | 0.001 | −0.203 | 0.205 |
| Residual variability (%GSD) | 0.036 (8.7) | N/A |
| *R*2 | 0.9957 | N/A |
| Sample #43 | Slope | 1.132 | 0.976 | 1.289 |
| Intercept | −0.281 | −0.585 | 0.022 |
| Residual variability (%GSD) | 0.097 (25.0) | N/A |
| *R*2 | 0.9857 | N/A |
| Sample #47 | Slope | 0.975 | 0.841 | 1.109 |
| Intercept | 0.099 | −0.195 | 0.393 |
| Residual variability (%GSD) | 0.107 (27.9) | N/A |
| *R*2 | 0.9814 | N/A |
| A/Brisbane/02/2018 | Sample #15 | Slope | 0.997 | 0.980 | 1.013 |
| Intercept | 0.018 | −0.017 | 0.054 |
| Residual variability (%GSD) | 0.013 (3.1) | N/A |
| *R*2 | 0.9997 | N/A |
| Sample #49 | Slope | 1.041 | 0.902 | 1.180 |
| Intercept | −0.018 | −0.333 | 0.296 |
| Residual variability (%GSD) | 0.111 (29.1) | N/A |
| *R*2 | 0.9824` | N/A |
| B/Maryland/15/2016 | Sample #14 | Slope | 0.975 | 0.937 | 1.012 |
| Intercept | 0.066 | −0.009 | 0.141 |
| Residual variability (%GSD) | 0.023 (5.5) | N/A |
| *R*2 | 0.9989 | N/A |
| Sample #35 | Slope | 0.899 | 0.829 | 0.968 |
| Intercept | 0.353 | 0.208 | 0.499 |
| Residual variability (%GSD) | 0.043 (10.4) | N/A |
| *R*2 | 0.9955 | N/A |
| B/Phuket/3073/2013 | Sample #14 | Slope | 0.921 | 0.880 | 0.961 |
| Intercept | 0.240 | 0.154 | 0.325 |
| Residual variability (%GSD) | 0.032 (7.6) | N/A |
| *R*2 | 0.9981 | N/A |
| Sample #16 | Slope | 0.993 | 0.983 | 1.003 |
| Intercept | 0.036 | 0.015 | 0.057 |
| Residual variability (%GSD) | 0.008 (1.8) | N/A |
| *R*2 | 0.9999 | N/A |

%GSD, percent geometric standard deviation; HAI, hemagglutination inhibition; LCL, lower confidence limit; N/A, not applicable; *R*2, coefficient of determination; UCL, upper confidence limit.

**Table S3.** Results of linearity regression parameters of VLP HAI assay for four homologous seasonal influenza strains (A/Kansas, A/Brisbane, B/Maryland, and B/Phuket) and drifted strains (A/California, A/Cardiff, A/Netherlands, and A/Tokyo).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Strain** | **Sample** | **Parameter** | **Estimate** | **95% LCL** | **95% UCL** |
| A/Kansas/14/2017 | Sample #46 | Slope | 0.991 | 0.942 | 1.040 |
| Intercept | 0.049 | −0.066 | 0.164 |
| Residual variability (%GSD) | 0.049 (11.8) | N/A |
| *R*2 | 0.9969 | N/A |
| Sample #47 | Slope | 0.999 | 0.990 | 1.008 |
| Intercept | 0.006 | −0.015 | 0.027 |
| Residual variability (%GSD) | 0.009 (2.1) | N/A |
| *R*2 | 0.9999 | N/A |
| A/Brisbane/02/2018 | Sample #15 | Slope | 0.986 | 0.960 | 1.012 |
| Intercept | 0.075 | 0.019 | 0.130 |
| Residual variability (%GSD) | 0.021 (5.0) | N/A |
| *R*2 | 0.9993 | N/A |
| Sample #49 (1:2 diluted)  | Slope | 1.008 | 0.970 | 1.047 |
| Intercept | −0.000 | −0.084 | 0.084 |
| Residual variability (%GSD) | 0.031 (7.3) | N/A |
| *R*2 | 0.9985 | N/A |
| B/Maryland/15/2016 | Sample #14 | Slope | 1.071 | 0.957 | 1.186 |
| Intercept | −0.261 | −0.487 | −0.036 |
| Residual variability (%GSD) | 0.052 (12.7) | N/A |
| *R*2 | 0.9941 | N/A |
| Sample #45 (1:2 diluted)  | Slope | 0.996 | 0.973 | 1.018 |
| Intercept | 0.011 | −0.038 | 0.060 |
| Residual variability (%GSD) | 0.018 (4.2) | N/A |
| *R*2 | 0.9995 | N/A |
| B/Phuket/3073/2013 | Sample #15 | Slope | 1.036 | 0.964 | 1.107 |
| Intercept | −0.063 | −0.205 | 0.078 |
| Residual variability (%GSD) | 0.044 (10.8) | N/A |
| *R*2 | 0.9964 | N/A |
| Sample #16 | Slope | 1.140 | 1.019 | 1.261 |
| Intercept | −0.331 | −0.573 | −0.089 |
| Residual variability (%GSD) | 0.075 (18.9) | N/A |
| *R*2 | 0.9915 | N/A |
| A/California/94/2019 | Sample #43 (1:4 diluted)  | Slope | 0.932 | 0.907 | 0.957 |
| Intercept | 0.249 | 0.191 | 0.306 |
| Residual variability (%GSD) | 0.025 (5.9) | N/A |
| *R*2 | 0.9991 | N/A |
|  Sample #50 (1:4 diluted)  | Slope | 0.955 | 0.884 | 1.026 |
| Intercept | 0.172 | 0.018 | 0.325 |
| Residual variability (%GSD) | 0.044 (10.6) | N/A |
| *R*2 | 0.9959 | N/A |
| A/Cardiff/0508/2019 |  Sample #43 | Slope | 1.014 | 0.894 | 1.133 |
| Intercept | 0.031 | −0.228 | 0.290 |
| Residual variability (%GSD) | 0.095 (24.6) | N/A |
| *R*2 | 0.9862 | N/A |
|  Sample #53 | Slope | 0.957 | 0.863 | 1.052 |
| Intercept | 0.233 | 0.025 | 0.441 |
| Residual variability (%GSD) | 0.076 (19.0) | N/A |
| *R*2 | 0.9903 | N/A |
| A/Netherlands/1268/2019 |  Sample #43 (1:2 diluted)  | Slope | 0.978 | 0.892 | 1.063 |
| Intercept | 0.126 | −0.061 | 0.312 |
| Residual variability (%GSD) | 0.053 (13.0) | N/A |
| *R*2 | 0.9942 | N/A |
| Sample #52 | Slope | 0.992 | 0.972 | 1.011 |
| Intercept | 0.041 | −0.001 | 0.082 |
| Residual variability (%GSD) | 0.015 (3.6) | N/A |
| *R*2 | 0.9996 | N/A |
| A/Tokyo/EH1801/2018 |  Sample #43 (1:2 diluted)  | Slope | 1.005 | 0.977 | 1.033 |
| Intercept | −0.007 | −0.068 | 0.053 |
| Residual variability (%GSD) | 0.022 (5.3) | N/A |
| *R*2 | 0.9992 | N/A |
| Sample #51 (1:2 diluted)  | Slope | 0.966 | 0.798 | 1.133 |
| Intercept | 0.171 | −0.172 | 0.513 |
| Residual variability (%GSD) | 0.104 (27.0) | N/A |
| *R*2 | 0.9777 | N/A |

%GSD, percent geometric standard deviation; HAI, hemagglutination inhibition; LCL, lower confidence limit; N/A, not applicable; *R*2, coefficient of determination; UCL, upper confidence limit; VLP, virus-like particle.

**Table S4**. Accuracy (% Relative bias) and precision (%GCV) of the HAI titers linearity with A/Kansas/14/2017 egg-derived virus/VLP.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Dilution** | **1** | **2** | **4** | **8** | **16** | **1** | **2** | **4** | **8** | **16** | **32** | **64** | **1** | **2** | **4** | **8** | **16** | **32** | **64** | **128** |
| A/Kansas/14/2017 | Egg-derived |  | Sample #42 | Sample #43 | Sample #47 |
| % Relative bias | −0.0 | −2.8 | 15.5 | 0.0 | −2.8 | 0.0 | −0.0 | 15.5 | 15.5 | 9.1 | −35.2 | −42.2 | 0.0 | −5.6 | −5.6 | 18.9 | 58.7 | 58.7 | −5.6 | −5.6 |
| Total %GCV | 20.5 | 10.2 | 31.4 | 20.5 | 9.9 | 33.7 | 36.8 | 0.0 | 0.0 | 20.2 | 33.7 | 0.0 | 27.1 | 0.0 | 0.0 | 31.7 | 28.2 | 34.0 | 0.0 | 0.0 |
|  |
| VLP |  | **1** | **2** | **4** | **8** | **16** | **32** | **64** | **128** | **256** | **1** | **2** | **4** | **8** | **16** | **32** | **64** | **128** | **256** |
|  | Sample #46 | Sample #47 |
| % Relative bias | 0.0 | 0.0 | 0.0 | 5.9 | 33.5 | 22.4 | 0.0 | 0.0 | 5.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 5.9 | 0.0 | 0.0 | 0.0 |
| Total %GCV | 0.0 | 0.0 | 0.0 | 20.5 | 36.0 | 34.0 | 0.0 | 0.0 | 20.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 20.5 | 0.0 | 0.0 | 0.0 |

$$\% Relative bias=100 × \frac{(Observed overall HAI GMT-Expected HAI GMT)}{Expected HAI GMT}$$

%GCV, percent geometric coefficient of variation; GMT, geometric mean titer; HAI, hemagglutination inhibition; VLP, virus-like particle.

**Table S5.** Accuracy (% Relative Bias) and precision (%GCV) of the HAI titers linearity with A/Brisbane/02/2018, B/Maryland/15/2016, B/Phuket/3073/2013, A/California/94/2019, A/Cardiff/0508/2019, A/Netherlands/1268/2019, and A/Tokyo/EH1801/2018 virus strains.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Dilution** | **1** | **2** | **4** | **8** | **16** | **32** | **64** | **128** | **256** | **1** | **2** | **4** | **8** | **16** | **32** | **64** | **128** |
| A/Brisbane/02/2018 | Egg-derived |  | Sample #15 | Sample #49 |
| % Relative bias | 0.0 | 0.0 | 9.1 | −0.0 | 2.9 | 2.9 | 2.9 | 2.9 | – | 0.0 | 12.2 | 41.4 | 41.4 | 54.2 | 45.6 | −10.9 | −18.3 |
| Total %GCV | 10.2 | 10.2 | 13.6 | 22.1 | 0.0 | 0.0 | 0.0 | 0.0 | – | 34.4 | 34.0 | 22.8 | 27.5 | 10.2 | 21.7 | 17.5 | 9.9 |
|  |  | Sample #15 |  Sample #49 (1:2 diluted)  |
| VLP | % Relative bias | 0.0 | 9.1 | 15.5 | 15.5 | 15.5 | 15.5 | 9.1 | 12.2 | – | 0.0 | 0.0 | 12.2 | 18.9 | 2.9 | 0.0 | 0.0 | 0.0 |
| Total %GCV | 30.7 | 20.5 | 0.0 | 0.0 | 0.0 | 0.0 | 20.2 | 10.5 | – | 0.0 | 0.0 | 27.5 | 28.2 | 10.2 | 0.0 | 0.0 | 0.0 |
| B/Maryland/15/2016 | Egg-derived |  | Sample #14 |  Sample #35 |
| % Relative bias | 0.0 | 2.9 | 0.0 | 0.0 | 12.2 | 0.0 | 15.5 | – | – | 0.0 | 26.0 | 41.4 | 58.7 | 58.7 | 58.7 | 58.7 | – |
| Total %GCV | 0.0 | 10.2 | 0.0 | 0.0 | 31.8  | 0.0 | 23.2 | – | – | 27.5 | 35.9 | 22.9 | 0.0 | 0.0 | 0.0 | 0.0 | – |
| VLP |  | Sample #14 | Sample #45 (1:2 diluted)  |
| % Relative bias | 0.0 | −29.3 | −29.3 | −29.3 | −29.3 | −29.3 | – | – | – | 0.0 | 0.0 | −5.6 | −0.0 | 9.1 | 0.0 | 0.0 | 0.0 |
| Total %GCV | 37.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | – | – | – | 0.0 | 0.0 | 20.5 | 32.8 | 44.0 | 0.0 | 0.0 | 0.0 |
| B/Phuket/3073/2013 | Egg-derived |  | Sample #14 | Sample #16 |
| % Relative Bias | 0.0 | 9.1 | 15.5 | 15.5 | 15.5 | 15.5 | 49.8 | 54.2 | – | 0.0 | 5.9 | 5.9 | 5.9 | 5.9 | 5.9 | 5.9 | 5.9 |
| Total %GCV | 26.1 | 20.5 | 0.0 | 0.0 | 0.0 | 0.0 | 39.3 | 41.5 | – | 20.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| VLP |  | Sample #15 | Sample #16 |
| % Relative bias | 0.0 | 5.9 | 5.9 | 5.9 | 5.9 | 5.9 | −20.6 | – | – | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | −33.3 | −47.0 | – |
| Total %GCV | 20.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 49.3 | – | – | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 54.5 | 20.5 | – |
| A/California/94/2019 | VLP |  |  Sample #43 (1:4 diluted)  | Sample #50 (1:4 diluted)  |
| % Relative bias | 0.0 | 18.9 | 18.9 | 26.0 | 22.4 | 22.4 | 37.4 | 45.6 | 63.4 | 0.0 | 15.5 | 18.9 | 33.5 | 33.5 | 9.1 | 33.5 | – |
| Total %GCV | 47.3 | 10.2 | 10.2 | 10.2 | 0.0 | 0.0 | 31.8 | 34.9 | 37.1 | 44.9 | 28.8 | 31.8 | 0.0 | 16.2 | 49.0 | 0.0 | – |
| A/Cardiff/0508/2019 |  |  Sample #43 | Sample #53 |
| % Relative bias | 0.0 | 2.9 | 22.4 | 54.2 | 58.7 | −2.8 | 0.0 | 0.0 | – | 0.0 | 26.0 | 54.2 | 49.8 | 83.4 | 41.4 | 45.6 | 29.7 |
| Total %GCV | 0.0 | 10.0 | 32.2 | 39.3 | 40.0 | 10.5 | 0.0 | 0.0 | – | 15.6 | 36.1 | 35.9 | 40.0 | 10.2 | 41.5 | 39.3 | 43.0 |
| A/Netherlands/1268/2019 |  | Sample #43 (1:2 diluted)  | Sample #52 |
| % Relative bias | 0.0 | 22.4 | 29.7 | 29.7 | 29.7 | 2.9 | 29.7 | – | – | 0.0 | 2.9 | 5.9 | 9.1 | 12.2 | 2.9 | 5.9 | 5.9 |
| Total %GCV | 32.4 | 20.5 | 0.0 | 0.0 | 0.0 | 40.0 | 0.0 | – | – | 20.5 | 10.2 | 0.0 | 10.2 | 15.5 | 10.5 | 0.0 | 0.0 |
| A/Tokyo/EH1801/2018 |  | Sample #43 (1:2 diluted)  | Sample #51 (1:2 diluted)  |
| % Relative bias | 0.0 | 0.0 | 0.0 | 12.2 | 0.0 | −5.6 | 0.0 | 0.0 | – | 0.0 | 12.2 | 41.4 | 63.4 | 63.4 | −5.6 | 33.5 | – |
| Total %GCV | 0.0 | 0.0 | 0.0 | 29.1 | 0.0 | 20.5 | 0.0 | 0.0 | – | 33.7 | 32.5 | 41.1 | 22.6 | 22.5 | 20.5 | 37.5 | – |

$$\% Relative bias=100 × \frac{(Observed overall HAI GMT-Expected HAI GMT)}{Expected HAI GMT}$$

%GCV, percent geometric coefficient of variation; GMT, geometric mean titer; HAI, hemagglutination inhibition; VLP, virus-like particle.

**Table S6**. Assay robustness – effect of human RBC suspension storage time using four homologous seasonal influenza strains (A/Kansas, A/Brisbane, B/Maryland, and B/Phuket).

|  |  |  |
| --- | --- | --- |
| **Strain** | **HAI assay type** | **% Difference from baseline1** |
| **0.75% RBC stored for2 weeks** | **0.75% RBC from 10% RBC stored for 2 weeks** |
| A/Kansas/14/2017  | Egg-derived | −50.0–88.8 | −5.6–100.0 |
| VLP | −52.8–0.0 | −50.0–100.0 |
| A/Brisbane/02/2018 | Egg-derived | −50.0–2.9 | −29.3–100.0 |
| VLP | −50.0–2.9 | −50.0–15.5 |
| B/Maryland/15/2016 | Egg-derived | −50.0–9.1 | −48.5–100.0 |
| VLP | −50.0–100.0 | −29.3–88.8 |
| B/Phuket/3073/2013 | Egg-derived | −50.0–33.5 | −50.0–5.9 |
| VLP | −64.6–0.0 | −50.0–0.0 |

$$\% Difference=100 × \frac{(Testing HAI GMT -Baseline Overall HAI GMT)}{Baseline Overall HAI GMT}$$

1Baseline values were overall HAI GMT from the precision assay runs utilizing fresh 0.75% RBCs stored at 2 to 8 °C for less than a week (≤7 days).

GMT, geometric mean titer; HAI, hemagglutination inhibition; RBC, red blood cells; VLP, virus-like particle.

**Table S7.** Assayrobustness – effect of plate reading time (incubation time) using four homologous seasonal influenza strains (A/Kansas, A/Brisbane, B/Maryland, and B/Phuket).

|  |  |  |
| --- | --- | --- |
| **Strain** | **HAI assay type** | **% Difference (Range) at varying plate reading time relative to standard 90 min** |
| **75 min** | **120 min** | **150 min** |
| A/Kansas/14/2017  | Egg-derived | −29.3–0.0 | 0.0–41.4 | 0.0–100.0 |
| VLP | −29.3–0.0 | 0.0–68.2 | 0.0–100.0 |
| A/Brisbane/02/2018 | Egg-derived | 0.0–41.4 | 0.0–41.4 | 0.0–41.4 |
| VLP | −40.5–0.0 | 0.0–68.2 | 0.0–100.0 |
| B/Maryland/15/2016 | Egg-derived | −15.9–41.4 | 0.0–68.2 | 0.0–100.0 |
| VLP | −15.9–41.4 | 0.0–41.4 | 0.0–41.4 |
| B/Phuket/3073/2013 | Egg-derived | −15.9–41.4 | 0.0–41.4 | 0.0–41.4 |
| VLP | −15.9–0.0 | 0.0–68.2 | 0.0–68.2 |

$$\% Difference=100 × \frac{(Testing HAI GMT -Baseline Overall HAI GMT)}{Baseline Overall HAI GMT}$$

GMT, geometric mean titer; HAI, hemagglutination inhibition; VLP, virus-like particle.

**Table S8.** Assayrobustness in terms of HAI GMT % difference from baseline1 – effect of combined serum–egg-derived virus/VLP incubation time and plate reading time using four homologous seasonal influenza strains (A/Kansas, A/Brisbane, B/Maryland, and B/Phuket).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Serum-virus/VLP incubation time** | **Plate reading time (min)** | **A/Kansas/14/2017** | **A/Brisbane/02/2018** | **B/Maryland/15/2016** | **B/Phuket/3073/2013** |
|  |  | Egg-derived | VLP | Egg-derived | VLP | Egg-derived | VLP | Egg-derived | VLP |
| 50 min | 75 | −50.0–0.0 | −50.0–41.4 | −50.0–0.0 | −50.0–41.4 | −50.0–0.0 | −50.0–0.0 | −50.0–0.0 | −50.0–0.0 |
| 90 | −50.0–0.0 | −50.0–41.4 | −50.0–0.0 | −50.0–41.4 | −50.0–0.0 | −50.0–0.0 | −50.0–41.4 | −50.0–0.0 |
| 120 | −50.0–0.0 | −50.0–100.0 | −50.0–41.4 | −50.0–41.4 | −50.0–0.0 | −50.0–0.0 | −50.0–41.4 | −50.0–0.0 |
| 150 | −50.0–0.0 | −50.0–100.0 | −50.0–41.4 | −50.0–41.4 | −50.0–0.0 | −50.0–0.0 | −50.0–100.0 | −50.0–0.0 |
| 60 min | 75 | −50.0–0.0 | −29.3–0.0 | 0.0–41.4 | −50.0–0.0 | −50.0–0.0 | −50.0–0.0 | −50.0–0.0 | −50.0–0.0 |
| 90 | – | – | – | – | – | – | – | – |
| 120 | 0.0–100.0 | 0.0–100.0 | 0.0–100.0 | 0.0 | 0.0–100.0 | 0.0 | 0.0–100.0 | 0.0–41.4 |
| 150 | 0.0–100.0 | 0.0–100.0 | 0.0–100.0 | 0.0–41.4 | 0.0–100.0 | 0.0–100.0 | 0.0–100.0 | 0.0–41.4 |
| 70 min | 75 | −50.0–100.0 | −50.0–100.0 | −29.3–100.0 | −50.0–100.0 | −29.3–100.0 | 0.0–100.0 | −50.0–100.0 | −29.3–41.4 |
| 90 | −29.3–100.0 | −50.0–100.0 | 0.0–100.0 | −50.0–100.0 | 0.0–100.0 | 0.0–100.0 | 0.0–100.0 | 0.0–41.4 |
| 120 | −29.3–100.0 | −50.0–100.0 | 0.0–100.0 | −50.0–100.0 | 0.0–100.0 | 0.0–100.0 | 0.0–100.0 | 0.0–100.0 |
| 150 | −29.3–100.0 | −50.0–100.0 | 0.0–100.0 | −50.0–100.0 | 0.0–100.0 | 0.0–100.0 | 0.0–100.0 | 0.0–100.0 |

$$\% Difference=100 × \frac{(Testing HAI GMT -Baseline Overall HAI GMT)}{Baseline Overall HAI GMT}$$

Geometric mean titer (GMT) was defined as the antilog of the mean of the log-transformed HAI titers for a given treatment group [21].

1Baseline HAI GMTs were from assay runs in which serum-virus/VLP incubation time was 1 h and the plate reading time was 90 min after RBC addition.

GMT, geometric mean titer; HAI, hemagglutination inhibition; VLP, virus-like particle.

**Table S9.** Stability of RDE-treated samples in egg-derived and VLP HAI assay using four homologous seasonal influenza strains (A/Kansas, A/Brisbane, B/Maryland, and B/Phuket).

|  |  |  |
| --- | --- | --- |
| **Strain** | **HAI assay type** | **% Difference from baseline1** |
| **Stored at 2 to 8 °C for1 month** | **Stored at 2 to 8 °C for2 months** | **Stored at ≤−20 °C for2 months** | **Two freeze/thaw cycles** |
| A/Kansas/14/2017  | Egg-derived | −52.8–78.2 | −75.0–0.0 | −5.6–535.0 | −42.2–100.0 |
| VLP | −70.3–41.4 | −70.3–100.0 | −50.0–100.0 | −5.6–100.0 |
| A/Brisbane/02/2018 | Egg-derived | −50.0–94.3 | −50.0–58.7 | −50.0–100.0 | −50.0–88.8 |
| VLP | −56.7–0.0 | −56.7–78.2 | −50.0–100.0 | −29.3–78.2 |
| B/Maryland/15/2016 | Egg-derived | −50.0–41.4 | −50.0–100.0 | −20.6–182.8 | −43.9–100.0 |
| VLP | −50.0–41.4 | −50.0–88.8 | −75.0–49.8 | −25.1–41.4 |
| B/Phuket/3073/2013 | Egg-derived | −50.0–88.8 | −50.0–15.5 | −35.2–100.0 | −33.3–100.0 |
| VLP | −50.0–94.3 | −50.0–0.0 | −29.3–100.0 | −50.0–41.4 |

$$\% Difference=100 × \frac{(Testing HAI GMT -Baseline Overall HAI GMT)}{Baseline Overall HAI GMT}$$

1Baseline values were overall HAI GMT results from the precision assay runs in which RDE-treated samples were stored at 2 to 8 °C for ≤7 days before testing.

GMT, geometric mean titer; HAI, hemagglutination inhibition; RDE, receptor-destroying enzyme; VLP, virus-like particle.

**Table S10.** Total %GCV for singleton titers, paired replicates, and random replicates for A/Kansas/14/2017 VLP HAI assay.

|  |  |  |  |
| --- | --- | --- | --- |
| **No.** | **Sample ID** | **%GCV** | **% Difference** |
| **Singleton titers** | **Paired replicates** | **Random replicates 1** | **Random replicates 2** | **Singleton titers vs. Paired replicates** | **Random 1vs. Paired replicates** | **Random 2vs. Paired replicates** |
| 1 | Sample #3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 2 | Sample #3 (1:2)  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 | Sample #15 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 4 | Sample #16 | 21.7 | 20.5 | 20.5 | 20.5 | 5.4 | 0.0 | 0.0 |
| 5 | Sample #19 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 6 | Sample #20 | 35.3 | 34.9 | 34.9 | 34.9 | 1.3 | 0.0 | 0.0 |
| 7 | Sample #21 | 35.5 | 35.9 | 35.9 | 35.9 | −1.0 | 0.0 | 0.0 |
| 8 | Sample #23 | 21.7 | 20.5 | 20.5 | 20.5 | 5.4 | 0.0 | 0.0 |
| 9 | Sample #24 | 36.2 | 32.2 | 37.0 | 42.8 | 12.4 | 14.8 | 32.8 |
| 10 | Sample #25 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 11 | Sample #26 | 37.5 | 35.6 | 38.6 | 36.9 | 5.4 | 8.4 | 3.6 |
| 12 | Sample #34 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 13 | Sample #35 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 14 | Sample #37 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 15 | Sample #39 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 16 | Sample #43 | 14.3 | 10.2 | 20.5 | 20.5 | 40.4 | 101.6 | 101.6 |
| 17 | Sample #46 Dil. 1  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 18 | Sample #46 Dil. 2  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 19 | Sample #46 Dil. 3  | 21.7 | 20.5 | 0.0 | 0.0 | 5.4 | −100.0 | −100.0 |
| 20 | Sample #46 Dil. 4  | 38.9 | 36.0 | 20.5 | 20.5 | 8.1 | −42.9 | −42.9 |
| 21 | Sample #46 Dil. 5  | 38.6 | 34.0 | 41.5 | 37.5 | 13.3 | 21.8 | 10.2 |
| 22 | Sample #46 Dil. 6  | 0.0 | 0.0 | 34.9 | 40.0 | 0.0 | 0.0 | 0.0 |
| 23 | Sample #46 Dil. 7  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 24 | Sample #46 Dil. 8  | 21.7 | 20.5 | 0.0 | 0.0 | 5.4 | −100.0 | −100.0 |
| 25 | Sample #46 Dil. 9  | 39.9 | 40.0 | 20.5 | 20.5 | −0.3 | −48.6 | −48.6 |
| 26 | Sample #46 Dil. 10  | 0.0 | 0.0 | 40.0 | 40.0 | 0.0 | 0.0 | 0.0 |
| 27 | Sample #47 Dil. 1  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 28 | Sample #47 Dil. 2  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 29 | Sample #47 Dil. 3  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 30 | Sample #47 Dil. 4  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 31 | Sample #47 Dil. 5  | 21.7 | 20.5 | 0.0 | 0.0 | 5.4 | −100.0 | −100.0 |
| 32 | Sample #47 Dil. 6  | 0.0 | 0.0 | 20.5 | 20.5 | 0.0 | 0.0 | 0.0 |
| 33 | Sample #47 Dil. 7  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 34 | Sample #47 Dil. 8  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 35 | Sample #47 Dil. 9  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 36 | Sample #47 Dil. 10  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

$$\% Difference=100 × \frac{(Paired or Random \%GCV-Singleton \%GCV)}{Singleton \%GCV}$$

%GCV, percent geometric coefficient of variation; HAI, hemagglutination inhibition; VLP, virus-like particle.

**Table S11.** Total %GCV for singleton titers, paired replicates, and random replicates for A/Brisbane/02/2018 VLP HAI assay.

|  |  |  |  |
| --- | --- | --- | --- |
| **No.** | **Sample ID** | **%GCV** | **% Difference** |
| **Singleton titers** | **Paired replicates** | **Random replicates 1** | **Random replicates 2** | **Singleton titers vs. Paired replicates** | **Random 1vs. Paired replicates** | **Random 2vs. Paired replicates** |
| 1 | Sample #3 | 34.6 | 33.7 | 33.7 | 33.7 | 2.6 | 0.0 | 0.0 |
| 2 | Sample #3 (1:2)  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 | Sample #13 | 19.8 | 13.6 | 20.5 | 20.5 | 45.8 | 51.6 | 51.6 |
| 4 | Sample #14 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 5 | Sample #15 Dil. 1  | 33.4 | 30.7 | 33.0 | 33.7 | 8.6 | 7.5 | 9.7 |
| 6 | Sample #15 Dil. 2  | 21.7 | 20.5 | 20.5 | 20.5 | 5.4 | 0.0 | 0.0 |
| 7 | Sample #15 Dil. 3  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 8 | Sample #15 Dil. 4  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 9 | Sample #15 Dil. 5  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 10 | Sample #15 Dil. 6  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 11 | Sample #15 Dil. 7  | 21.1 | 20.2 | 20.2 | 20.2 | 4.5 | 0.0 | 0.0 |
| 12 | Sample #15 Dil. 8  | 14.3 | 10.2 | 0.0 | 0.0 | 40.4 | −100.0 | −100.0 |
| 13 | Sample #15 Dil. 9  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 14 | Sample #17 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 15 | Sample #18 | 30.0 | 28.4 | 32.1 | 32.1 | 5.7 | 13.0 | 13.0 |
| 16 | Sample #19 | 32.5 | 26.7 | 44.3 | 27.5 | 21.5 | 65.8 | 2.9 |
| 17 | Sample #20 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 18 | Sample #22 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 19 | Sample #23 | 14.2 | 10.0 | 20.2 | 20.2 | 41.8 | 101.5 | 101.5 |
| 20 | Sample #24 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 21 | Sample #25 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 22 | Sample #26 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 23 | Sample #49 Dil. 1  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 24 | Sample #49 Dil. 2  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 25 | Sample #49 Dil. 3  | 26.9 | 27.5 | 27.5 | 27.5 | −2.3 | 0.0 | 0.0 |
| 26 | Sample #49 Dil. 4  | 31.5 | 28.2 | 32.1 | 35.9 | 11.7 | 14.0 | 27.3 |
| 27 | Sample #49 Dil. 5  | 14.3 | 10.2 | 20.5 | 20.5 | 40.4 | 101.6 | 101.6 |
| 28 | Sample #49 Dil. 6  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 29 | Sample #49 Dil. 7  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 30 | Sample #49 Dil. 8  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 31 | Sample #49 Dil. 9 | 30.0 | 24.2 | 32.1 | 35.9 | 24.3 | 33.0 | 48.5 |
| 32 | Sample #49 Dil. 10  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 33 | Sample #37 | 50.7 | 48.7 | 54.0 | 54.0 | 4.1 | 11.0 | 11.0 |
| 34 | Sample #38 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 35 | Sample #39 | 21.7 | 16.5 | 0.0 | 0.0 | 31.2 | −100.0 | −100.0 |
| 36 | Sample #48 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

$$\% Difference=100 × \frac{(Paired or Random \%GCV-Singleton \%GCV)}{Singleton \%GCV}$$

%GCV, percent geometric coefficient of variation; HAI, hemagglutination inhibition; VLP, virus-like particle.

**Table S12.** Total %GCV for singleton titers, paired replicates, and random replicates for B/Maryland/15/2016 VLP HAI assay.

|  |  |  |  |
| --- | --- | --- | --- |
| **No.** | **Sample ID** | **%GCV** | **% Difference** |
| **Singleton titers** | **Paired replicates** | **Random replicates 1** | **Random replicates 2** | **Singleton titers vs. Paired replicates** | **Random 1vs. Paired replicates** | **Random 2vs. Paired replicates** |
| 1 | Sample #3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 2 | Sample #3 (1:2)  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 | Sample #7 | 40.4 | 37.1 | 40.0 | 41.5 | 8.8 | 7.6 | 11.7 |
| 4 | Sample #12 | 32.8 | 31.8 | 31.8 | 31.8 | 3.1 | 0.0 | 0.0 |
| 5 | Sample #13 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 6 | Sample #14 Dil. 1  | 40.5 | 37.7 | 39.2 | 42.0 | 7.6 | 4.0 | 11.4 |
| 7 | Sample #14 Dil. 2  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 8 | Sample #14 Dil. 3  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 9 | BRH1452659 Dil. 4 (Sample #14) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 10 | Sample #14 Dil. 5  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 11 | Sample #14 Dil. 6  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 12 | Sample #14 Dil. 7  | 56.0 | 55.4 | 55.4 | 55.4 | 1.1 | 0.0 | 0.0 |
| 13 | Sample #15 | 31.7 | 30.1 | 28.7 | 34.9 | 5.4 | −4.7 | 15.9 |
| 14 | Sample #16 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 15 | Sample #17 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 16 | Sample #19 | 27.1 | 24.4 | 20.2 | 31.8 | 10.9 | −17.3 | 29.9 |
| 17 | Sample #21 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 18 | Sample #22 | 35.5 | 32.4 | 34.9 | 35.9 | 9.4 | 7.5 | 10.6 |
| 19 | Sample #23 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 20 | Sample #24 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 21 | Sample #26 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 22 | Sample #45 Dil. 1  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 23 | Sample #45 Dil. 2  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 24 | Sample #45 Dil. 3  | 21.7 | 20.5 | 20.5 | 20.5 | 5.4 | 0.0 | 0.0 |
| 25 | Sample #45 Dil. 4  | 36.4 | 32.9 | 35.3 | 20.5 | 10.8 | 7.2 | −37.5 |
| 26 | Sample #45 Dil. 5  | 48.3 | 44.1 | 61.0 | 46.8 | 9.6 | 38.3 | 6.3 |
| 27 | Sample #45 Dil. 6  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 28 | Sample #45 Dil. 7  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 29 | Sample #45 Dil. 8  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 30 | Sample #45 Dil. 9  | 21.7 | 20.5 | 20.5 | 20.5 | 5.4 | 0.0 | 0.0 |
| 31 | Sample #45 Dil. 10  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 32 | Sample #36 | 14.2 | 10.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 33 | Sample #37 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 34 |  Sample #38 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 35 | Sample #39 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 36 |  Sample #44 | 38.3 | 37.1 | 37.5 | 39.7 | 3.3 | 1.2 | 7.1 |

$$\% Difference=100 × \frac{(Paired or Random \%GCV-Singleton \%GCV)}{Singleton \%GCV}$$

%GCV, percent geometric coefficient of variation; HAI, hemagglutination inhibition; VLP, virus-like particle.

**Table S13.** Total %GCV for singleton titers, paired replicates, and random replicates for B/Phuket/3073/2013 VLP HAI assay.

|  |  |  |  |
| --- | --- | --- | --- |
| No. | **Sample ID** | **%GCV** | **%Difference** |
| **Singleton titers** | **Paired replicates** | **Random replicates 1** | **Random replicates 2** | **Singleton titers vs. Paired replicates** | **Random 1vs. Paired replicates** | **Random 2vs. Paired replicates** |
| 1 | Sample #3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 2 | Sample #3 (1:2)  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 | Sample #13 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 4 | Sample #14 | 21.7 | 20.5 | 20.5 | 20.5 | 5.4 | 0.0 | 0.0 |
| 5 | Sample #15 Dil. 1  | 21.7 | 20.5 | 20.5 | 20.5 | 5.4 | 0.0 | 0.0 |
| 6 | Sample #15 Dil. 2  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 7 | Sample #15 Dil. 3  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 8 | Sample #15 Dil. 4  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 9 | Sample #15 Dil. 5  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 10 | Sample #15 Dil. 6  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 11 | Sample #15 Dil. 7  | 50.2 | 49.3 | 49.3 | 49.3 | 1.7 | 0.0 | 0.0 |
| 12 | Sample #15 Dil. 8  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 13 | Sample #15 Dil. 9  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 14 | Sample #16 Dil. 1  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 15 | Sample #16 Dil. 2  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 16 | Sample #16 Dil. 3  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 17 | Sample #16 Dil. 4  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 18 | Sample #16 Dil. 5  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 19 | Sample #16 Dil. 6  | 57.1 | 54.5 | 55.4 | 55.4 | 4.6 | 1.6 | 1.6 |
| 20 | Sample #16 Dil. 7  | 21.7 | 20.5 | 20.5 | 20.5 | 5.4 | 0.0 | 0.0 |
| 21 | Sample #16 Dil. 8  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 22 | Sample #16 Dil. 9  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 23 | Sample #17 | 14.3 | 10.2 | 20.5 | 20.5 | 40.4 | 101.6 | 101.6 |
| 24 | Sample #18 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 25 | Sample #19 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 26 | Sample #20 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 27 | Sample #21 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 28 | Sample #23 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 29 | Sample #24 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 30 | Sample #34 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 31 | Sample #35 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 32 | Sample #36 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 33 | Sample #37 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 34 | Sample #38 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 35 | Sample #39 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 36 |  Sample #40 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

$$\% Difference=100 × \frac{(Paired or Random \%GCV-Singleton \%GCV)}{Singleton \%GCV}$$

%GCV, percent geometric coefficient of variation; HAI, hemagglutination inhibition; VLP, virus-like particle.