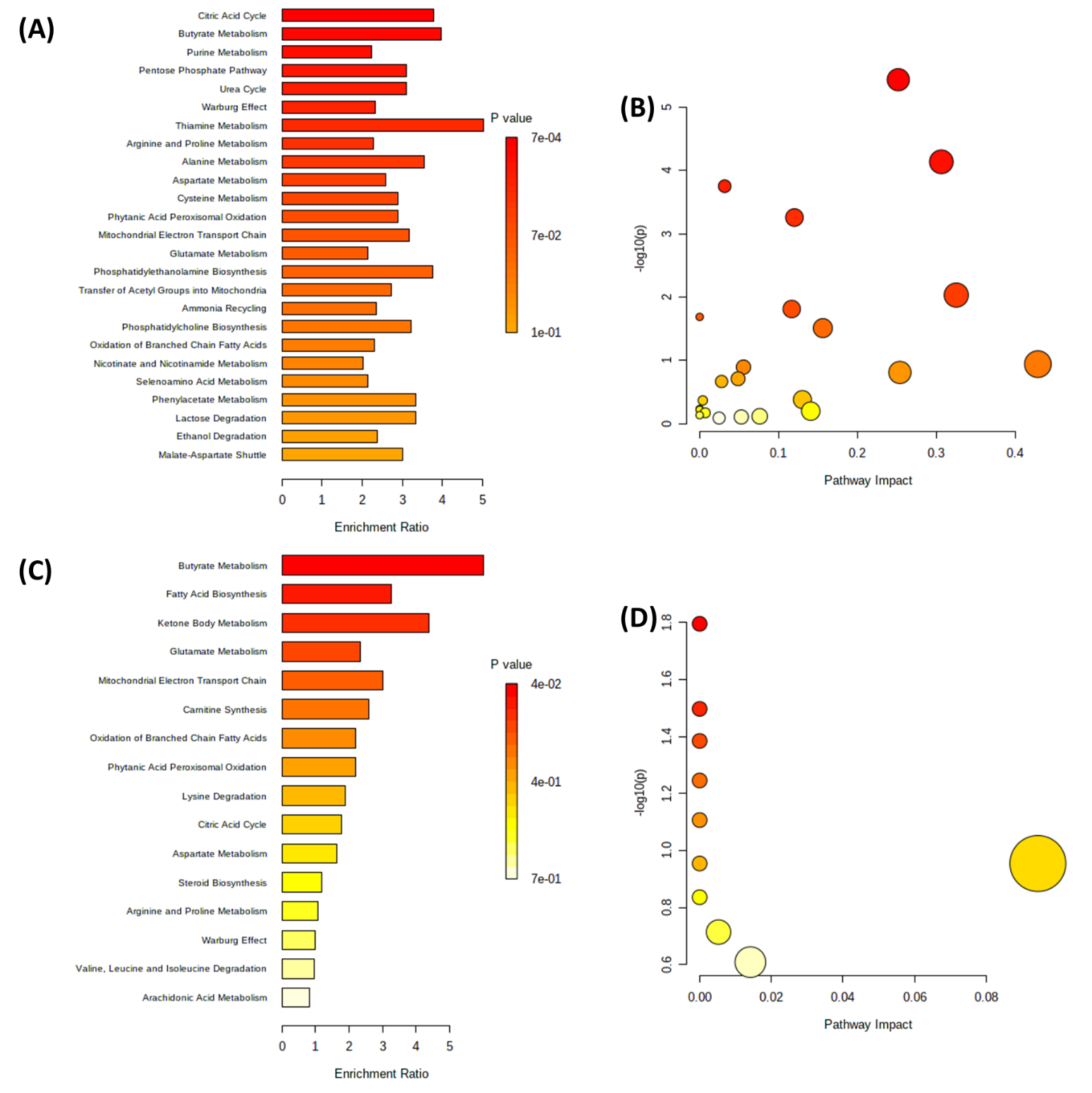


**Supplementary Figure S1.** PCA overview of the central carbon metabolite data analyzed in the nasal wash (A-C, R2*X* = 0.656, and Q2 = 0.476, DCrit value = 1.2648), oral swab (D-F, R2*X* = 0.276, and Q2 = 0.092, DCrit value = 1.2365), and rectal swab (G-I, R2*X* = 0.595, and Q2 = 0.459, DCrit value = 1.2365) samples pre-infection, during viral shedding, and post-viral shedding. (A, D, G) PLS-DA Score Scatter plot of metabolite data. (B, E, H) PLS-DA Loading Scatter plot of metabolite data. (C, F, I) DModX plot of metabolite data. The ellipse on the loadings scatter plots represents the 95% Hoteling’s threshold. *Note, the ellipse presented in Figures S2A, S2D, and S2G represents Hotelling's T2 confidence limit (95%). Note: The colored circles in panel “A”, “D”, and “G” represent each analyzed sample, with the white circles representing the distribution of metabolite features between these groups.*

**Supplementary Table S1.** Identified significant metabolite clusters (p ≤ 0.1) during viral shedding and post-shedding using the central carbon metabolism metabolite dataset.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample Type** | **Cluster name** | **Cluster size** | **P-value** | **FDR** | **Key compound** | **HMDB** | **KEGG ID** | **Altered metabolites** |
| Nasal Wash | Indoles | 4 | 0.0075 | 0.00005 | Indoline-2-carboxylate | HMDB0002285 | NA | 1 |
| Adenine Nucleotides | 5 | 0.0346 | 0.0066 | Adenosine 5-monophosphate | HMDB0000045 | C00020 | 1 |
| Succinates | 5 | 0.0681 | 0.0066 | Succinic semialdehyde | HMDB0001259 | C00232 | 2 |
| Deoxycytosine Nucleotides | 3 | 0.0939 | 0.007 | 2-Deoxycytidine 5-diphosphate | HMDB0001245 | C00705 | 2 |
| Dicarboxylic Acids | 5 | 0.0190 | 0.0095 | Citramalic acid | HMDB0000426 | C00815 | 0 |
| Glutarates |  | 0.0848 | 0.013 | 3-Methylglutaric acid | HMDB0000752 | NA | 0 |
| Guanine Nucleotides | 6 | 0.0647 | 0.024 | Deoxyguanosine 5-triphosphate | HMDB0001440 | C00286 | 3 |
| Hydroxy Acids | 4 | 0.0190 | 0.024 | Mevalonic acid | HMDB0000227 | C00418 | 1 |
| Hydroxybenzoates | 6 | 0.0886 | 0.028 | m-Hydroxybenzoic acid | HMDB0002466 | C00587 | 3 |
| Pentoses | 3 | 0.0086 | 0.061 | L-Arabinose | HMDB0000646 | C11476 | 2 |
| Pentanols | 2 | 0.0716 | 0.061 | Isopentyl acetate | HMDB0031528 | C12296 | 1 |
| Pentose phosphates | 5 | 0.0650 | 0.061 | 2-Deoxyribose 5-phosphate | HMDB0001031 | C00673 | 1 |
| Sialic Acids | 1 | 0.0778 | 0.065 | N-Acetylneuraminic acid | HMDB0000230 | C19910 | 1 |
| Tricarboxylic Acids | 5 | 0.0157 | 0.12 | trans-Aconitic acid | HMDB0000958 | C02341 | 2 |
| Oral Swab | Succinates | 5 | 0.039 | 1 | Argininosuccinic acid | HMDB0000052 | C03406 | 2 |
| Rectal Swab | Hexose phosphates | 4 | 0.0076 | 0.33 | D-Galactosamine | NA | C02262 | 2 |
| Hydroxy acids | 4 | 0.027 | 0.59 | Lactic acid | HMDB0144295 | C01432 | 2 |

FDR: False Discovery Rate; HMDB: Human Metabolome Database; KEGG: Kyoto Encyclopedia of Genes and Genomes



**Supplementary Figure S2.** Enrichment analysis of the identified metabolites of importance from nasal wash samples. (A) Metabolite enrichment analysis. (B) Metabolite pathway impact analysis. (C) Lipid enrichment analysis. (D) Lipid pathway impact analysis.



**Supplementary Figure S3:** Boxplot representing the data of individual significant metabolites from the key metabolism changes in a SARS-CoV-2 ferret model during viral shedding and post-shedding events. Normalized metabolite expression is determined as the mean ± the standard error.

**Supplementary Table S2.** Significant metabolites identified from central carbon metabolism metabolite dataset.

| **Metabolite** | **log2 (FC)** | **-log10 (p-value)** | **KEGG ID** | **HMDB ID** | **Elevated in** |
| --- | --- | --- | --- | --- | --- |
| L-Cystine | 2.7682 | 2.1375 | C01420 | HMDB0000192 | SA01 |
| L-Methionine | 2.5291 | 1.5963 | C01733 | HMDB0033951 |
| L-Histidine | 2.0048 | 3.2117 | C00135 | HMDB0000177 |
| Pyridoxal hydrochloride | 1.8407 | 2.4058 | C00250 | HMDB0001545 |
| Cytidine 5-diphosphate | 1.1293 | 1.481 | C00112 | HMDB0001546 |
| L-Glutamine | 0.76587 | 9.3871 | C00064 | HMDB0000641 |
| L-Threonine | 0.74125 | 3.3936 | C00188 | HMDB0000167 |
| L-Homoserine | 0.64845 | 2.9521 | C00263 | HMDB0000719 |
| L-Dihydroorotic acid | 0.62133 | 2.0999 | C00337 | HMDB0003349 |
| 2-Deoxyribose 5-phosphate | 0.58836 | 5.2058 | C00673 | HMDB0001031 |
| Itaconic acid | -0.62578 | 2.5257 | C00490 | HMDB0002092 | VIC01 |
| D-Gluconic acid | -0.69118 | 1.9507 | C00257 | HMDB0000625 |
| N-Formyl-L-Tyrosine | -0.77537 | 2.0569 | No result | No result |
| Shikimic acid | -0.78933 | 2.5124 | C00493 | HMDB0003070 |
| N-Acetyl-alpha-D-glucosamine 1-phosphate | -0.8396 | 3.2117 | C04256 | HMDB0001367 |
| N-Acetyl-D-glucosamine 6-phosphate | -0.8396 | 3.2117 | C00357 | HMDB0001062 |
| N-Acetyl D-galactosamine | -0.8726 | 1.5963 | C01132 | HMDB0000853 |
| Ketovaleric acid | -0.94453 | 2.477 | C06255 | HMDB0001865 |
| L-Malic acid | -1.0265 | 2.1663 | C00711 | HMDB0000744 |
| 3-Methylglutaric acid | -1.0438 | 3.2427 | No result | HMDB0000752 |
| Cellobiose | -1.053 | 1.3492 | C06422 | HMDB0000055 |
| 4-Guanidobutyric acid | -1.0534 | 2.1375 | C01035 | HMDB0003464 |
| L-Hydroxyglutaric acid | -1.1278 | 1.912 | C02630 | HMDB0059655 |
| Mevalonic acid | -1.1278 | 1.912 | C00418 | HMDB0000227 |
| Dihydroxyacetone phosphate | -1.2088 | 1.5963 | C00111 | HMDB0001473 |
| 2-3-Dihydroxyisovalerate | -1.3219 | 1.3492 | C04039 | No result |
| alpha-D-Glucose-1-phosphate | -1.4665 | 1.3535 | C00103 | HMDB0001586 | VIC01 |
| Oxamic acid | -1.4678 | 5.0099 | C01444 | No result |
| Homocitrate | -1.4685 | 1.3492 | C01251 | HMDB0003518 |
| D-Fructose 6-phosphate | -1.6229 | 1.5051 | C00085 | HMDB0000124 |
| 2-Deoxyinosine | -1.6479 | 1.4177 | C05512 | HMDB0000071 |
| L-Glutamic acid | -1.7373 | 2.1375 | C00025 | HMDB0000148 |
| 4-Methyl-2-oxovaleric acid | -1.8268 | 2.6782 | C00233 | HMDB0000695 |
| 2-3-Dihydroxybenzoic acid | -1.8476 | 2.6638 | C00196 | HMDB0000397 |
| Uridine 5-monophosphate | -1.9414 | 2.4376 | C00105 | HMDB0000288 |
| D-Ribulose 1,5-biphosphate | -1.9674 | 2.4058 | C01182 | No result |
| beta-Nicotinamide mononucleotide | -1.9977 | 1.6314 | C00455 | HMDB0000229 |
| 5-Hydroxy-3-indoleacetic acid | -2.0265 | 2.1426 | C05635 | HMDB0000763 |
| Salicylic acid | -2.0703 | 2.4058 | C00805 | HMDB0001895 |
| 2-Deoxyadenosine | -2.1486 | 1.8156 | C00559 | HMDB0000101 |
| Orotic acid | -2.3856 | 2.5257 | C00295 | HMDB0000226 |
| Glyceric acid | -2.4357 | 6.9069 | C00258 | HMDB0000139 |
| 4-Hydroxybenzoic acid | -2.4671 | 2.5257 | C00156 | HMDB0000500 |
| Xanthosine | -2.5255 | 2.5257 | C01762 | HMDB0000299 |
| m-Hydroxybenzoic acid | -2.8056 | 2.5257 | C00587 | HMDB0002466 |
| DL-2-Aminoadipic acid | -3.1699 | 3.2117 | C00956 | HMDB0000510 |
| 3-Hydroxyphenylacetic acid | -3.1823 | 2.8092 | C05593 | HMDB0000440 |
| Arabinose-5-phosphate | -3.3687 | 4.5317 | C01112 | HMDB0011734 |

**Supplementary Table S3.** Significant metabolites identified from untargeted metabolomics using LC-QToF-MS.

| **Putatively Identified Metabolites** | **Mass Error (ppm)** | **log2 (FC)** | **-log10 (p-value)** | **KEGG ID** | **HMDB ID** | **Elevated in** |
| --- | --- | --- | --- | --- | --- | --- |
| L-Serine | -7.61 | 4.1948 | 4.3568 | C00065 | HMDB0000187 | SA01 |
| L-Leucine | 1.52 | 3.8207 | 1.3328 | C00123 | HMDB0000687 |
| L-Isoleucine | 1.52 | 3.8207 | 1.3328 | C00407 | HMDB0000172 |
| Arachidonic Acid | -10.83 | 3.8104 | 1.4151 | #N/A | #N/A |
| L-Alanine | 4.48 | 3.7569 | 1.976 | C00041 | HMDB0000161 |
| leukotriene-C4 | -3.19 | 3.1122 | 2.09 | C02166 | #N/A |
| N10-Formyltetrahydrofolic acid | -7.39 | 3.0032 | 3.2109 | #N/A | HMDB0000972 |
| 4-Hydroxybutanoic acid | 6.72 | 2.0549 | 2.6513 | C00989 | #N/A |
| 4-Hydroxyphenylpyruvic acid | -0.55 | 1.963 | 2.6513 | C01179 | #N/A |
| L-Dopa | -5.07 | 1.743 | 1.6468 | C00355 | HMDB0000181 |
| Thiophene | 3.56 | 1.4386 | 1.6468 | #N/A | HMDB0029718 |
| 5,10-Methylenetetrahydrofolate | -0.87 | 1.3491 | 1.7646 | C00143 | #N/A |
| L-Formylkynurenine | 16.90 | 1.2267 | 2.7231 | C02700 | HMDB0060485 |
| L-Tryptophan | 3.43 | 1.2154 | 1.361 | C00078 | HMDB0000929 |
| Nicotinamide adenine dinucleotide | 1.06 | 1.1301 | 1.3328 | C00003 | #N/A |
| D-Glutamine | 5.47 | 0.68765 | 2.6513 | C00819 | HMDB0003423 |
| L-threo-7,8-Dihydrobiopterin | 0.418 | 0.66428 | 1.3666 | C20263 | #N/A |
| 2,5-Dihydroxypyridine | 0.90 | -0.6266 | 2.6513 | C01059 | #N/A | VIC01 |
| N2-Acetyl-L-ornithine | 2.30 | -1.2455 | 2.6513 | C00437 | #N/A |
| Niacinamide | 1.53 | -1.5095 | 1.3666 | C00153 | HMDB0001406 |
| Nicotine imine | 7.61 | -1.5764 | 1.7116 | #N/A | HMDB0001010 |
| 2-Hydroxyphenylacetic acid | -0.29 | -1.6213 | 1.4151 | C05852 | HMDB0000669 |
| 2,3,6-Trihydroxypyridine | 2.36 | -1.6277 | 2.3912 | C03458 | #N/A |
| Quinic acid | -0.52 | -2.0971 | 2.0255 | C00296 | #N/A |
| 2-Oxoarginine | -24.51 | -2.2389 | 1.7726 | C03771 | HMDB0004225 |
| Tryptamine | 1.57 | -2.2553 | 1.5488 | C00398 | HMDB0000303 |
| 2-amino-tetradecanoic acid | 4.40 | -2.4455 | 3.4059 | #N/A | #N/A |
| enalaprilat (anhydrous) | 0.51 | -2.5619 | 1.6438 | C11720 | HMDB0041886 |
| trans-3-Hydroxycotinine glucuronide | 2.81 | -2.642 | 1.5187 | #N/A | HMDB0001204 |
| **Putatively Identified Metabolites** | **Mass Error (ppm)** | **log2 (FC)** | **-log10 (p-value)** | **KEGG ID** | **HMDB ID** | **Elevated in** |
| 2-amino tridecanoic acid | 3.58 | -2.7872 | 2.9742 | #N/A | #N/A | VIC01 |
| (Z)-5-Oxohex-2-enedioate | 0.00 | -3.3279 | 2.0398 | C03453 | #N/A |
| 2'-Deoxyuridine | 2.63 | -3.3498 | 2.0204 | C00526 | #N/A |
| Maleamic acid | -0.87 | -3.4589 | 3.1193 | C01596 | #N/A |
| 5-Hydroxy-L-tryptophan | 0.49 | -3.4709 | 3.1564 | C00643 | HMDB0000472 |
| N-Acetyl-L-glutamic acid | 1.59 | -3.5539 | 4.9153 | C00624 | #N/A |
| 5'-Butyrylphosphoinosine | -2.87 | -3.6568 | 4.2144 | C06435 | #N/A |
| 2-Keto-6-acetamidocaproate | 2.36 | -3.9522 | 3.2715 | C05548 | HMDB0012150 |
| 5-Hydroxyindoleacetylglycine | -0.03 | -4.0211 | 1.9645 | C05832 | HMDB0004185 |
| 4-(2-Aminophenyl)-2,4-dioxobutanoic acid | -0.97 | -4.0493 | 3.2687 | C01252 | #N/A |
| Thymidine | 0.94 | -4.3172 | 1.4488 | C00214 | HMDB0000273 |
| L-Erythrulose | 11.17 | -6.2344 | 2.1279 | C02045 | HMDB0006293 |

**Supplementary Table S4.** Significant lipids identified from untargeted lipidomics using LC-QToF-MS

| **Putatively Identified Lipids** | **Mass Error (ppm)** | **log2 (FC)** | **-log10 (p-value)** | **Elevated in** |
| --- | --- | --- | --- | --- |
| PE(13:0/18:3(6Z,9Z,12Z)) | -5.23 | 1.9716 | 3.3921 | SA01 |
| PE(14:0/22:2(13Z,16Z)) | -0.28 | 0.92763 | 1.4637 |
| PE(15:1(9Z)/22:4(7Z,10Z,13Z,16Z)) | -2.41 | 0.92124 | 1.35 |
| PE(12:0/18:2(9Z,12Z)) | -4.11 | 0.90112 | 1.35 |
| PE(12:0/17:0) | -6.41 | 0.76407 | 1.35 |
| PE(16:1(9Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z)) | 1.63 | -1.1496 | 1.35 | VIC01 |
| PE(12:0/20:4(5Z,8Z,11Z,14Z)) | -1.33 | -1.2432 | 1.35 |
| PE(18:4(6Z,9Z,12Z,15Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z)) | -0.65 | -1.3902 | 1.4541 |
| PE(14:0/20:5(5Z,8Z,11Z,14Z,17Z)) | 1.18 | -1.4124 | 1.4183 |
| PE(15:1(9Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z)) | 1.19 | -2.0691 | 1.7454 |



**Supplementary Figure S4** Boxplot representing the data of individual significant metabolites from the key metabolism changes in a SARS-CoV-2 ferret model infected with the SA01 isolate and VIC01 isolate during virus shedding. Normalized metabolite expression is determined as the mean ± the standard error.

**Supplementary Table S5:** Mutations (>20% Frequency) in VIC01 and SA01 isolates relative to Wuhan-Hu-1 (NC\_045512)

|  |  |  |  |
| --- | --- | --- | --- |
| Isolate | Mutation | Effect | Frequency (%) |
| VIC01 | T19065C | Silent | 99.81 |
|  | T22303G | S247R in Spike | 99.94 |
|  | G26144T | G251V in ORF3a | 99.83 |
|  | 29750Del (10nt) | 10nt Deletion in 3’ UTR | 76.51 |
| SA01 | C3037T | Silent | 99.41 |
|  | C17074T | L5604F in orf1ab | 99.94 |
|  | 26284Del | Loss of V14 in Envelope | 87.16 |
|  | C27213T | Silent | 97.88 |
|  | T27384C | Silent | 99.82 |