

## Supporting Information

### A novel immunoassay technique using principal component analysis for enhanced detection of emerging viral variants

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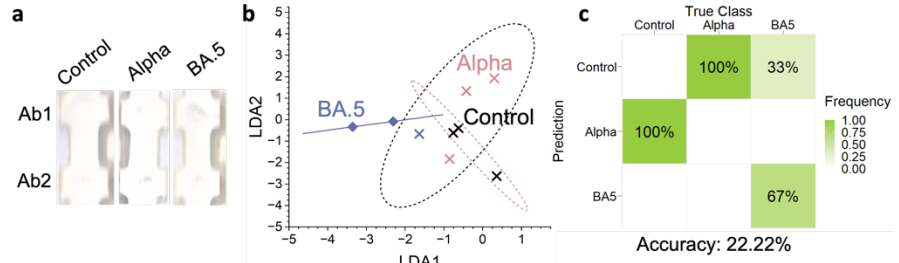
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Alpha	319	RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFK	378
BA.5	328	RVQPTESIVRFPNITNLCPFDEVFNATRFASVYAWNRKRISNCVADYSVLYNFAPFFAFK	387
BA.1	328	RVQPTESIVRFPNITNLCPFDEVFNATRFASVYAWNRKRISNCVADYSVLYNLAPFFTFK	387
Alpha	379	CYGVSP TKLNDLCFTNVYADSFVIRGDEV RQIAPGQTGKIADYNYKLPDDFTGCVIAWNS	438
BA.5	388	CYGVSP TKLNDLCFTNVYADSFVIRGNEVSQIAPGQTGNIADYNYKLPDDFTGCVIAWNS	447
BA.1	388	CYGVSP TKLNDLCFTNVYADSFVIRGDEV RQIAPGQTGNIADYNYKLPDDFTGCVIAWNS	447
Alpha	439	NNLDSKVG GNYNYLRYLFRKSNLKP FERDISTE IYQAGSTPCNGVEGFNCYFPLQSYGFQ	498
BA.5	448	NKLDSKVG GNYNYRYLFRKSNLKP FERDISTE IYQAGNKPCNGVAGVNCYFPLQSYGFR	507
BA.1	448	NKLDSKVS GNYNYLRYLFRKSNLKP FERDISTE IYQAGNKPCNGVAGFNCYFPLQSYSFR	507
Alpha	499	PTNGVGYQP YRWWVLSFELLHAPATVCGPKKSTNLVKNKCVNF	541
BA.5	508	PTYGVGHQP YRWWVLSFELLHAPATVCGPKKSTNLVKNKCVNF	550
BA.1	508	PTYGVGHQP YRWWVLSFELLHAPATVCGPKKSTNLVKNKCVNF	550

**Supporting Information Figure S1. Sequences for spike RBD protein of Alpha, Omicron BA.5, and Omicron BA.1 Similarities relative to alpha in highlighted in orange.**

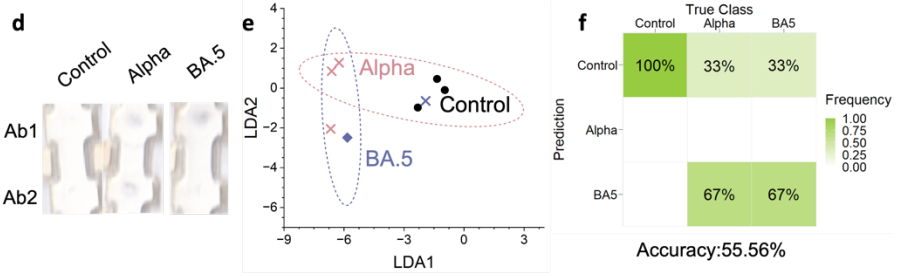
First Trial

1. Casein
2. HS, RB, GNS-Ab1, target
3. HS, RB, NP-Ab2 IgG
4. Casein



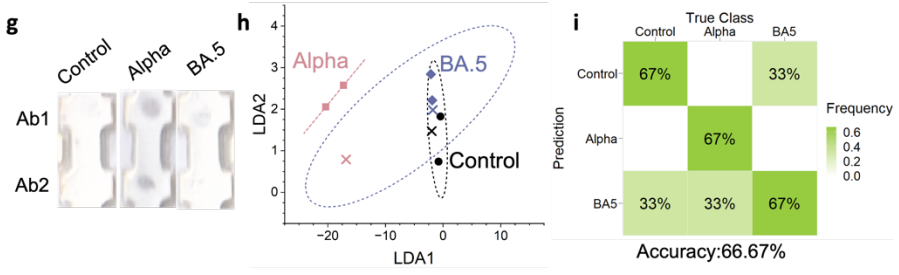
Second Trial

1. HS, RB, GNS-Ab1, Quencher, target
2. HS, RB, NP-Ab2 IgG
3. Casein



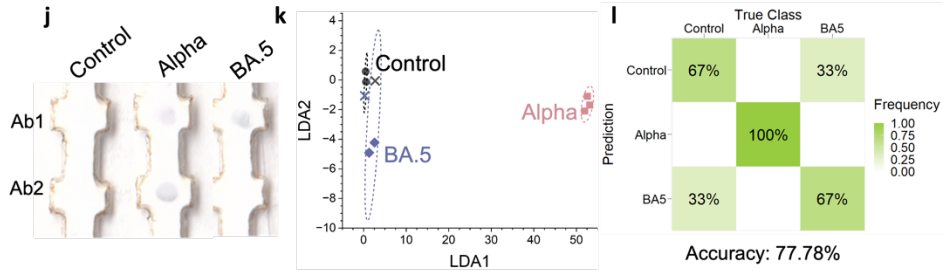
Third Trial

1. HS, RB, GNS-Ab1, Tris Buffer, target
2. HS, RB, NP-Ab2 IgG
3. Casein



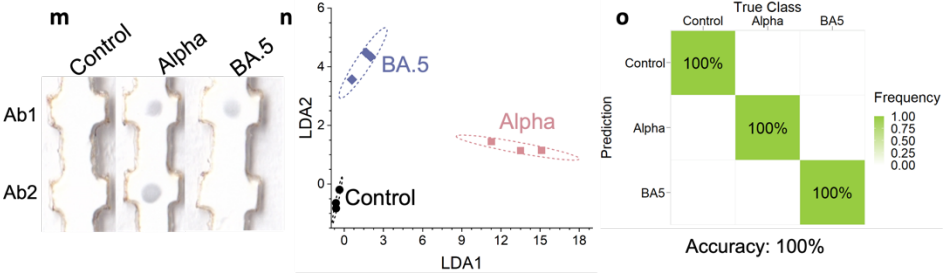
Fourth Trial

1. HS, RB, GNS-Ab1, Tris Buffer, target
2. Casein
3. HS, RB, NP-Ab2 IgG
4. Casein



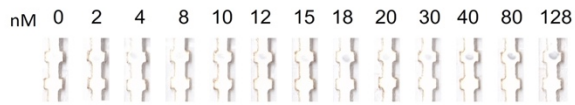
Fifth Trial

1. HS, RB, GNS-Ab1, Tris Buffer, target
2. Casein
3. HS, RB, NP-Ab2 IgG
4. Higher Volume- Casein



**Supporting Information Figure S2. ML trials used to iterate on different running conditions and resulting LDA and confusion matrix showing the test accuracy. For each trial it results in strip images for the strips run with the negative control, Alpha, and BA.5 (a, d, g, j, m); LDA plots of the resulting image analysis (b, e, h, k, n) where misclassified classes are shown as X's; and confusion matrices (c, f, l, o) where diagonals are correctly classified and off diagonal incorrectly classified classes.**

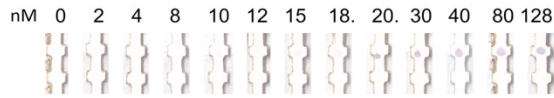
**a** GNS-Ab1/Ab1 run with alpha



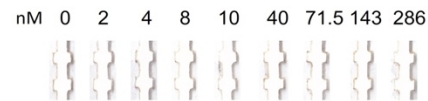
**b** GNS-Ab1/Ab1 run with BA.5



**c** NP-Ab2/Ab2 run with alpha



**d** NP-Ab2/Ab2 run with BA.5

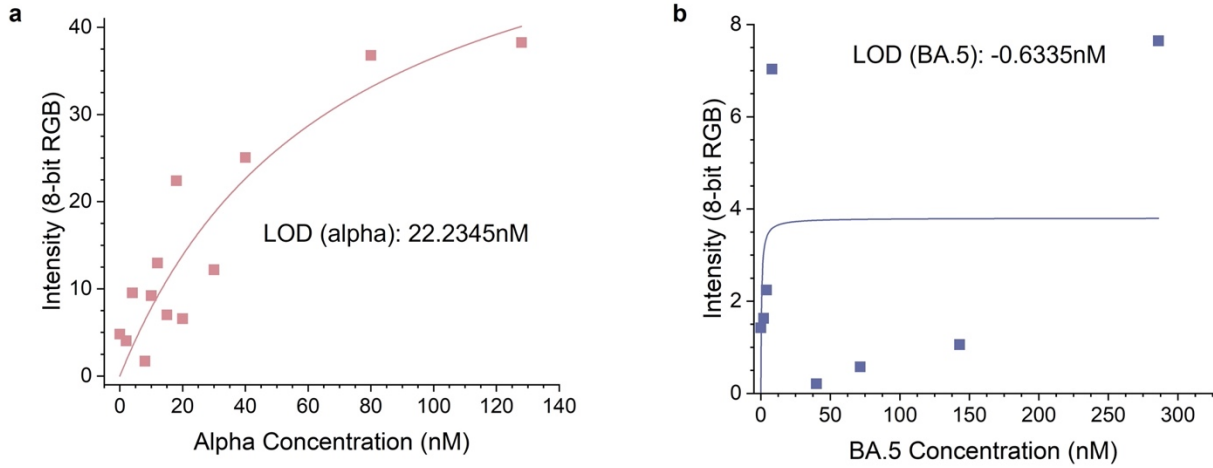


**e**

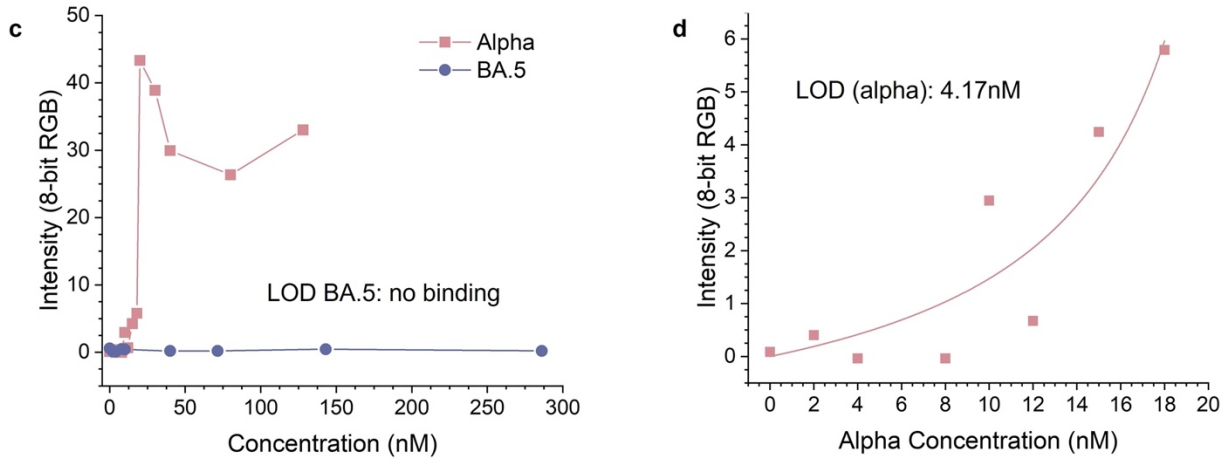
Concentration (nM)	GNS-Ab1/Ab1			NP-Ab2/Ab2		
	R	G	B	R	G	B
0	249.1	249.08	250.54	253.95	253.71	253.83
2	250.12	250.13	251.16	253.67	253.34	252.59
4	242.79	242.69	246.47	254.84	253.16	254
8	252.74	252.47	252.84	254.96	253.04	254
10	243.9	243.97	245.5	253.13	247.98	252.15
12	238.02	237.36	241.25	253.55	251.63	252.47
15	245.75	245.3	247.51	251.39	246.09	250.31
18	227.29	226.8	231.86	252.24	246.54	251.51
20	246.37	246.17	247.94	215.67	198.68	212.54
30	240.13	239.71	244.43	224.7	208.7	221.04
40	224.47	223.86	228.58	231.4	215.96	229.17
80	216.96	216.3	221.08	232.92	220.14	231.43
128	217.37	217.28	222.22	222.58	209.74	221.99
0	253.67	253.45	253.56	253.44	253.38	253.41
2	251.93	252.02	252.71	254.62	253.3	253.98
4	251.96	251.54	252.32	253.87	252.41	252.25
8	246.34	246.62	249.58	254.88	253.74	252.93
10	244.94	244.65	247.05	254.91	253.5	253.39
40	254.02	253.5	251.65	254.64	253.57	253.7
71.5	253.72	253.64	253.68	254.89	253.11	254.02
143	253.56	252.88	251.37	254.86	253.44	253.33
286	242.87	242.79	246.14	254.55	253.42	253.58

Supporting Information Figure S3. a-d) Strips run for LODs in SI Figure S7. Antigen concentrations in nM. e) RGB values for LOD strips.

**GNS-Ab1/Ab1**



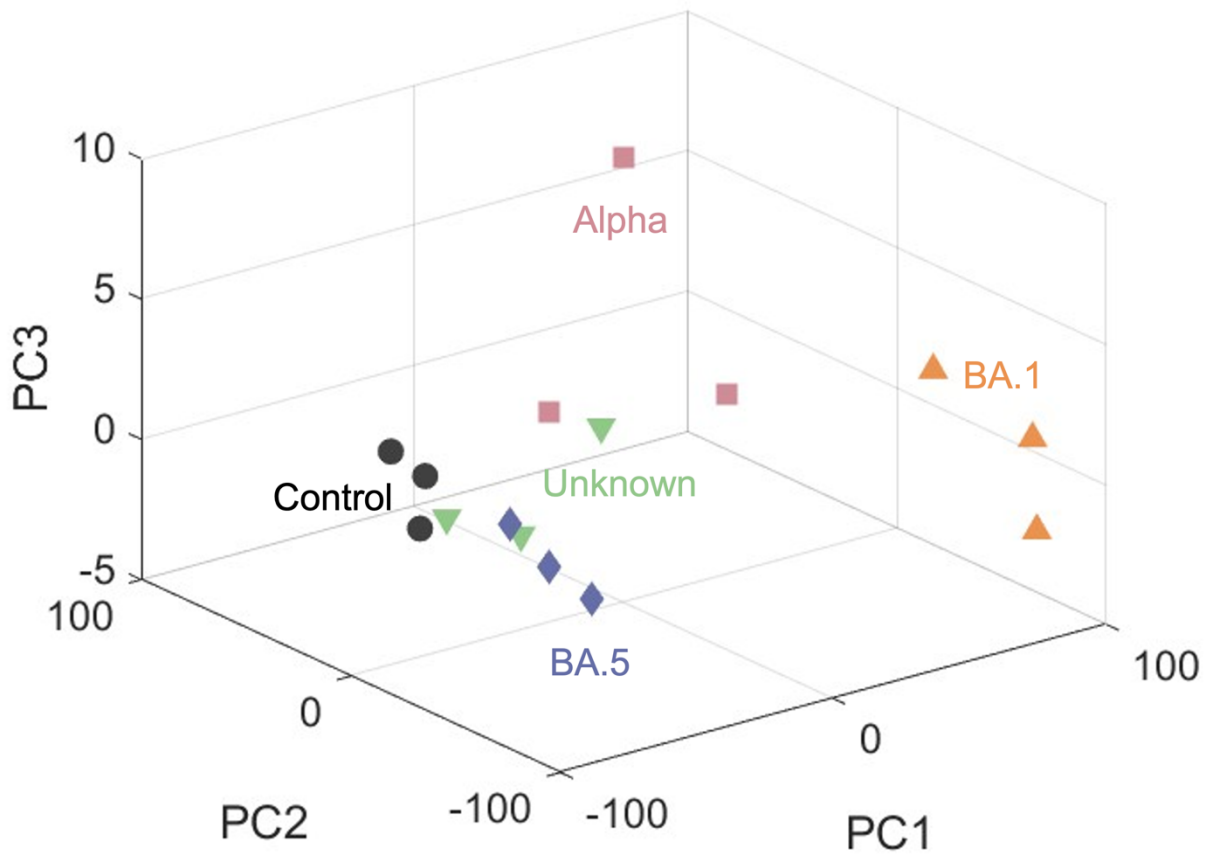
**NP-Ab2/Ab2**



**Supporting Information Figure S4. Concentration dependence of the strip signals for LOD calculations (points) and fit to a Langmuir equation (line):  $y = (k_1x)/(1+k_2x)$ . a) GNS-Ab1/Ab1 run with alpha; b) GNS-Ab1/Ab1 run with BA.5; c) NP-Ab2/Ab2 with alpha (pink squares) and BA.5 (blue circles); d) zoom in of non-hook region for NP-Ab2/Ab2 with alpha. Fits: For Ab1 with alpha,  $k_1 = 0.8927$  and  $k_2 = 0.0144$ . For Ab2 with alpha,  $k_1 = 0.0436$  and  $k_2 = -0.049$ . For BA.5, reliable Keff values could not be determined.**

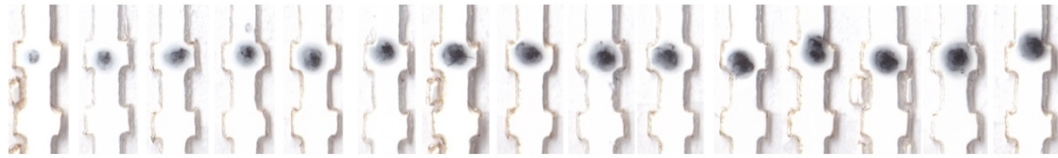


Supporting Information Figure S5. BA.1 unknowns run in triplicates.



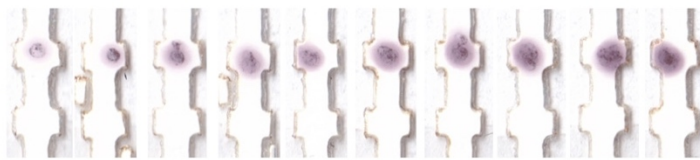
Supporting Information Figure S6. PCA in 3D of unknowns run (control, alpha, and BA.5, green triangles) and BA.1 (orange triangles).

**Blue GNS-Ab2**



Number of 0.2 $\mu$ L Spots	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
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**Red NP-Ab1**



Number of 0.2 $\mu$ L Spots	1	2	3	4	5	6	7	8	9	10
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**Supporting Information Figure S7. Spotted Blue GNS-Ab2 and Red NP-Ab1 in aliquots for measuring the range of possible RGB values.**