Supporting Information

An approach to use machine learning to optimize paper immunoassays for SARS-CoV-2 IgG and IgM antibodies

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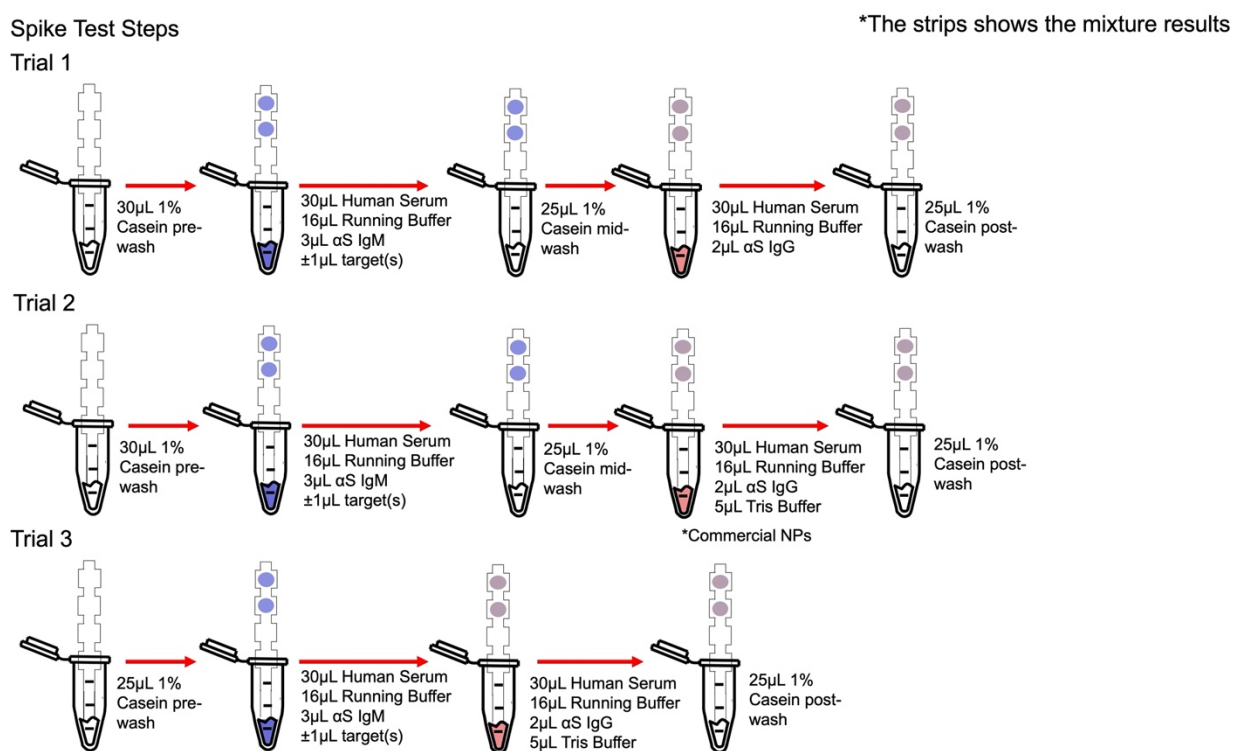[2]School for the Environment, University of Massachusetts Boston, Boston, MA 02125

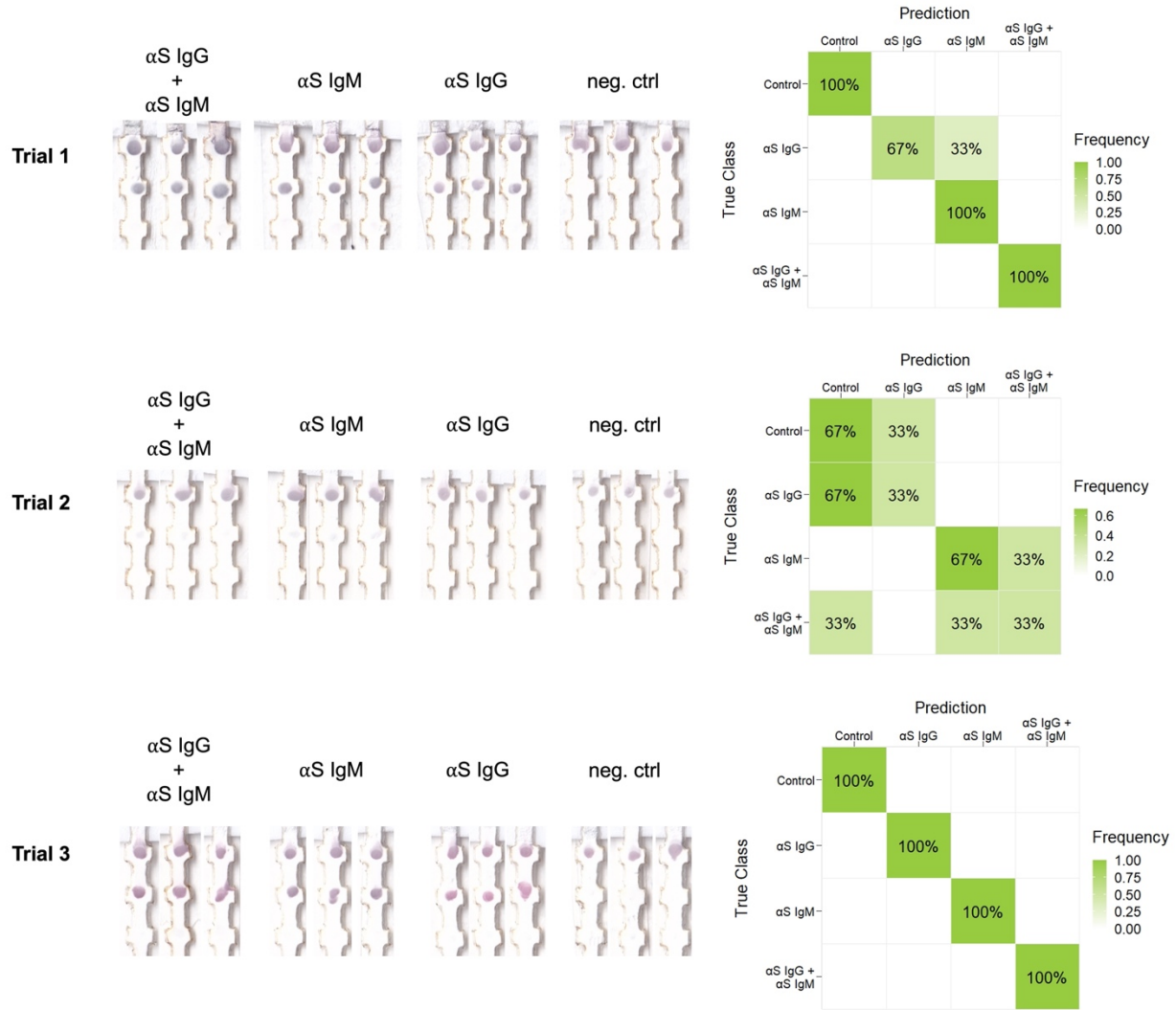**Figure S1. Workflow for 4X4, details on conditions for each trial.**

Figure S2. Triplicates of Trials 1-3 for the S IgG, S IgM assay, and corresponding 4x4 confusion matrices.
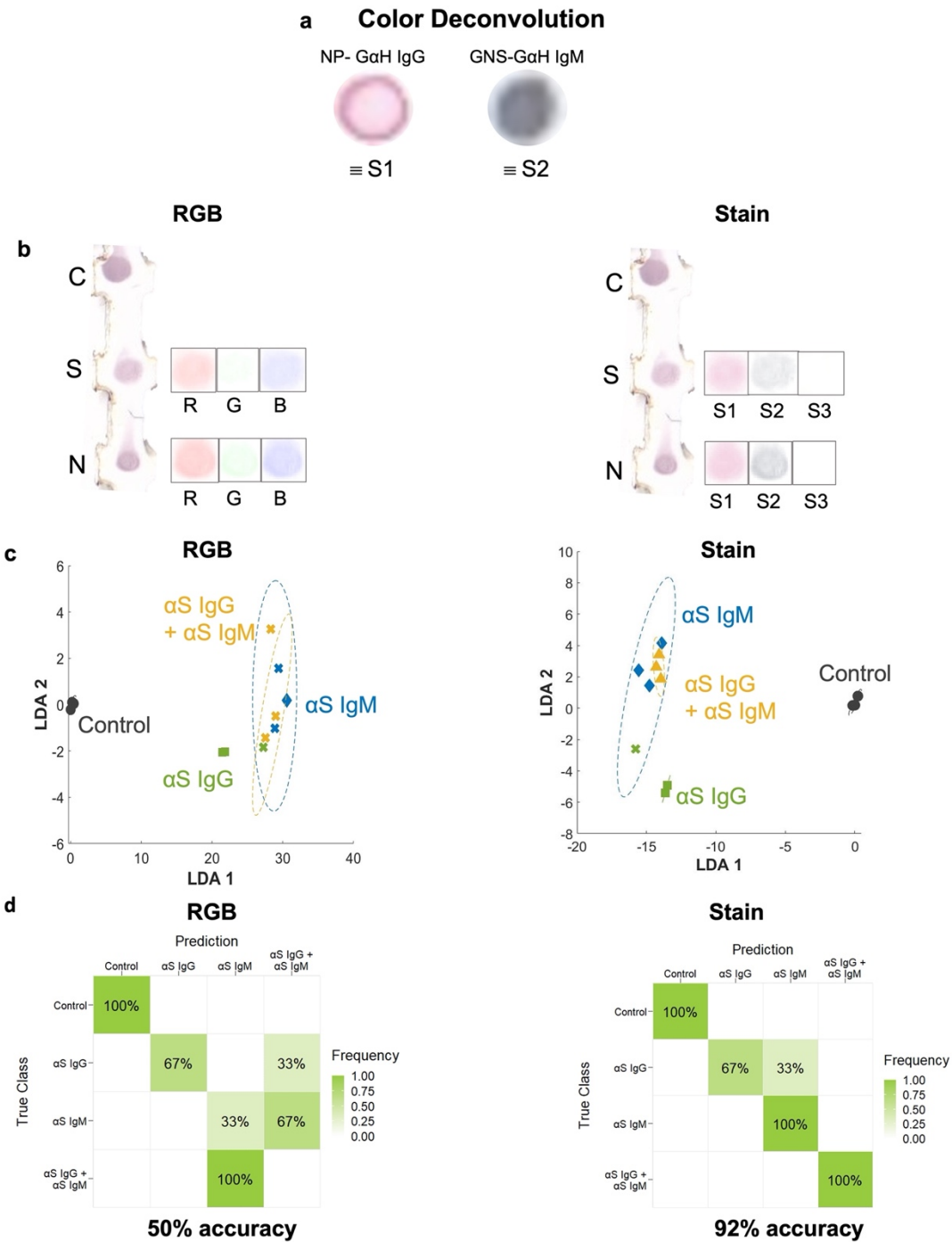
Figure S3. Stain deconvolution compared to RGB analysis for LDA. a) test lines where pure red nanospheres vs. blue gold nanostars. b) Deconvolution of test areas for a strip run with S IgG + S IgM by RGB analysis (left) vs stain vectors (right). c) LDA of the RGB (left) vs. stain vectors (right). d) The resulting confusion matrices for RGB analysis has an accuracy of 50% (left) vs. stain vectors for LDA which has an accuracy of 92% (right).
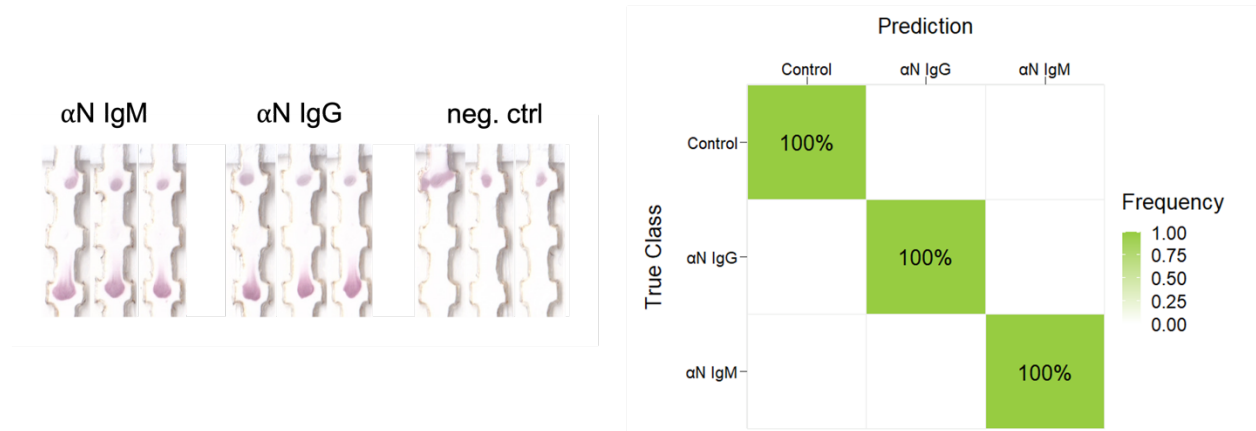
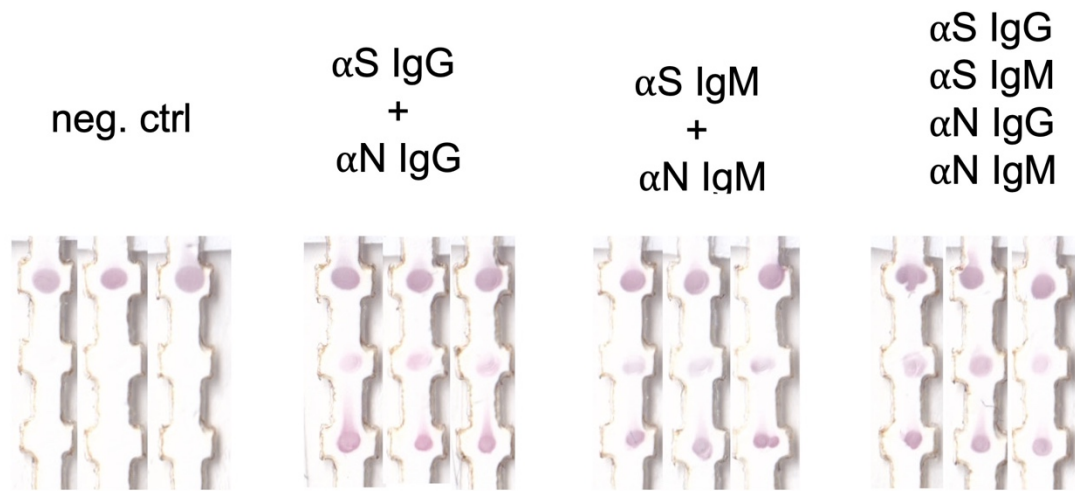**Figure S4. Triplicates of strips run with αN IgG, αN IgM, and negative control.**
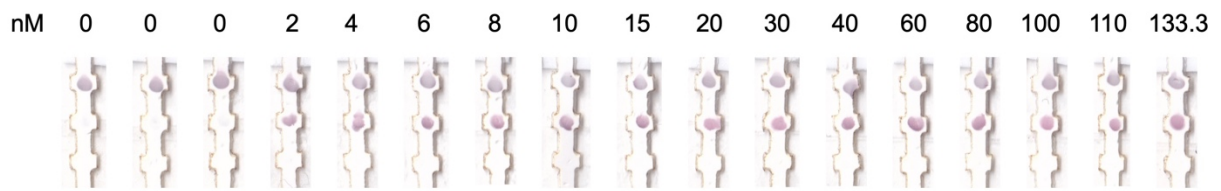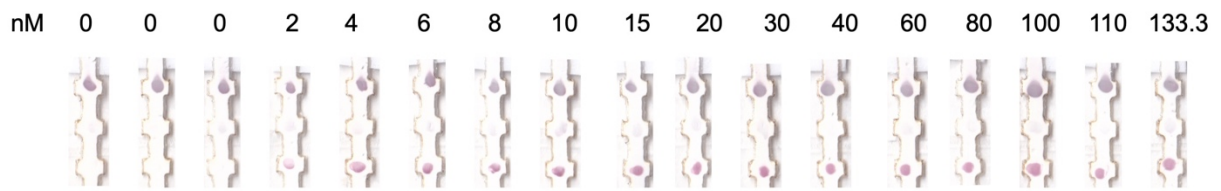
Figure S5. Triplicates for the remaining categories to make the 9X9: αS IgG + αN IgG, αS IgM + αN IgM, αS IgG + αS IgM + αN IgG +αN IgM. **Confusion matrix using RGB analysis vs. stain analysis.**
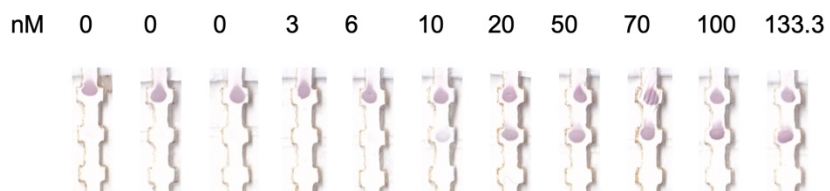
S IgG

nM 0 0 0 2 4 6 8 10 15 20 30 40 60 80 100 110 133.3

N IgG

nM 0 0 0 2 4 6 8 10 15 20 30 40 60 80 100 110 133.3

S IgM

nM 0 0 0 3 6 10 20 50 70 100 133.3

N IgM

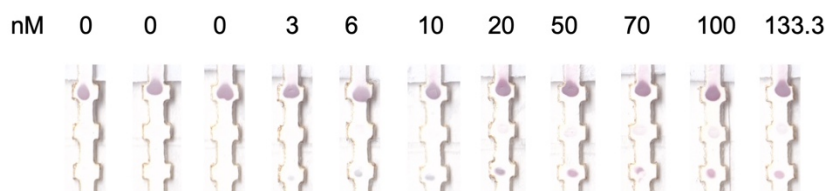nM 0 0 0 3 6 10 20 50 70 100 133.3

Figure S6. Strips run to calculate the LOD for αS IgG, αN IgG, αS IgM, αN IgM.
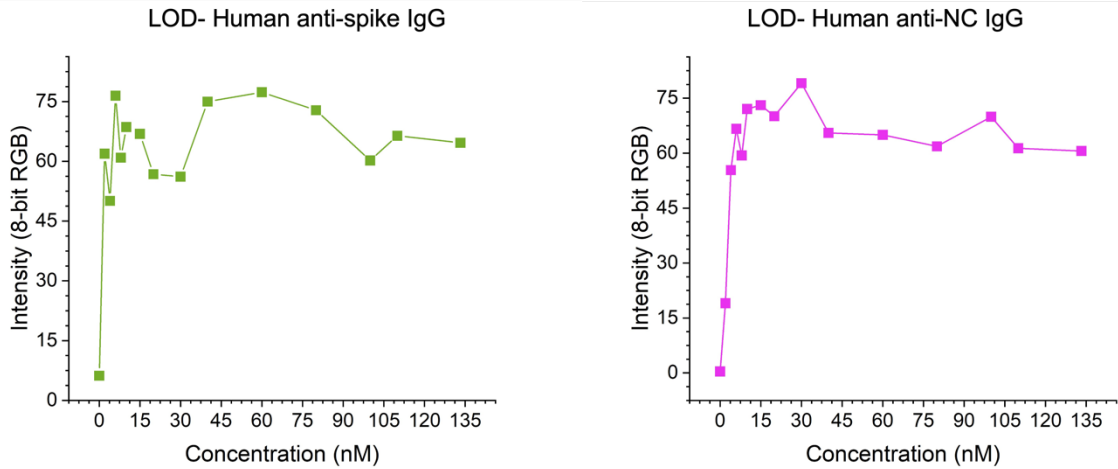
Figure S7. Dose response curves for the full concentration range of αS IgG and αN IgG, including the hook region.

Additional materials provided in Supporting Information: Matlab scripts used to analyze data for LDA.

Classification.m
Classifier.m