Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Porous Stamp-based Reagent Patterning for Lateral Flow Immunoassays

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I. Limitations of manual pipetting for patterning antibody on nitrocellulose

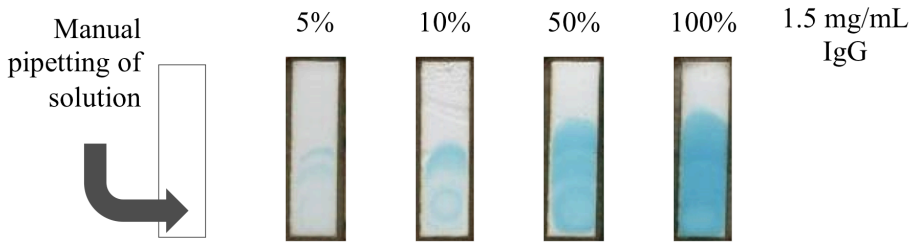
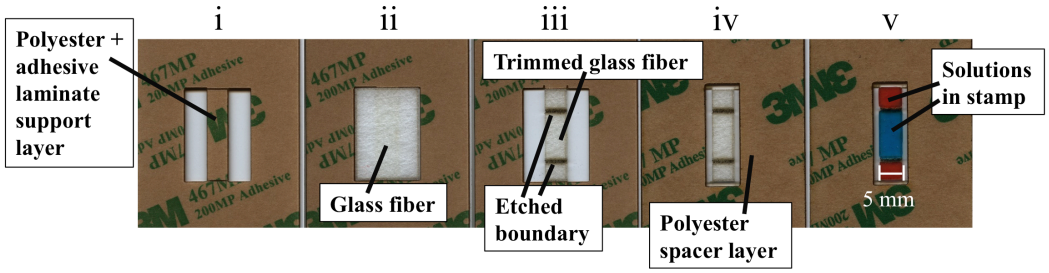


Figure S1. Manual pipetting of an antibody solution does not produce a uniform extended region of antibody on nitrocellulose. Although manual pipetting can be used to create small uniform circular patterns, the method does not enable the creation of larger rectilinear regions of antibody that have been found to be useful in assays (either a single extended region or multiple smaller regions). The four patterns resulted from manual pipetting of different dilutions of a stock 1.5 mg/mL IgG solution (the goat anti-mouse IgG described in the article), drying overnight in a desiccator, and subsequent protein staining. The volume of solution pipetted corresponded to the approximate pore volume of the nitrocellulose substrate. For all cases, note the non-uniform antibody patterns due to the high affinity of antibody for the nitrocellulose. Though solution traversed the length of the nitrocellulose strip, the solution was effectively depleted of antibody at the downstream side of the strip. This served as motivation for us to develop a cost-effective method for patterning rectilinear regions of antibody on nitrocellulose, without the substantial capital cost of a liquid dispensing system, as presented in the article.

II. Additional description of porous stamp fabrication



i. Cut the polyester/adhesive laminate using a CO_2 laser to produce the support layer for the stamp
ii. Apply a single piece of glass fiber to the exposed adhesive on the support layer of the stamp
iii. Remove glass fiber material using a CO_2 laser to define the separate pads within the stamp
iv. Apply a polyester spacer layer around glass fiber stamp
v. Apply solutions to appropriate pads of the stamp (blue and red dye used above)

Figure S2. Image series of the streamlined stamp fabrication process. (i) First, polyester/adhesive laminate is cut using a CO_2 laser to produce the support layer for the stamp. (ii) Next, a single piece of glass fiber is applied to the exposed adhesive on the support layer of the stamp. (iii) Select glass fiber material is removed using a CO_2 laser in order to define the separate pads within the stamp. (iv) Then, a polyester spacer layer is applied onto the area around the glass fiber stamp. (v) Finally, solutions are applied to appropriate pads of the stamp (blue and red dye used above) and the substrate may be stamped.

III. Additional description of label application

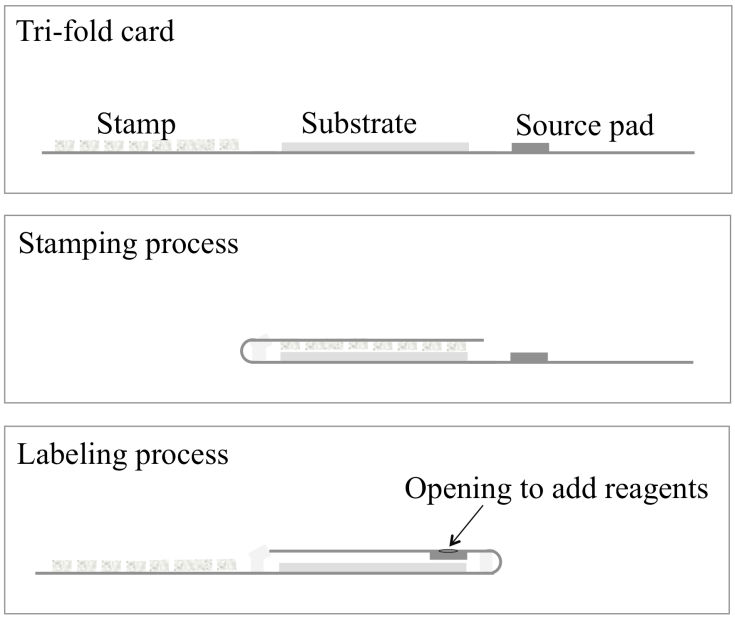


Figure S3. Schematic of a tri-fold card for stamping and subsequent label application. (Top) Side view of the open card. The left panel contains the stamp, the center panel contains the substrate (wicking pad is not shown), and the right panel contains the source pad. (Middle) During the stamping process, the left panel is folded over such that the stamp makes contact with the substrate. (Right) During the labeling process, the right panel is folded over such that the source pad makes contact with the downstream side of the substrate. (The left panel may be removed for easier handling.) An opening, located in the polyester/adhesive backing of the right panel and positioned above the source pad, enables additional reagent applications to the source pad.

IV. Image data demonstrating the requirement for adequate buffer regions in the porous stamp

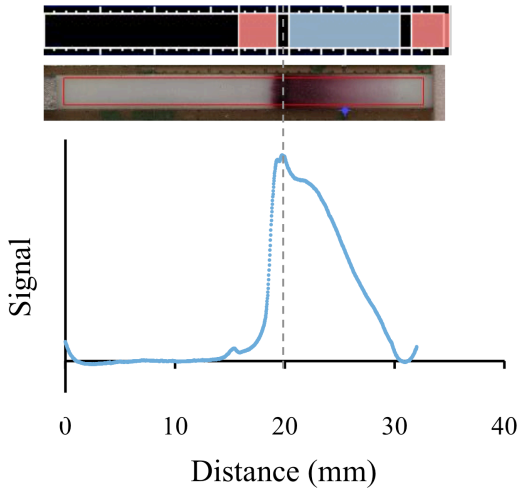


Figure S4. Buffer-filled pads of adequate volume are required within the stamp to restrict the lateral flow of the protein solution in the substrate. When the extended region stamp did not include the additional buffer used in the experiment of Figure 3, the antibody solution was wicked laterally to upstream regions of the nitrocellulose substrate. The result was a gradient in the protein distribution, and the presence of antibody in a region of the substrate not intended to contain protein, as shown in the plot above of the average signal profile over replicates ($N = 6$). Profiles have been shifted such that the average substrate background is zero. An image of a representative strip (signal data from the strip was extracted from the region outlined by the red rectangle) and an overlay of the multi-pad stamp on the substrate (from the DraftSight design file) are shown above the plot.

V. Full set of image data for different antibody concentrations visualized using staining

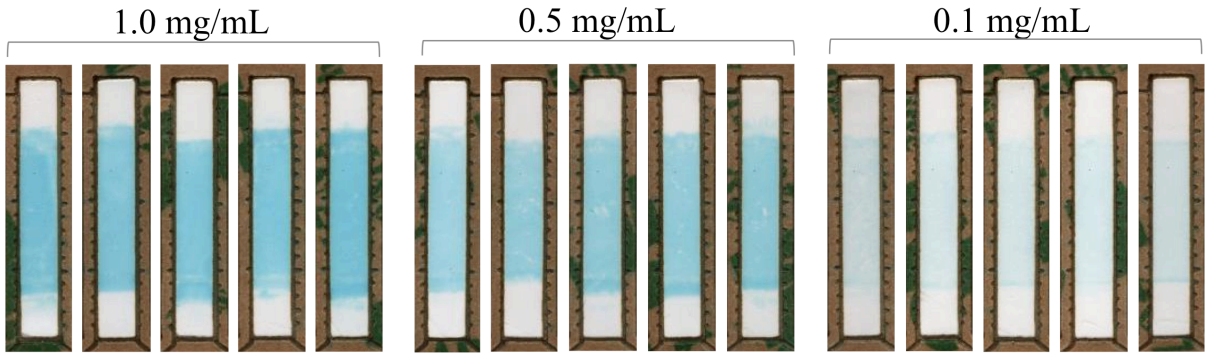


Figure S5. Images of strips patterned with three different concentrations of antibody. (Left) The protein solution consisted of an antibody at a concentration of 1 mg/mL. (Middle) The protein solution consisted of an antibody at a concentration of 0.5 mg/mL. (Right) The protein solution consisted of an antibody at a concentration of 0.1 mg/mL.

VI. Stamp reuse experiments

Stamp reuse was evaluated. The stamp was composed of two glass fiber pads, 5.5 mm in length and 5 mm wide, with a 1 mm space between the pads. One pad was filled with 0.75 mg/mL of unconjugated goat anti-mouse IgG antibody (~16 µL), and the other pad was filled with PBS (~16 µL). After stamping, the pads were refilled with either protein or PBS (3 µL), and the nitrocellulose substrate was replaced. The process was repeated three times, for a total of four uses per stamp. The protein-stamped nitrocellulose substrates were dried overnight, and visualized the next day following the Pierce™ Reversible Protein Stain Kit for Nitrocellulose Membranes protocol. After staining, the nitrocellulose substrates were allowed to dry for 15 min and were then scanned. On each substrate, the average grayscale intensity in a 2.4 mm × 4 mm region of interest (ROI) was extracted. The ROI was positioned using a fiducial mark located in the polyester backing at the midpoint of the nitrocellulose strip length. The intensity values were transformed to signal by subtracting each intensity value from the maximum intensity value possible in a 16-bit image, 65,536, and dividing by that value. The boundaries between the protein and no protein regions were also characterized by extracting profiles of the average grayscale intensity as a function of position along the length of the nitrocellulose substrates. Custom MATLAB (Natick, MA) code was used to complete the analysis.

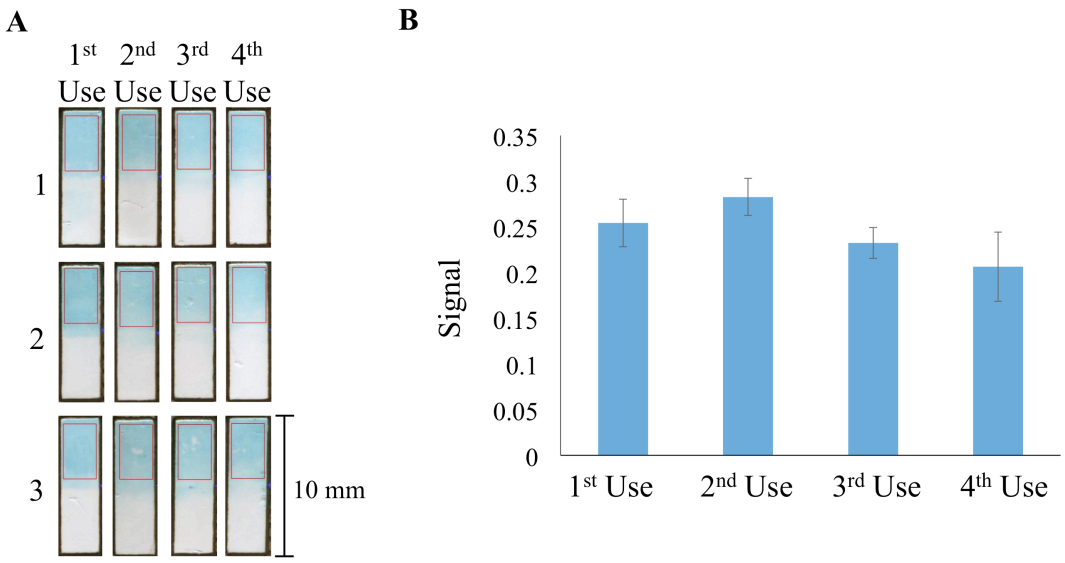


Figure S6. Protein patterns created using new and reused stamps. (A) Image series of protein-stamped nitrocellulose (3 mm × 10 mm). The first column of images corresponds to patterns created using new stamps. Each adjacent column of images, moving from left to right, depicts patterns created from reused stamps, with the number of times used indicated by the label at the top of the column. Each of the three stamps was used a total of four times. The average grayscale intensity was extracted from the region of interest outlined by the red rectangle (2.4 mm × 4 mm) for each substrate. (B) The average signal and standard deviation across replicates ($N = 3$) for the protein patterns created from new and reused stamps. The pairwise % difference in signals among replicates ($N = 3$) of the first time

used stamp ranged between 4% and 21%. By comparison, the % difference in average signals for successive stamp reuses ranged between 11% and 18%.

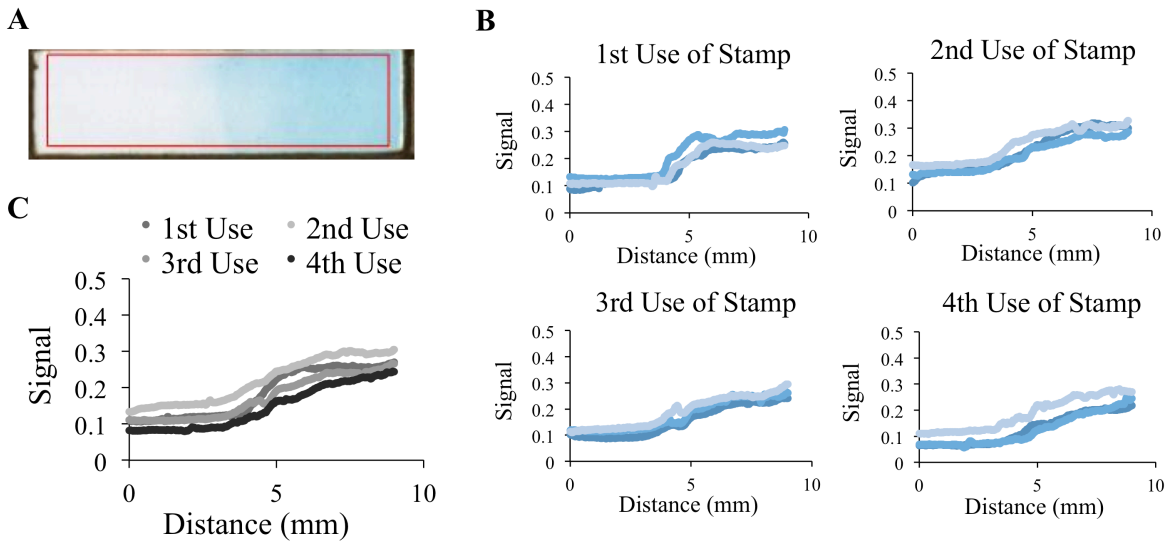


Figure S7. Signal profiles across regions with and without protein created using new and reused stamps. (A) The image shows a typical stamped protein pattern after staining. The red rectangular region was used to extract the average grayscale intensity versus distance along the length of the substrate. (B) The four plots show profiles of signal versus distance along the length of the nitrocellulose substrate for patterns created using new and reused stamps. The three profiles on each plot correspond to replicates. (C) The plot shows average signal profiles across replicates for patterns created with new and reused stamps.

VII. Full set of image data for immunoassay demonstration

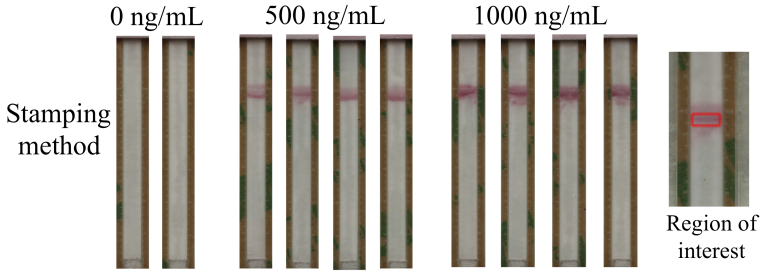


Figure S8. Images of replicate data for the immunoassay demonstration using stamping to pattern the capture antibody on nitrocellulose. The analyte concentrations were 0 ng/mL, 500 ng/mL, and 1000 ng/mL. The image on the far right shows the region of interest in one of the strips. Intensity values were extracted using a custom MATLAB program as described in Materials and Methods.

VIII. Comparison of immunoassay signal magnitude using the stamping method vs. using manual pipetting

For comparison, the immunoassay was implemented using substrates in which the anti-mouse capture antibody was applied directly onto the nitrocellulose using a micropipette. In this procedure, 0.75 μL of 1.5 mg/mL of anti-mouse IgG antibody was pipetted in the same location as the stamped capture antibody. Further processing of the nitrocellulose was the same as for the stamped capture antibody cases. Four replicates were performed for each non-zero analyte concentration and two replicates for the zero concentration.

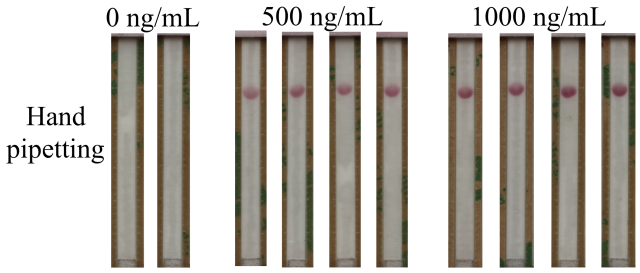


Figure S9. Images of replicate data for the immunoassay demonstration based on manual pipetting to pattern the capture antibody on nitrocellulose. The analyte concentrations were 0 ng/mL, 500 ng/mL, and 1000 ng/mL.

For the side-by-side immunoassay signal comparison using the two patterning methods, ImageJ (W. Rasband, NIH) was used to extract the green channel intensity from the image data. Regions of interest (ROIs) of 2.3 mm^2, were located about the most intense region of signal (also using fiducial marks on the test card as a guide). A rectangular ROI was used for the immunoassay data based on the stamping method, while an oval ROI was used for the immunoassay data based on the manual-pipetting method. These choices were motivated by the shape of label distribution in the detection region in each case. The average signal was calculated as described in Materials and Methods. For each of the two non-zero analyte concentrations, the average signal in the immunoassay based on stamping the capture antibody was comparable to the average signal in the immunoassay based on manually pipetting the capture antibody, with the two differing by less than 5%.

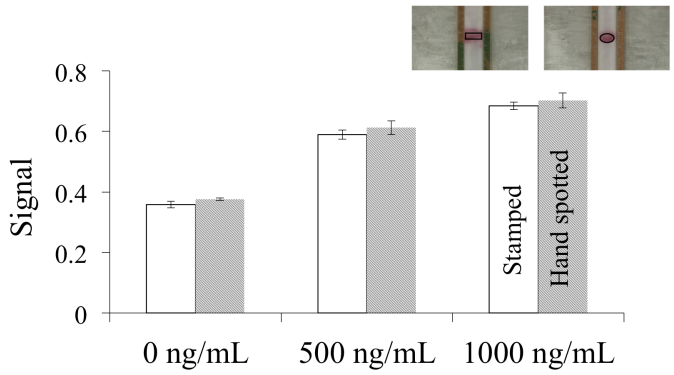


Figure S10. Comparison of the signals from the immunoassay demonstrations, in which the stamping and the manual-pipetting methods were used to pattern capture antibodies onto nitrocellulose substrates. The bar chart shows signal vs. analyte concentration for stamping (white) and manual pipetting (gray). The inset images illustrate representative rectangular and oval regions of interest (of the same area) for the stamping and manual-pipetting methods, respectively, in 1000 ng/mL strips. The error bars represent the standard deviation ($N = 4$ for the non-zero analyte concentrations and $N = 2$ for the zero concentration). Note that the signals are comparable between the stamping and the manual-pipetting methods (to within 5%). The manual-pipetting method, capable of achieving high concentrations of capture antibody within nitrocellulose, serves as a gold-standard method of comparison for the stamping method in this respect.