Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Material for:

Fast Immunoassay for Microfluidic Western Blotting by Direct Deposition of Reagents onto Capture Membrane

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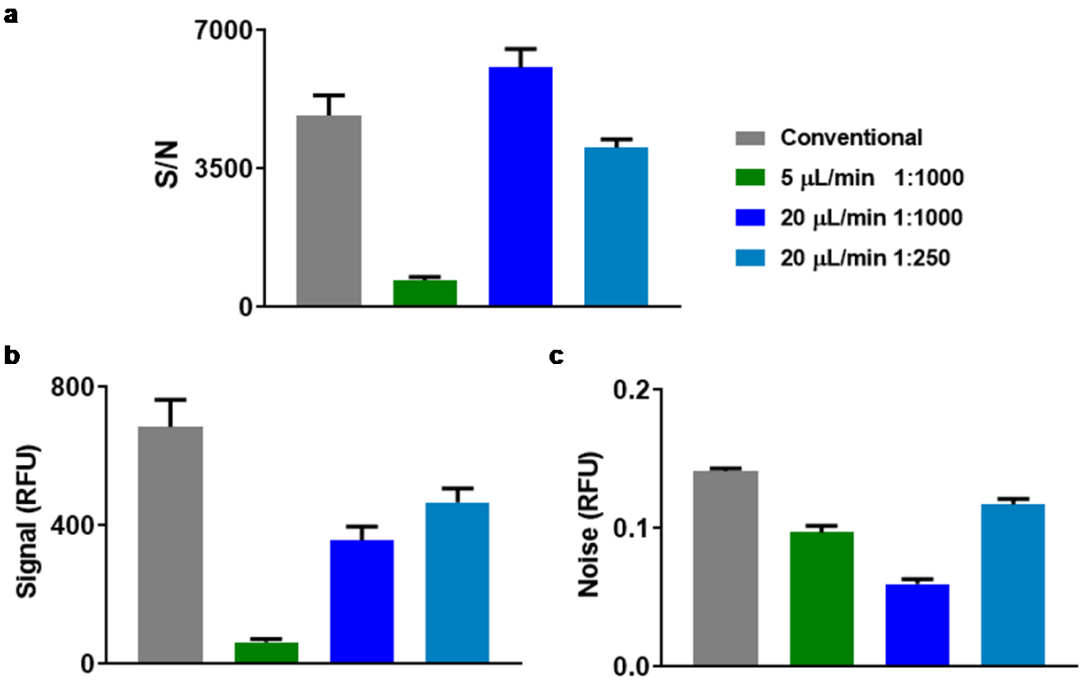

Figure S1. Investigation of primary antibody deposition parameters where all steps followed the microscale immunoassay method except for secondary antibody incubation which was applied with a traditional incubation step. Investigation of antibody deposition indicated that flow rates from 5-20 µL/min with stage speeds from 2-8 mm/min were adequate for completely saturating the width of a deposited protein trace. Primary antibody deposition was investigated between parameters of 5 and 20 µL/min, and the results shown here were at the selected stage speed of 4 mm/min for 50 µg/mL actin dot blotted with n=4 protein spots. The S/N (a), signal (b), and noise (c) values calculated and plotted are compared for selection a of deposition parameters that give the most comparable result to the traditional immunoassay. As shown, increasing the concentration of the primary antibody does not improve the S/N ratio within the selected deposition parameters.

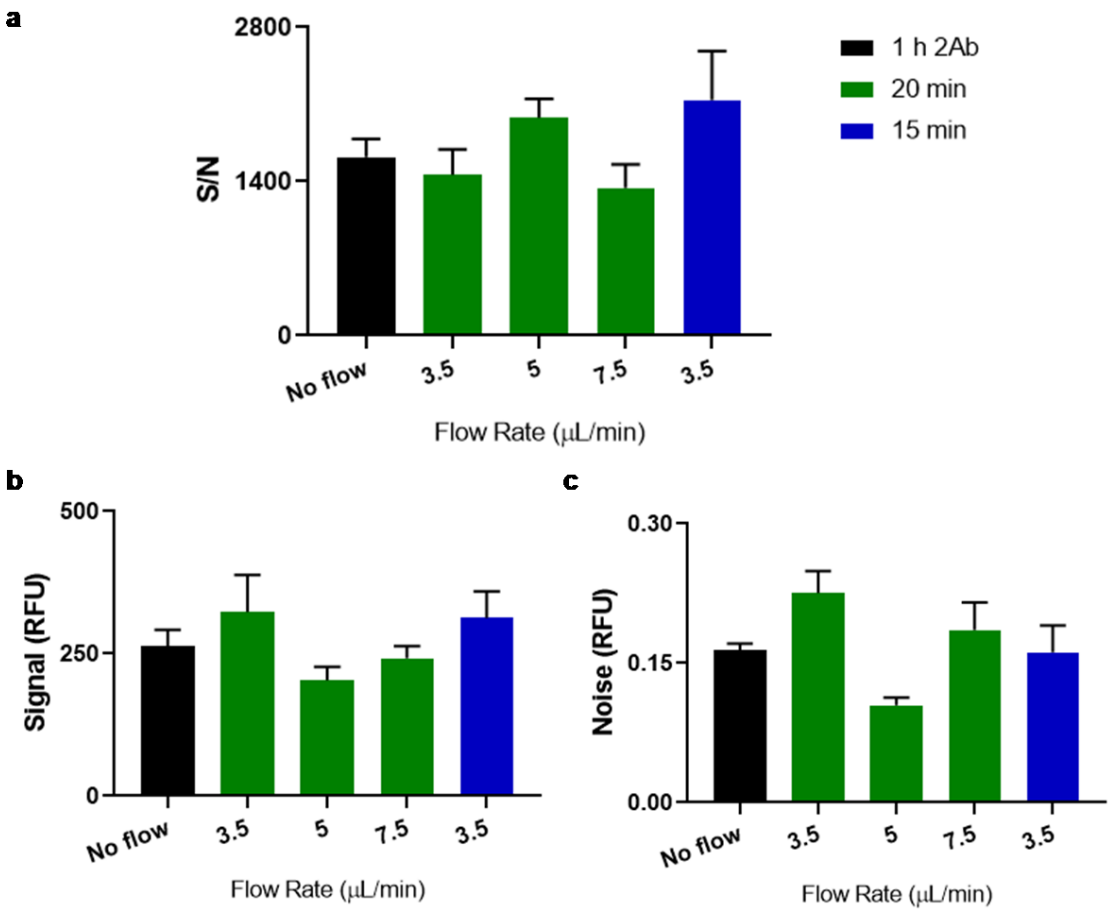

Figure S2. Investigation of secondary antibody deposition parameters where all steps are applied via the microscale, fast immunoassay method. Detection of actin 50 µg/mL dot blotted with n=4 protein spots was investigated with flow rates ranging from 3.5-7.5 µL/min with a fast stage speed of 70 mm/min that repeated movement along the x-axis. This deposition approach produced better binding with acceptable background and noise levels. The S/N (a), signal (b), and noise (c) are compared with a modified, microscale 2 h immunoassay that had previously been determined to give comparable results to a traditional immunoassay.

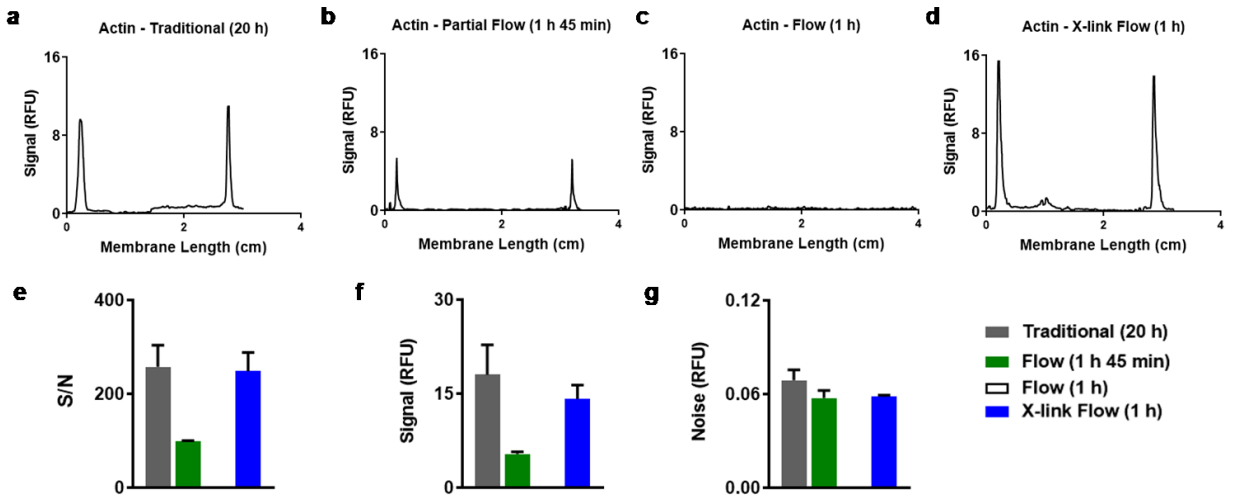

Figure S3. Comparison of a traditional immunoassay (a) with initial protein loss from non-crosslinked, partial flow immunoassay method (b), a non-crosslinked flow immunoassay (c), and a cross-linked flow immunoassay (d) for microchip Western blotting of 3.3 µg/mL actin. The S/N (e), signal (f), and noise (g) values calculated and plotted with n=3 injected and separated protein peaks.

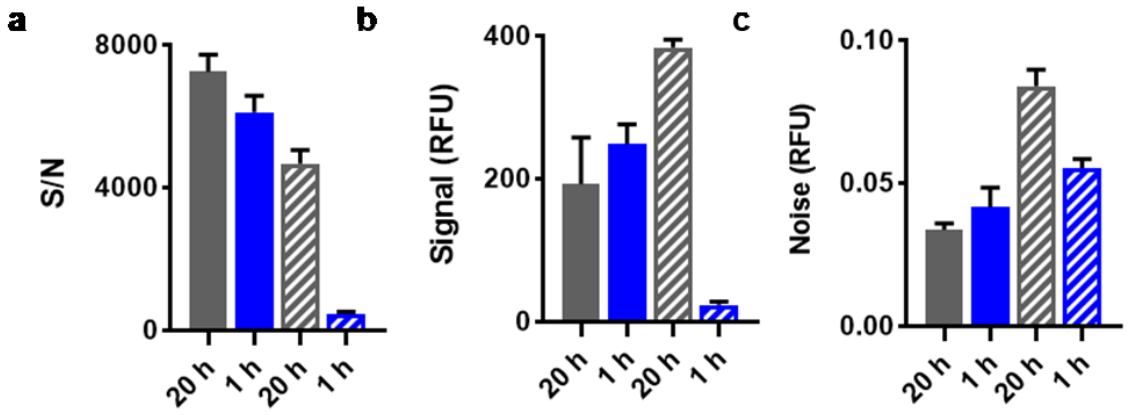

Figure S4. Comparison of dot blots of actin diluted in water (solid) versus dot blots of actin diluted in electrophoresis gel (hatched) with 3.3 µg/mL actin. The S/N (a), signal (b), and noise (c) values calculated and plotted demonstrating the effect on protein binding and protein loss seen with the non-crosslinked fast immunoassay.