Paper-based RNA extraction, *in situ* isothermal amplification, and lateral flow detection for low-cost, rapid diagnosis of Influenza A (H1N1) from clinical specimens

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SUPPORTING INFORMATION

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Table S1: Primer sequences

RT-PCR	**Fwd** **Rev** **Probe**	**5' - gtgctataaacaccagcctycca** **5' - cgggatattccttaatcctgtrgc** **5'NED - cagaatatacatccRGtcacaattgga -MGB**
RT-LAMP	**F3** **B3** **FIP** **BIP** **LF** **LB**	**5' - gctaagagagcaattgagc** **5' - atgtaggatttgctgagct** **5' - cgagtcatgattgggccatgacagtgtcatcatttgaaaggttt** **5' - aaggtgtaacggcagcatgtccgaatttccttttttaactagccat** **5'FAM-acttgtcttggggaatatctc** **5'Biotin-atgctggagcaaaaagct**

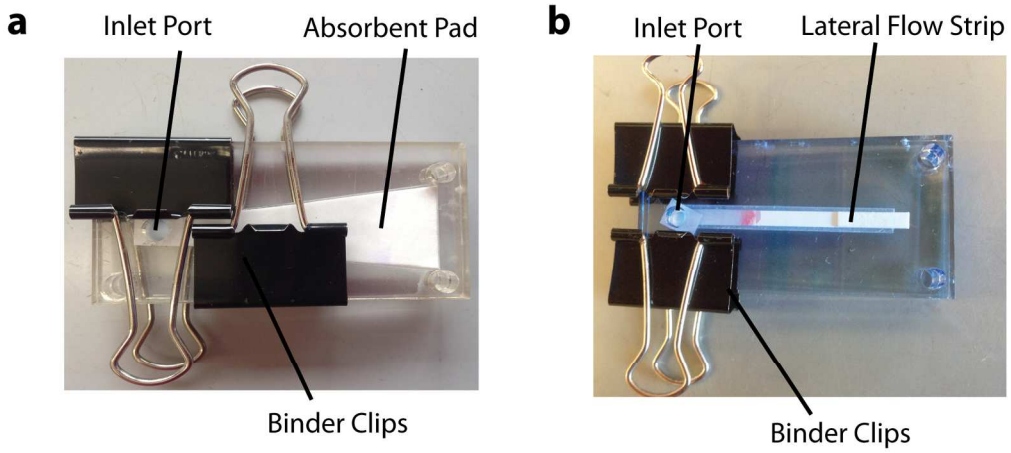

Figure S1: a) Extraction setup. b) LFD elution setup

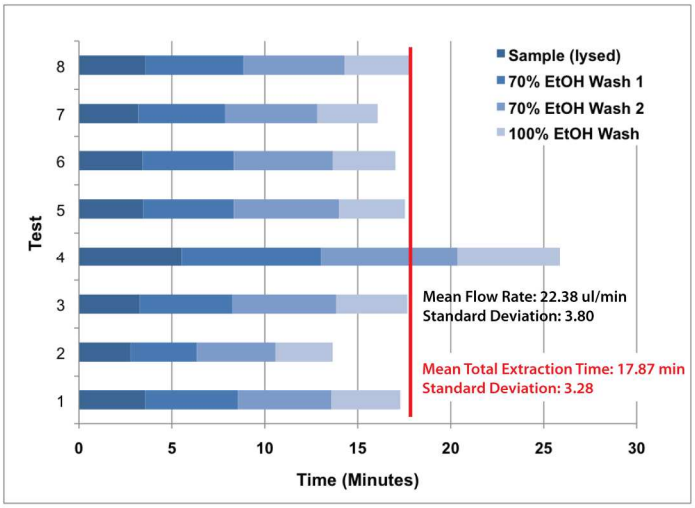

Figure S2: Flow rates and times for extraction. Mean flow rate = 22.38 µl/min, SD = 3.8. Mean total extraction time = 17.87 min, SD = 3.28 (n=8).

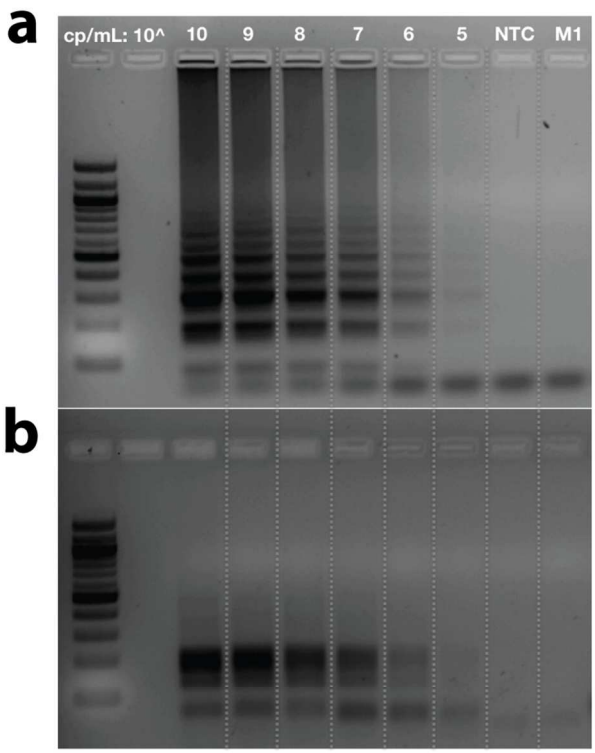

Figure S3: RT-LAMP product restriction enzyme digest. a) 2% Agarose gel electrophoresis of RT-LAMP products. First Lane = 100bp DNA ladder, 10 = 10^10 cp/mL, 9 = 10^9 cp/mL, etc. NTC = no template control. M1 = M1 gene in-vitro transcribed standards, 10^10 cp/mL. b) HindIII digestion of RT-LAMP products

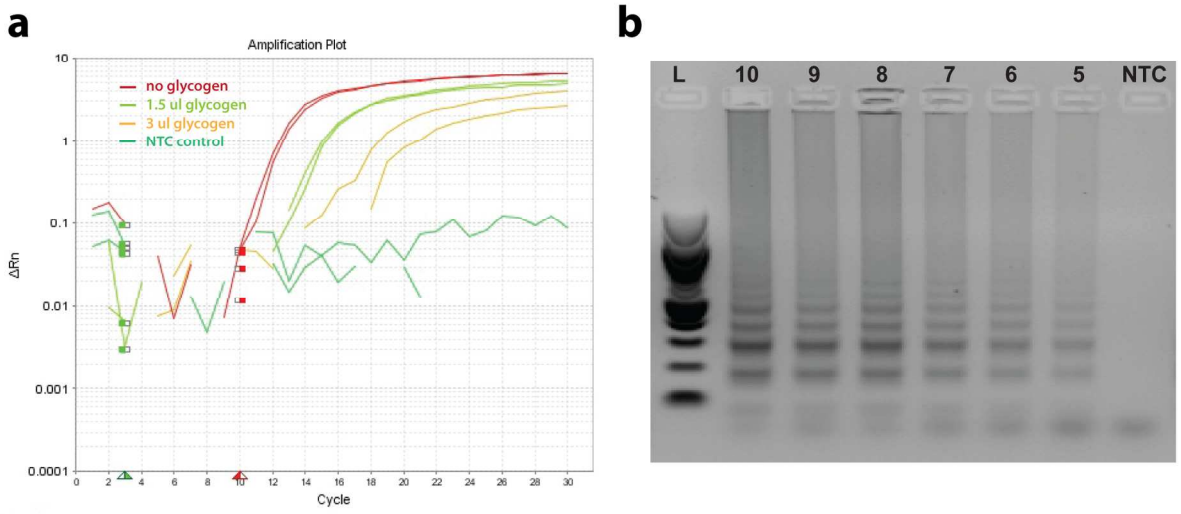

Figure S4: Effects of glycogen on RT-LAMP reaction. a) Real-time RT-LAMP amplification with increasing amounts of Glycoblue causing greater delays in amplification. b) Agarose gel electrophoresis of paper extracted RNA + RT-LAMP *in situ* products for 23 min at 65 °C. L= 100bp DNA ladder, 10 = 10^10 cp/mL, 9 = 10^9 cp/mL, etc. NTC = no template control.

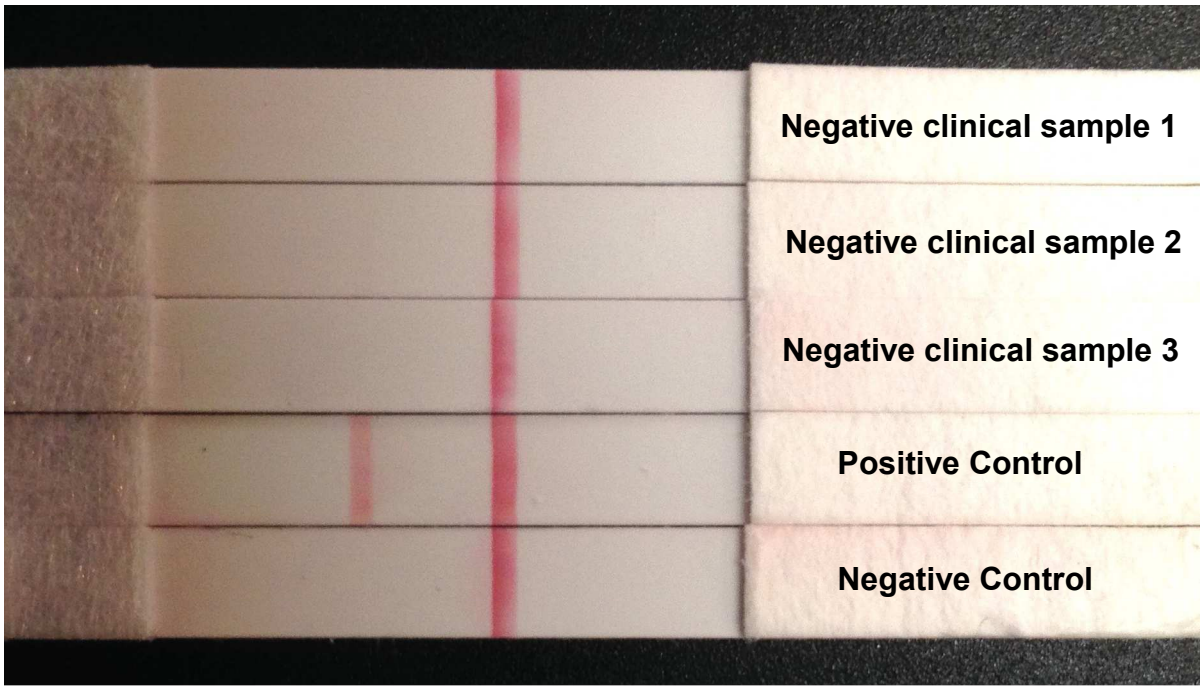

Figure S5: Lateral flow detection strips from negative clinical samples that were PES-extracted and *in situ* RT-LAMP amplified. Three known H1N1-negative clinical samples from patients exhibiting symptoms of respiratory illness at the time of specimen collection were chosen at random. Prior laboratory testing indicated that sample 1 was Influenza B-positive, and samples 2 and 3 were Respiratory Syncytial Virus (RSV)-positive. None were detected by our assay, demonstrating our H1N1 strain-specificity. 10^9 cp/mL H1N1 RNA and no RNA samples were run alongside the samples as positive and negative controls.