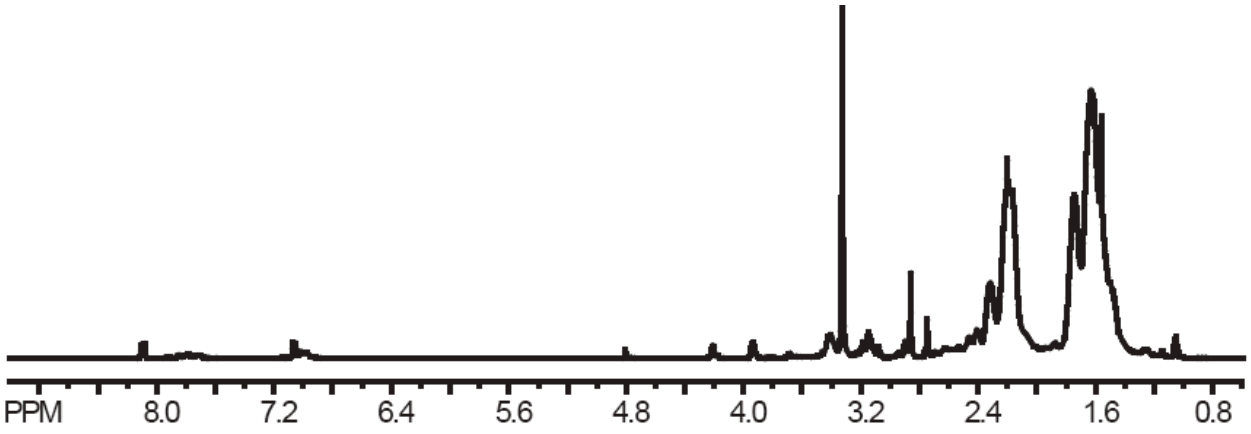
Supporting Information for "Antigen detection using polymerization-based amplification"

Macroinitiator synthesis and characterization:

Coupling of Irgacure 2959 initiators (Ciba) and flu antibodies (Inverness Medical-Biostar) to poly(acrylic acid co-acrylamide) backbone polymers (200,000 MW, Aldrich) was accomplished using a similar strategy as previously described (Nature Materials 2008, Vol. 7, 52-56) with alterations as necessitated by the differences between avidin (the previous molecular recognition element) and the viral type-specific flu antibodies. The concentration of the coupling reagent EDC was decreased by 15% to prevent gelation. In addition, the coupling of initiators and the coupling of antibodies were done in sequential steps to allow estimation of the number of antibodies per macroinitiator. With avidin, HABA assays were used for this purpose. Since an analogous assay for these antibodies does not exist, absorbance of the product at 280 nm and an extinction coefficient empirically determined from a standard curve of unconjugated antibodies were used (220,000 $M^{-1}cm^{-1}$ in 0.1 M MES, 0.5 M NaCl buffer, pH 6). Since the initiator also absorbs at this wavelength, its extinction coefficient at 280 nm was empirically determined (11,900 $M^{-1}cm^{-1}$ in 0.1 M MES, 0.5 M NaCl buffer, pH 6). The extinction coefficient of the initiator at 300 nm (7100 $M^{-1}cm^{-1}$ in 0.1 M MES, 0.5 M NaCl buffer, pH 6) is useful since absorbance at this wavelength by the antibodies was negligible compared to absorbance by the initiators. The couplings were done sequentially and UV absorbance measurements were made after each step. Unconjugated antibodies were separated from antibodies coupled to the macroinitiator using a 300,000 MWCO filter.

Product	A(280)	A(300)
Initator + backbone 100 μg/ml	0.8	0.5
Initator + backbone 160 μg/ml	1.2	0.7
Antibody + initator + backbone 100 μg/ml	0.6	0.3
Antibody + initator + backbone 160 μg/ml	1.0	0.5

Macroinitiator NMR spectrum:



1H NMR spectra were collected using samples comprised of 20 mg of the macroinitiator dissolved in 0.7 ml of deuterium oxide. The water peak was pre-saturated for collection of the spectra.

Flu assay protocol:

Biostar OIA Flu A/B tests were performed as directed in the package insert. Briefly, extraction of nucleoproteins was accomplished by incubation of an analyte solution containing the virus (influenza A/Hong Kong/68 and/or influenza B/Panama45/90) with the sample diluent and one drop of Reagent 1 (a pH 7 buffered solution containing a surfactant and a reducing agent) for three minutes. For standard enzymatic detection, one drop of Reagent 2 (flu antibody-horseradish peroxidase conjugates) was added to the analyte solution, and two drops of the combined solutions were placed on each test surface. After 6 minutes, the surfaces were washed with the wash buffer provided in the kit. The surfaces were developed via a 6-minute incubation with the substrate solution (a pH 5.5 aqueous solution of tetramethylbenzidine (TMB) and hydrogen peroxide). Following a thorough wash and blotting, surfaces were inspected for a color change from gold to blue. For detection using polymerization-based amplification, the extraction step was as described above. In the above protocol, Reagent 2 was replaced by a 50 ml of a 10 mg/ml solution of a macrophotoinitiator in pH 7.2 phosphate buffered saline. Two drops of the combined solutions were placed in contact with each test surface for 6 minutes. After washing with the wash buffer included in the Biostar OIA Flu A/B test kit, 50 ml of an argon-purged monomer solution (97% by weight hydroxyethyl acrylate and 3% by weight ethyleneglycol dimethacrylate crosslinker, each triply distilled to remove inhibitors) was pipetted over the entire test surface. Polymerization from spots containing captured flu nucleoproteins and bound macrophotoinitiator was accomplished using a 12 minute dose of 5 mW/cm^2 UV light centered around 365 nm from a Blak-Ray B Series-100A lamp.