Supplementary Information:

Methods: Visualization of ATPS with a Triggered Well

For these visualization experiments, a 10 mm in diameter and 10 mm in height transparent Pyrex cloning cylinder (Corning Inc., Corning, NY) open on both ends was used as the well. The bottom of the well was sealed with Parafilm and 400 mL of a mixed phase ATPS containing dye and dextran-coated gold nanoparticles were added to fill the well. Subsequently, the top of the well was sealed with Parafilm, and the ATPS was left at room temperature to phase separate. Fluid flow out of the well was triggered by first puncturing the bottom of the well with a needle, placing the entire well assembly on top of an 8 x 70 mm strip of fiberglass paper, and then puncturing the upper seal (Fig. S1a). Video and images of the flow were taken in a controlled lighting environment at various time points.

Results and Discussion: Visualization of ATPS in the Triggered Well Device

We used a cylindrical Pyrex well sealed with parafilm to physically contain the two phases of the ATPS prior to triggering fluid flow through the paper membrane. Incorporating a physical well into this device provides a time delay which allows the ATPS to first concentrate the analyte before flowing through the paper membrane. This controlled release bypasses the need for the user to extract the desired phase because the concentrated bottom phase containing the target biomarker flows first from the bottom of the well to the device detection zone. This triggered well device represents the scenario in which the two phases of the ATPS are completely separated prior to application to the paper membrane.

The triggered well device employs a method that differs from that of the paper well; a transparent cylindrical well is filled with the sample and completely sealed with parafilm (Fig. S1a). By exploiting the pressure difference between the inside and outside of the well, fluid flow can be triggered by puncturing the parafilm seals in the appropriate order. Fluid can only flow out of the well if the pressure inside the well is greater than the outside atmospheric pressure at the location of the bottom hole. After puncturing the bottom seal, a little fluid will flow but will quickly stop due to the decrease in the hydrostatic pressure and the decrease in the pressure of the overlying gas phase as it has expanded to a larger volume. Once the upper seal is punctured, fluid will continue to flow as the pressure inside the container at the location of the hole will always be greater than atmospheric pressure due to the hydrostatic pressure adding to the atmospheric pressure now constantly present in the overlying gas phase. Overall, this process is analogous to puncturing a hole on top of a water jug to dispense fluid.

This system has successfully demonstrated its application as a triggered delay and its ability to allow each phase to flow out of the well sequentially without mixing. After allowing both a 1:1 and 9:1 PEG-salt solution to phase separate within the triggered well and then puncturing the parafilm seals to initiate flow, we can clearly see the interface between the two phases as it flows out of the well and onto the paper (Figs. S1b and S1c, and Triggered Well Supplementary Videos, ESI). The triggered well represents the situation in which the two phases of the ATPS are completely separated prior to fluid flow. However, our 3-D paper well achieves results similar to the triggered well and does not require a time delay for the phases to separate – instead, the phases separate as they flow through the layers of the paper well.

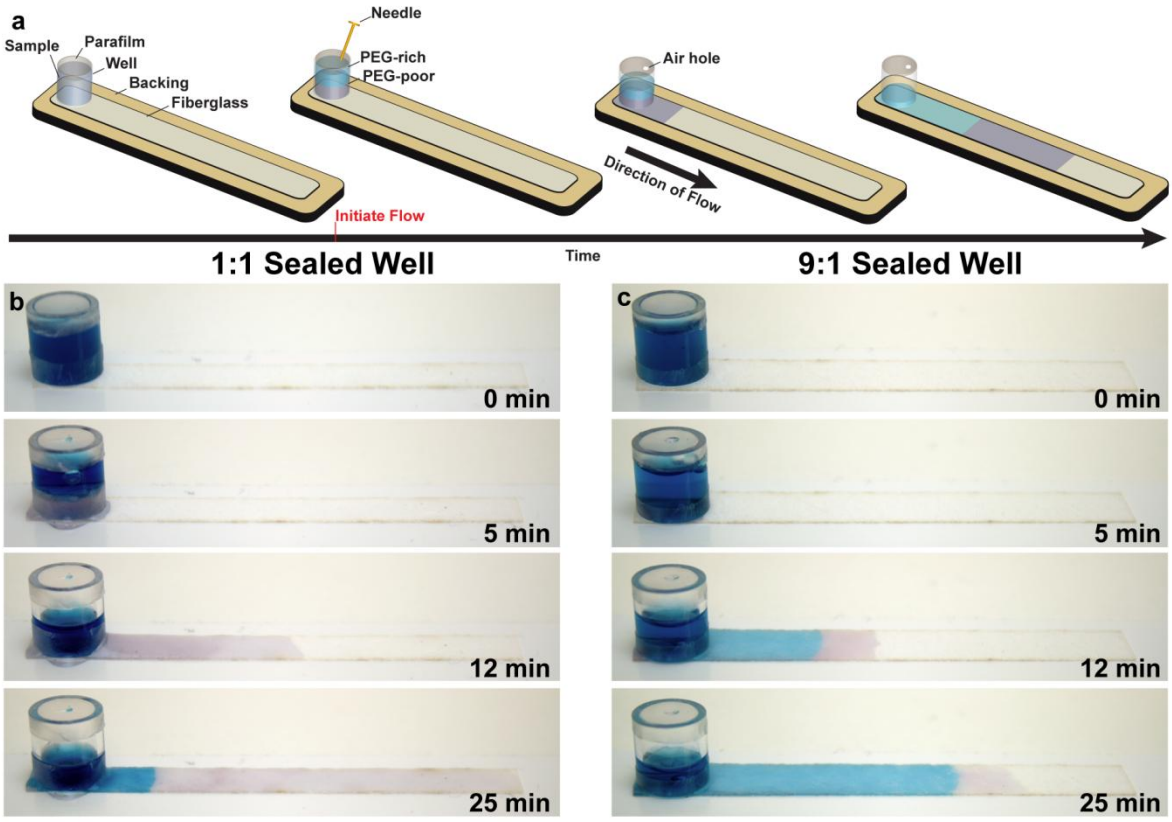

Figure S1: *The triggered well allows for ordered flow of the PEG-poor phase followed by the PEG-rich phase through the paper membrane.* (a) Flow is initiated along the strip only when the upper parafilm seal is punctured. (b) The 1:1 PEG-salt ATPS phase separated in 2 minutes and exhibited intact phases after flowing onto the paper membrane. (c) The 9:1 PEG-salt ATPS phase separated in 5 minutes, and there is a smaller purple volume as the bottom phase volume is reduced.

Supplementary Videos

Paper Membrane with 1:1 Volume Ratio ATPS: The mixed 1:1 volume ratio ATPS phase separates as it flows through the paper membrane. Flow and phase separation are completed around 3.5 minutes. The PEG-rich phase is visualized with Brilliant Blue FCF dye while the PEG-poor phase is visualized with the dextran-coated gold nanoparticles.

Filename: Membrane_1to1.mov

Keywords: Aqueous two phase system, lateral-flow immunoassay, paper diagnostic device

Paper Membrane with 9:1 Volume Ratio ATPS: The 9:1 volume ratio ATPS phase separates as it flows through the paper membrane. Flow and phase separation finish around 5 minutes. Dark clumps of aggregated gold can be seen in the blue PEG-rich phase due to inefficient phase separation.

Filename: Membrane_9to1.mov

Keywords: Aqueous two phase system, lateral-flow immunoassay, paper diagnostic device

3-D Paper Well with 1:1 Volume Ratio ATPS: The 1:1 volume ratio ATPS phase separates as it flows through the 3-D paper well. Phase separation occurs in less than 30 seconds within the paper well. Running buffer is then added to drive the PEG-poor phase out of the well.

Filename: 3DPaperWell_1to1.mov

Keywords: Aqueous two phase system, lateral-flow immunoassay, 3-D paper diagnostic device

3-D Paper Well with 9:1 Volume Ratio ATPS: The 9:1 volume ratio ATPS phase separates as it flows through the 3-D paper well. Phase separation occurs in less than 35 seconds within the paper well. After the addition of running buffer, the phases do not appear to mix.

Filename: 3DPaperWell_9to1.mov

Keywords: Aqueous two phase system, lateral-flow immunoassay, 3-D paper diagnostic device

Triggered Well with 1:1 Volume Ratio ATPS: The 1:1 volume ratio ATPS phase separates within the triggered well and flows onto the paper membrane after the seals are punctured. Phase separation takes approximately 5 minutes to occur in the well, and flow along the membrane is much slower than with the 3-D paper well.

Filename: TriggeredWell_1to1.mov

Keywords: Aqueous two phase system, lateral-flow immunoassay, 3-D diagnostic device

Triggered Well with 9:1 Volume Ratio ATPS: The 9:1 volume ratio ATPS phase separates within the triggered well and flows onto the paper membrane after the seals are punctured. Flow along the membrane is slower than with the 1:1 volume ratio ATPS due to the larger volume of the more viscous PEG-rich phase in the 9:1 volume ratio ATPS.

Filename: TriggeredWell_9to1.mov

Keywords: Aqueous two phase system, lateral-flow immunoassay, 3-D diagnostic device