Microfluidics Lateral Flow Test and Pregnancy Test Kit - Fundamentals

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What is a microfluidic device?

Microfluidics is a multidisciplinary field intersecting engineering, physics, chemistry, biochemistry, nanotechnology, and biotechnology, with practical applications to the design of systems in which low volumes of fluids are processed to achieve multiplexing, automation, and high-throughput screening. Microfluidic devices, also called "lab-on-a-chip", have gained a lot of attention and research in the past two decades has produced advancements in miniaturization, integration, cost-reduction, and automation within these microfluidic devices. Commonly, microfluidic devices have been fabricated using silicon, glass, and polymers. Recently paper-based microfluidic devices have gained popularity due to their portability, compactness, and ease of use without external instrumentation, making them an attractive option for point-of-care (POC) devices, or devices that can be used in low-resource settings. POC devices are meant to be affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable. A common, simple, paper-based microfluidic device is a lateral flow test (LFT). Table 1 is a comparison of LFTs versus a lab-based test called an enzyme-linked immunosorbent assay (ELISA) (basically an immunoassay in a well, which will be explained later).

ELISA Test	Lateral Flow Test
Long time to receive results	Quick results (less than 20 minutes)
Requires trained personnel	Anyone can use (simply add sample/water)
Tests can be expensive	Cheap to produce (less than $1)
Requires equipment/instrumentation	No equipment/instrumentation (wicks sample)
Requires electricity	No electricity required
Produces quantitative results	Only produces yes or no results

Table 1. List of pros and cons of lab-based ELISA versus lateral flow tests.

LFTs are made out of porous materials (typically glass fiber, nitrocellulose, and cellulose papers) that are lower cost than traditional materials used in microfluidic devices. LFTs are also user-friendly, equipment free, and produce rapid results, which make them ideal for use at the POC. However, LFTs can lack the sensitivity and specificity of lab-based tests.

LFTs can be used to diagnose infectious diseases such as malaria in low-resource settings in developing countries. An example of a well-known LFT is a simple dipstick pregnancy test, similar to the example shown in Figure 1. In this pregnancy test, as in most LFTs, there is a source or sample pad, a dried conjugate pad, a reaction membrane, an absorbent wicking pad, plastic barriers to maintain one-dimensional flow, and sometimes a housing that is typically made out of plastic.

This test works like a traditional immunoassay. The sample is applied to one end of the test and rehydrates labeled detection antibodies (such as gold nanoparticle conjugated antibodies) previously dried onto the conjugate pad. The rehydrated antibodies bind to the target analyte in the sample as both flow together down the strip to the capture region consisting of a test and control line (we will revisit the control line during the lab). Non-labeled antibodies specific for the analyte are immobilized at the test line and "capture" the analyte bound to gold labeled antibodies, creating a visually detectable signal at the test line (this complex can be seen in

Figure 1). For the control line, keep in mind that not all of the labeled detection antibodies bind to the analyte, and some can flow past the test line. The wicking pad acts to continue to draw the sample through the LFT and the capture region by capillary action once the reaction membrane has been "fully wetted". A diagram of the LFT immunoassay process is displayed in Figure 1.

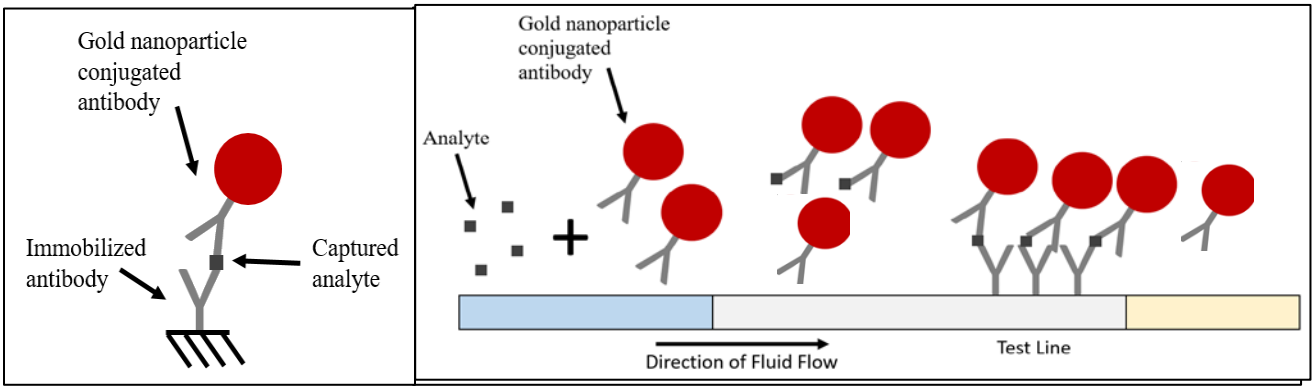


Figure 1. (Left) Diagram of a labeled captured analyte. The analyte is bound to the gold nanoparticle conjugated antibody and is captured by the immobilized antibody at the test line. (Right) Schematic of common immunoassay lateral flow test process. Analyte from the sample rehydrates and binds to gold nanoparticle conjugated antibodies. This compound flows down the device to be captured by the immobilized antibodies at the test line. The collected gold nanoparticles can be visually detected.

How does fluid flow in a paper-based microfluidic device?

Fluid transport through porous materials is by capillary action. Have you ever seen water flow through a small glass tube? Capillary flow is due to the cohesive liquid-liquid forces (the net attraction of the molecules in the liquid) that creates both surface tension (liquid molecules at the boundary layer that have half of the interactions with other liquid molecules as liquid molecules in the bulk fluid) and adhesive forces (liquid to solid adhesive interaction).

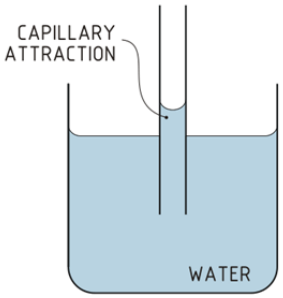
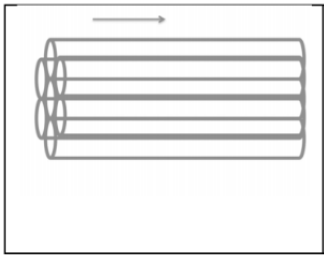


Figure 2. Demonstration of capillary action that draws liquid up capillary tube.

Now picture a porous material as not one capillary glass tube, but an ordered bundle of parallel capillary tubes. This is an ideal view of a porous material. Figure 3 shows a comparison of the ideal view and actual view of a porous material (nitrocellulose). The average pore diameter of

commonly used nitrocellulose is 8 µm, which can be compared to the average diameter of a human red blood cell of 6 to 8 µm.



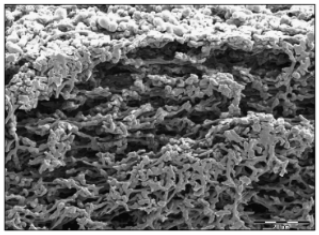


Figure 3. (Left) Side-view of a bundle of capillaries, which can be idealized as a porous material. (Right) Microscopic image of a cross-section of nitrocellulose membrane. Image from J. Osborn.

Laminar flow occurs at low Reynolds numbers. In paper-based microfluidic devices, materials used typically have a membrane pore diameter ranging from 5 to 10 µm, which results in Reynolds numbers << 1.

Recall the equation for Reynolds number (Re), where ρ is density, v is the fluid velocity, l is the characteristic linear dimension, and µ is the fluid viscosity.

$$Re = \frac{\rho v l}{\mu}$$

What do you think the characteristic linear dimension is for a porous material?

Answer: pore size

Calculate the Reynolds number of water flowing through nitrocellulose (pore size 8 µm). You can assume that fluid traveled 2 cm in 100 seconds. Also assume the viscosity of water is 1.00 mPa•s.

Answer: Reynolds Number (Re) = 0.00160

In the case of a fully wettable capillary, the Lucas-Washburn equation can correlate fluid travel distance versus time. The equation is shown below, where t is the time for a liquid of viscosity (μ) and surface tension (γ) to travel a distance (L) into the capillary with pore diameter (D). However, the Lucas-Washburn equation is only applicable if the fluid is flowing down the capillary in one dimension and contains an infinite source.

$$L^2 = \frac{\gamma D t}{4\mu}$$

How can you simplify the equation to compare two different distances of water traveling through a capillary (L_1, L_2) in terms of time (t_1, t_2)? Answer should be a ratio.

$$\frac{L_2}{L_1} = \sqrt{\frac{t_2}{t_1}}$$

NOTE: L_1 and L_2 are the distance from L=0 (start). Therefore, the Lucas-Washburn equation predicts the ratio of flow times will get smaller (flow times will slow down) with distance away from the start

Now that you have been introduced to how fluid flows through a porous material and the variables that are important to fluid flow, predict if there would be a difference in capillary flow time for two different widths of strips. Explain why or why not.

Answer: NO. The Lucas-Washburn equation predicts dependence only on fluid properties, pore size, and length travelled. No effect of sample width.

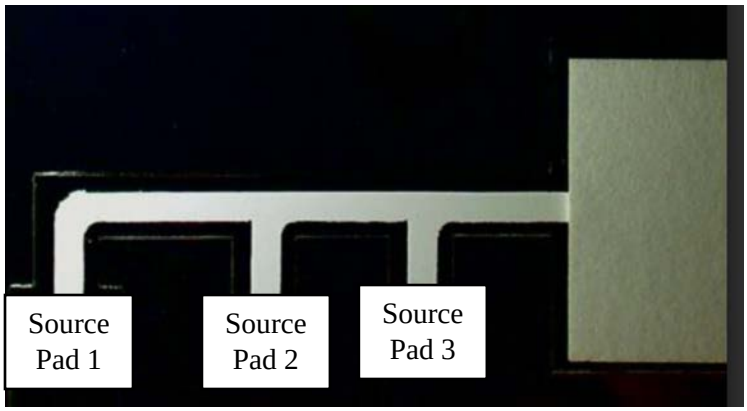
What about for the difference in capillary flow time for the same distance, L, traveled into the strip for two fluids of different viscosities?

Answer: YES. The Lucas-Washburn equation predicts a dependence on fluid properties (including viscosity and surface tension). The more viscous liquid would move more slowly.

An important parameter in the design of a lateral flow test is the time the analyte in the sample has to interact and bind with the reagents placed on the "test line" such as in the pregnancy test. Describe the easiest way that you can modify the device design to increase the time that the analyte in the sample spends within the test line zone.

Answer: Increase the distance of the test line from the start. The Lucas-Washburn Equation predicts that flow times will slow down with increasing distance from the start. The farther down the strip, the slower the capillary flow rate, and the greater the residence time of analytes in the test region.

Now hypothesize what would be the point of using different dimensions and strip geometries to transport multiple fluids flowing on one device that are meant to initiate multiple reactions at a wicking pad. An example of a strip with a different geometry that allows three different fluids to be added at three different source pads flowing to the wicking pad on the right is shown below. Specifically how would the example below be able to initiate two reactions?



Changing length dimensions and strip geometry will affect the timing of fluid delivery and volumes of the fluids being delivered in sequence. In this example, 2 and 3 will react before 1 arrives. Also, 1 and 2 will react prior to reaching 3. So multiple interactions can be studied with this more complicated delivery pattern.