Teacher's Preparatory Guide

Lab On A Slab

Overview: Microfluidics is a technique for manipulating liquid samples. With one drop of liquid sample, small devices and channels can be used with nanoparticles for sensitive detection of chemicals. In this lab, students will create a fluidic device with agar gel and use it to test and diagnose three "patient's" samples for pH, starch, and peroxide content.

Purpose: This lab is designed to help students understand fluid flow, capillary action, surface tension, and chemical tests used as sensors.

Time Required: Three 55-minute class periods or two 100-minute class periods

Level: High school chemistry or biology

Big Ideas in Nanoscale Science and Engineering:

- Forces and Interactions
- Models and Simulations
- Science and Technology
- Structure of Matter

Teacher Background A *lab-on-a-chip* is a very small, portable device that automatically controls the flow of a liquid sample to react with one or more chemicals or sensors. These devices can be used in many different environments without extensive training. The chips typically have sets of channels, sensors, mixing chambers, heaters, and valves that control how a sample moves, reacts, and is detected after it is put into the chip[1]. Multiple tests can automatically be done on a drop-sized sample of blood in minutes on a laptop-sized machine.

A lab-on–a-chip is a micro/nano sized device that can run several biochemical analyses (tests) at one time using very small samples – sometimes just a drop of blood or urine. They are also called Micro Total Analysis Systems - µTAS. In addition to medical applications, they can be used to detect toxins in the environment such as in water or the air. The most common area is in medical diagnostics and the most familiar ones are home pregnancy tests, drug tests, glucose monitoring, and strept tests. These devices are becoming very important as we seek ways for early disease detection and hazardous materials detection (important for Homeland Security). This interest has created a large demand for the development of easy-to-handle and inexpensive lab-on-a-chip devices that can work quickly and reliably. So why is nanotechnology important to this? It has the ability to make small devices (microfluidic channels in the micro and nanoscale dimensions) on chips capable of analyzing very small quantities of analyte.

Nanotechnology is playing an important role in lab-on-the-chip development and production. For example, researchers at the University of Alberta have created a small plastic chip[2] called the Domino to detect diseases and drug resistance in patients. Developed by using nanotechnology,

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this device is about the size of two postage stamps and will replace a fully equipped and expensive laboratory. The device is portable and much less expensive than a full laboratory ($5,000 versus several million dollars).

This lab uses *capillary action* to draw a liquid through a channel and expose it to several tests in order to show how different forces dominate and affect the behavior of a fluid as the channels they flow through get smaller. With capillary action, the sum of attractive forces between the molecules of liquid and the walls of the channel are greater than the downward pull of gravity. The liquid rises up the channel for a distance that is inversely proportional to the width. In other words, as the channel gets narrower, the height increases.

Capillary action **occurs when the molecules of a liquid in a small tube have both cohesive intermolecular forces and adhesive intermolecular forces**[3,4]. *Cohesive forces* are between two or more identical molecules, as with two water molecules in a droplet. *Adhesive forces* are between the water molecule and the molecules that make up the walls of the tube. When both of these forces are strong enough to overcome the weight of the thin column of water, the water is drawn up into the tube until either the adhesive or cohesive force equals the weight of the water column. At this balancing point, if the water were to try to climb any higher, the extra weight of water would cause the column to slip back down, so the equilibrium is reached.

The "Magic Crystal Tree" science toy[5] uses capillary action in a cardboard tree to draw up a solution of salt to its branches. The water evaporates, crystallizing the salts on the outside of the cardboard. Similarly, real trees use transpiration to move water and nutrients up their trunks.

Surface tension results from the fact that the cohesive intermolecular forces in water are greater than the adhesive intermolecular forces on the molecules around it. For example, water molecules experience more pull from the molecules inside the drop of water than they do from the air. This causes the water molecules to surround themselves with other water molecules, so that the fewest number of water molecules contact the air. The resulting shape (a sphere) has the least surface area compared to volume. A droplet on an attractive surface like glass will become a half sphere, still minimizing the air–water interactions as a result of maximizing the water–water and water–glass interactions.

In this lab, luminol[6] will detect the combination of alkali and hydrogen peroxide. Luminol is also used in forensic investigation of crime scenes[5] to detect the iron in blood stains.

Sources:

1. Science Daily. "Microfluidic Integrated Circuit Could Help Enable Home Diagnostic Tests." (accessed July, 2010) http://www.sciencedaily.com/releases/2010/04/100422170149.htm
2. Plastic Chip can perform twenty genetics tests from a single drop of blood (accessed June, 2012) http://www.technologynetworks.com/loac/news.aspx?id=140241
3. Wisegeek. "What Is Capillary Action?" (accessed July, 2010) http://www.wisegeek.com/what-is-capillary-action.htm
4. USGS Water Science For Schools. "Capillary Action." (accessed July, 2010) http://ga.water.usgs.gov/edu/capillaryaction.html
5. Instructables. "Grow your Own Magic Crystal Tree." (accessed July, 2010) http://www.instructables.com/id/Grow-your-own-Magic-Crystal-Tree-or-any-other-sha/
6. How Stuff Works. "How Luminol Works." (accessed July, 2010) http://science.howstuffworks.com/luminol4.htm

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Materials per class:

- hot plate
- saucepan (preferably with handle and pouring lip)
- refrigerator (*optional*, it mustn't get too cold or ice crystals will ruin the agar gel)
- filter paper, cut into small pieces (about 5 mm × 5 mm)
- hole punch
- tweezers
- agar, powered or granular (60 g)
- full range universal pH indicator solution (Wards Science #951 V 3101) or Hydrion 1–14 pH paper (Wards #15 V 2572)
- tincture of iodine (5 ml; e.g., 2% iodine in 47% ethanol and 51% water)
- 16, 20, 24, 30 and 33 gauge copper wire (or similar), at least 3m of each
- box of popsicle sticks and toothpicks (optional)
- glow sticks or luminol (test for peroxide) (0.2 g of powered luminol in 5 ml ethanol or 1 glow stick) (Wards #945 V 9800)
- starch solution (10 ml; e.g., Faultless liquid starch)
- vinegar (100 ml, household 5%)
- baking soda solution (20 g in 200 ml of water)
- hydrogen peroxide (20 ml, household 3% solution)
- distilled water (4 liters)
- 500 ml or 1 liter measuring jug
- 9 vials (15 ml glass) for 'patient samples'
- 3 small disposable cups to prepare sensor pads
- graduated cylinder
- 2 plastic pipettes, for making patient samples
- cardboard box (see *Advance Preparation* section)
- food coloring (one color)

Materials per student group:

- various pieces of wire
- paper clip
- toothpick
- popsicle stick
- wire cutters
- needle nose pliers
- 2 Hefty® 8-inch styrofoam plates (1 for each lab, other plates may not de-mold well)
- Sharpie® marker
- agar gel
- tweezers
- plastic cling wrap to seal agar gel overnight
- paper
- scissors
- 10 hole punch reinforcement rings
- 5 plastic pipettes (1 for 1st lab; 4 for 2nd lab)
- colored water in a small cup
- stopwatch

- sensor pads, four of each type (shape), organized in containers (see *Advance Preparation* section)
- distilled water in a small cup
- samples from 3 patients (see *Advance Preparation* section)
- plastic knife
- paper towels

Advance Preparation: Most materials should be available in a well stocked chemistry classroom. Agar powder can be purchased at a variety of scientific supply companies Flinn Scientifics, Carolina Biological; at food supply sites such as WillPowder and MoreBeer and at Asian food stores. Chemical indicators can be purchased at educational chemical supply companies, such as Wards Science (http://www.wardsci.com). Tincture of iodine and hydrogen peroxide can be purchased at most drug stores. Liquid starch, styrofoam plates, vinegar, and baking soda can be purchased at a supermarket. Glow sticks can be purchased from most craft stores. Needle nose pliers, wire cutters, and lengths of copper wire (variety of gauges from AWG 15 to 35) can be purchased from an electronics store.

1. ***Prepare the sensor pads the day before.***
   Each group needs at least 4 of each type of sensor. (Use different shapes to tell the sensors apart.)
   - Hole-punch the filter paper to get circles and put in a small cup. Add iodine to the cup to soak the circles (for the starch test).
   - Cut some filter paper into ~ 5 mm squares and put in another small cup. Add universal pH indicator to the cup to soak the squares (for the pH test).
   - Cut some filter paper into small triangles and put them in a small cup. Soak these in luminol solution for the peroxide test. (Luminol solution can be made from 0.2 g luminol in 5 ml ethanol, or by carefully cutting open a glow stick and using the liquid inside the inner glass vial to soak the filter papers.)

   Spread all sensors out to dry overnight in petri dishes or trays.
2. ***Make the patient samples.***
   Prepare three unknown 'patient samples' using these recipes:
   - *Patient A:* 10 ml of vinegar with 4 drops of starch solution
   - *Patient B:* 5 ml of hydrogen peroxide and 5 ml of baking soda solution
   - *Patient C:* 10 ml of baking soda solution with 4 drops of starch solution

   Put each sample into 3 separate labeled vials for the class to use.

3. ***Make a dark box.***
   To see the glow of luminol in the amounts used, you will need one or more 'dark boxes'—a simple cardboard box with a viewing slot. Use a cardboard box big enough to place over the styrofoam plates. Open the bottom by folding in the flaps and taping them in place. Cut a viewing slot in the center of the top of the box approx 3 cm × 15 cm. Check that the box is dark enough to see the pale *blue* glow of a positive luminol test through the viewing slot. (The color of glow may vary if using a glow stick to make the sensor pads.)
4. ***Premix the agar and water before the lab.***
   Recipe fills about 10 plates to a depth of 5 mm: Mix 10 g agar powder in 1 liter of cool water. When students are ready to pour, bring to a simmer in a saucepan while stirring for 5 minutes on a hot plate. (Adding agar to hot water works poorly.)

Safety: Students should wear goggles and gloves. Iodine and luminol are poisonous if ingested. Mild acidic and alkaline solutions can cause skin irritation. Read safety data sheets of chemicals.

Instructional Procedure:

Time	Activity
Day 1	
15 min	Follow *Guided Dialog* section (see below).
25 min	Students make channels with various materials (different thicknesses of wires or toothpicks) that are placed in the bottom of a foam plate, then covered with agar gel. Ask students to test at least four different ideas/materials to find the channels that best cause capillary action. Cover, and leave overnight in a refrigerator (one that is not so cold as to form ice crystals in the agar). If the activity is done in a longer class, agar gel takes about 10–15 minutes to set at this concentration.
5 min	Ask students to discuss what criteria should be used to test the channels. Make sure that the completeness of channel fill, the time taken, and how many drops to fill the channel are suggested as criteria.
5 min	Clean up.
Day 2	
15 min	Students remove agar from molds and carefully remove wire with tweezers. Have students drop dyed water onto the channel in the agar to observe which channels pull the liquid into them the best, using the criteria discussed the previous day. Remove liquid from channels by blotting with paper towels to retest or show other students a good channel.
10 min	Discuss with the class what channels worked best. *Thinner, deeper, open channels should work well. Air locked, wide channels will work poorly, if at all.* Explain the color change tests: • **Starch test:** iodine changes from brown to blue/black when starch is present. • **pH test:** pH paper turns red at pH 2 (acid), blue at pH 10 (alkaline), and green at pH 7 (neutral). (Distribute color charts if they came with the pH paper.) • **Peroxide test:** hydrogen peroxide makes luminol glow blue in the dark. *Note: The peroxide test only works well in an alkaline solution, but the test samples are set up so as to avoid the combination of an acidic peroxide sample. You may introduce the idea of confounding factors that cause a test to give false results, but it is beyond the scope of this document.*
5 min	Ask students to draw and label the design for their lab-on-a-slab. *A branched design is needed for 3 tests. Smooth, narrow, deep channels work well to wick the sample toward the sensor areas by capillary action.*
15 min	Students arrange 4 copies of their pattern using wires on a foam plate. (No sensor pads at this time—the hot agar solution would wash away the chemicals). Pour agar over designs and let set overnight. If the activity is done in a longer class, agar gel takes about 10–15 minutes to set at this concentration.
5 min	Clean up.

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Day 3	
20 min	Students carefully remove agar from the molds, trim, and put the agar slab channel-side up on the plate. Students place sensor pads on the agar in positions that will best test the fluid. *(See Advance Preparation section for instructions on making sensors. **Tests must be done within 10 minutes of placing the sensors on the slab** because the iodine paper slowly deactivates on the moist agar.)* Test the labs-on-a-slab by using a water control. Then test with unknown sample mixes (detailed in the *Advance Preparation* section). Diagnose each patient using the table on the 2nd page of the *Student Worksheet: Lab-on-a-Slab 2: Build Your Slab*. Label unclear results as "More testing needed."
10 min	Students list strengths and weaknesses of their design and suggest improvements.
10 min	Review concepts of capillary action and why shrinking the lab-on-a-chip concept is useful for medicine and defense.
5 min	Clean up.

Teaching Strategies: Groups of two or three students allows discussion and peer–peer teaching.

Guided Dialog: *Before* the lab, demonstrate surface tension by floating a paper clip on a cup of water. Lower the surface tension with a drop of soap. (The paper clip will sink to the bottom.) Then, introduce the science of capillary action and explain the meaning of these terms:

Surface tension *A property of liquids that cause them to ball up when dripped from a dropper. The shape of the liquid is the result of intermolecular forces. The attraction of the molecules to their neighbors is more than their attraction to the air so they form the lowest surface to volume ratio possible: a sphere.*

Cohesive force *When a substance is attracted to neighboring molecules of the same type as itself by intermolecular forces.*

Adhesive force *When a substance is attracted to neighboring molecules of a different type as itself by intermolecular forces.*

Capillary *A narrow tube that carries fluids (connect this with smallest blood vessels).*

pH *How acidic or basic a solution is; Acidic is pH 1–6, neutral water is pH 7 and basic/alkaline solutions are pH 8–14.*

Capillary action *When adhesion to different material is stronger than cohesion between molecules of liquid, the liquid is drawn up a capillary.*

Ask students questions to provoke thought and review what they already know:

1. What happens when you put the end of a paper towel in water? *Water is drawn into the towel.*
2. In this case, what is the *adhesion* force between? What is the *cohesion* force between? *The water molecules have cohesion forces between themselves and the adhesion force is between the water and paper towel material.*
3. Why might the water rise up the paper towel even though gravity is pulling it downwards? *The adhesion forces and cohesion forces are both greater than the gravity force pulling the column of fluid down. At the nanoscale, these intermolecular forces are stronger than gravity. As the column gets higher, the mass of the column increases until the force due to gravity matches either the cohesion force or the adhesion force, at which point the column stops rising.*

Cleanup: Do not pour excess hot agar solution down the sink. Instead, pour it into foam plates and discard it in the trash when set. Collect and store excess chemical solutions for future use or neutralize (e.g., acid with base, iodine with starch, etc.) and pour down the sink. Wipe benches.

Enhancing Understanding: Review this with students:

> Narrow channels can wick liquid along them by *capillary action*. This can be used to test a drop of sample for many things. This saves time and reduces the amount of equipment. The same process is how trees transport nutrients to their top leaves and is called *transpiration*.

Assessment:

Students should be able to express that liquids are drawn up thin channels/gaps by the way the molecules interact with the surface. These interactions are called *cohesion* and *adhesion*, and result in *surface tension* and *capillary action.*

Students should be able to list the strengths and weaknesses of their design and formulate ideas for improvement. Assessment will be based on participation in the lab and submission of (a) notes from the discussions (b) diagram of design with justification (c) results and justification of diagnosis (d) answers to questions (e) a critique of their lab-on-a-chip featuring strengths and weaknesses and ideas for improvement.

Resource: You may wish to use these resources either as background or as a resource for students to use in their inquiry-based design.

- Technology Review. Article on Shrinky Dink Fluidics. (accessed July, 2010) http://www.technologyreview.com/TR35/Profile.aspx?trid=764
- Grimes A., Breslauer D.N., Long M., Pegan J., Lee L.P. and Khine M., "Shrinky Dink Microfluidics." *Lab on a Chip*, 8 (2008): 170–172.
- Yang C.W.T., Ouellet E. and Lagally E.T., "Jello Chips for Microfluidics Education." *Anal. Chem.*, 82 (13) (2010): 5408–5414.
- How Stuff Works. "How Inkjet Printers Work." (accessed July, 2010) http://computer.howstuffworks.com/inkjet-printer3.htm
- Wikipedia: Lab-on-a-chip - http://en.wikipedia.org/wiki/Lab-on-a-chip
- Nanomedicine Issue of Nanooze: http://www.nanooze.org/english/pdfs/nanoozeissue_08.pdf

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- Lab-on-a-chip articles and videos at the Online Scientific Community http://www.technologynetworks.com/loac/(requies free registration but provides interesting articles on cutting edge science and engineering)
- Nanofluidics in Lab-on-a-chip devices: http://www.youtube.com/watch?v=O4lPkAlyIVw
- Real World: Using Lab on a Chip Technology to Identify Microorganisms: http://www.youtube.com/watch?v=r_RzRAzp3UM

Material safety and data sheets are available online at a variety of sources including
- http://www.msds.com/

National Science Education Standards (Grades 9–12)
Content Standard A: Science as Inquiry
- Abilities necessary to do scientific inquiry

Content Standard B: Physical Science
- Structure and properties of matter
- Chemical reactions

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