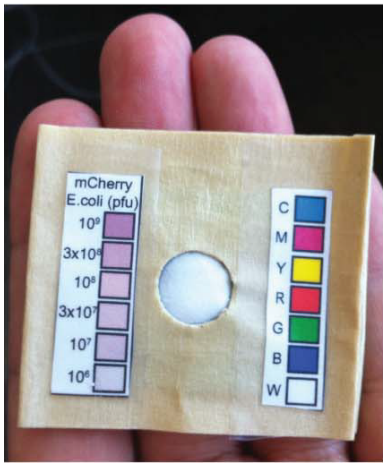
Paper and Tape Phage Detectors

Background



The article you will be reading for this assignment is an adaptation of a piece of scientific literature from a science journal. Scientists communicate their findings with each other by writing up their lab reports and submitting them to scientific journals. These journals are edited by experts in that particular field of study- articles submitted to 'Nature Cell Biology', for example, are selected and edited by a panel of experienced cell biologists. When an article goes through this process and is published in one of these peer-reviewed journals, it is considered to be primary literature. This means it is a direct reporting of the scientific research by the group that did that research. Primary literature makes up the backbone of communication in the scientific community. It is the most accurate and trustworthy source for science information and it is crucial that students are capable of reading and analyzing this material.

This article is an adaptation of a paper written by the Derda Research Group at the University of Alberta. It explores the development and testing of a portable experimental lab tool that could take the place of the Petri dish. The goal of the Derda Lab was to develop a device that could be produced at low cost and in low resource environments. Ideally, it could also be operated by untrained workers with limited biology knowledge. Students from Harry Ainlay high school worked on many of the steps of the development and testing of this product.

Your Activity

You will be answering questions on this article regarding the scientific method. Afterwards, you will be planning your own follow up experiment based on the experimental methods you have read about in this article.

Materials

- **Highlighter**
- **Paper and Tape Microorganism Detectors - physical or digital copy**

Assignment

Read the Abstract and Introduction

1. While you read, **highlight** any words or concepts you don't fully understand. When you are finished these sections, write a few words out here that you feel you need clarification on.

Summary

2. In three sentences, write out what these researchers intended to study.

3. Try to summarize this into a single **research question**. Details are not as important as getting at the heart of the scientific problem that exists. What question were scientists looking to answer?

4. **State** the main hypothesis of this research. This is NOT a question, but a statement that the scientists will attempt to find evidence for or against.

5. What was the **purpose** of the abstract?

6. After reading the introduction, do you feel you have a better understanding of why the researchers are approaching this problem? Write out 3-5 questions you have about how these researchers are going to go about doing their experiment.

Read the Experimental Design and Materials and Methods sections.

7. While you read, **highlight** any words or concepts you don't fully understand. Write any new words/concepts here that you don't understand.

8. In the experimental design section, write down at least 3 examples of problems the researchers identify that may interfere with their results. How do they intend to address these problem?

    a.

    b.

    c.

9. Though this is one piece of primary literature, there are 3 experiments that are being reported in this paper at the same time. First, the researchers are reporting on their process for creating their bacterial testing device. Then, they are reporting 2 separate experiments they did to test their device. For each of the following, identify the manipulated, responding, and at least 3 control variables.

    Testing Against a Traditional Agar Plate

    Manipulated Variable:

    Responding Variable:

    Controlled Variables:

Testing for Growth with Environmental Swabs

Manipulated Variable:

Responding Variable:

Controlled Variables:

10. Though the materials are not in an ordered list, work with your partner to brainstorm a shopping list for materials you would need to complete the **testing against a traditional agar plate** experiment. Think about quantities as well!

Read the Results and Conclusion sections.

11. Summarize the researchers findings in three sentences. Did they find what they set out to? Did they come across any problems?

12. Examine the graph in **Figure 5b**. Summarize the result this graph represents in 1 sentence.

13. Why are graphs such an effective way of displaying information?

14. What is the purpose of the conclusions section of this article?

15. Science discovery tends to lead to new opportunities for interesting and useful research. With your partner, brainstorm some potential future uses for this portable culture device. Would these be testable with an experiment?

Continuing Research

As mentioned above, scientists are continually building on each other's research. Small additions here and there eventually add up to big changes. Often these big research ideas bounce around the world to labs with similar focuses. It's not uncommon for a lab in Norway, a lab in Chile, and a lab in Australia to be working in close conjunction with each other in a new area of study.

Choose one of your potential uses for this technology from above. Using the guide on the next page as a framework, develop your ideas and begin to design an experiment to test your concept!

Your Assignment

Based on the extension experiment you've chosen to do, fill in the following experiment form.

1. **Experimental Question**:

**Before progressing, make sure this question is testable and scientific! Feel free to check this question with your teacher and/or peers before moving on.*

2. **Background Research** - Traditionally this would have to be heavily researched before moving on. For this activity, list some of the questions you would need to answer before you could proceed with your experiment. These could be theoretical questions or technical questions. List 3-5 questions total.

3. Identify the Experimental Variables.

Manipulated Variable:

Responding Variable:

Controlled Variables:

4. Write an If/Then **Hypothesis** Statement:

5. **Experimental Design** - Write a detailed procedure for how you might carry out your experiment. Make this procedure as repeatable as possible- write it as if your reader has a limited knowledge of science.

Paper and Tape Microorganism Detectors

Lab on a Chip: Portable devices for bacteria detection made from paper and tape

Maribel Funes-Huacca, Alyson Wu, Eszter Szepesvari, Pavithra Rajendran, Nicholas Kwan-Wong, Andrew Razgulin, Yi Shen, John Kagira, Robert Campbell and Ratmir Derda
University of Alberta, Edmonton, AB, Canada

Adapted from: Portable self-contained cultures for phage and bacteria made of paper and tape
DOI: 10.1039/c2lc40391a

Adapted to APL by: Quinn McCashin
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Abstract

Bacteria are common disease-causing microorganisms. Our lab has devised a method of creating inexpensive, portable, and functional devices to test for the growth and reproduction of bacteria. These devices were produced largely from low-cost materials that are readily available on a global scale. Verification of the durability and simplicity of production was carried out in a low-resource setting by high school students from Harry Ainlay school. The devices' ability to maintain humidity while allowing for gas exchange was confirmed. Devices were also established to have similar growth rates to traditional Petri-dish agar plates. Multiple environmental surfaces were swabbed and successfully cultured with the experimental paper and tape detectors. The fabrication and performance of the device has been verified in a low-resource laboratory setting in Nairobi, Kenya. This paper and tape device can be used as both an educational and diagnostic tool in low-resource environments worldwide.

Introduction

Point-of-care (POC) diagnostic devices are tests designed to be used in the field to quickly and accurately identify microorganisms, most commonly bacteria, that cause disease. One category of diagnostic device are those that detect the presence of living organisms using cell culture. Cell culture is the process of growing cells in a controlled environment.

Microorganisms are difficult to detect in small numbers so there is often a need for rapidly growing a population into a colony that is big enough to visualize. Tests like these are critical for identifying infectious diseases, especially in resource-limited areas. It may even be possible to use genetically engineered bacteria to test for other environmental factors (poisons or toxins, for example) using this style of diagnostic test.

Whatever the goal of the cell culture, it is necessary to have a simple, inexpensive way of culturing bacteria. The goal of this research is to design an inexpensive cell culture device and a simple procedure for using that device.

The common protocol for point-of-care testing is the **ASSURED** criteria. If we desire a test to be usable in a field environment, it must be: **A**ffordable, **S**ensitive, **S**pecific, **U**ser-friendly, **R**apid and robust, **E**quipment-free, and **D**elivered to those in need. Essentially, a point-of-care device has to be designed in a way that allows it to be created, used, and disposed of in a way that is inexpensive and requires very little training or technology.

Agar-filled Petri dishes are the traditional approach to culturing bacteria in a lab (Fig. 1). These dishes are expensive to create; they rely on aseptic conditions with controlled temperature and humidity to allow for proper growth of bacteria. Maintaining aseptic conditions (a sterile environment that is free from contact with microorganisms,) is very difficult in the field and without expensive laboratory equipment. Our goal was to keep the functional elements of other growth devices, but to replace the materials and assembly processes to make the device suitable for inexpensive and simple production.

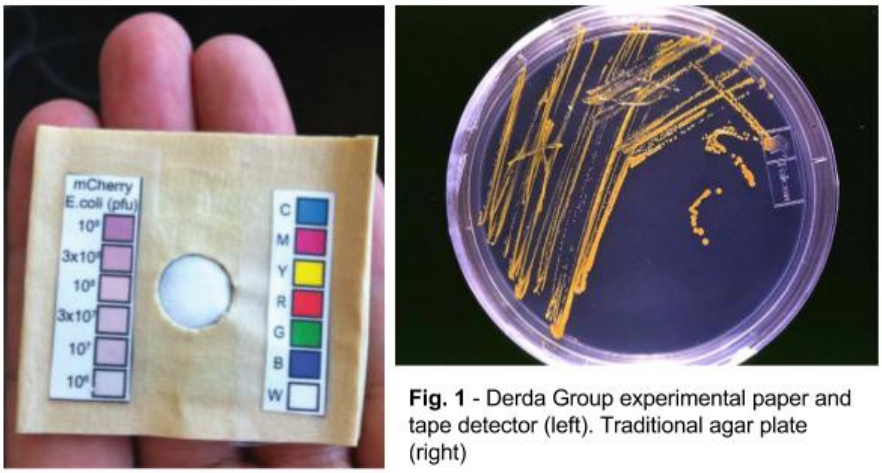
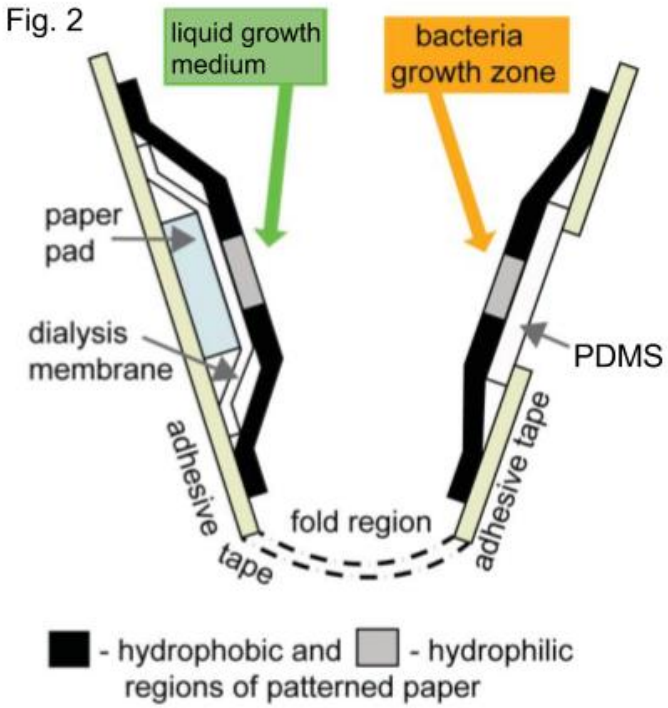


Fig. 1 - Derda Group experimental paper and tape detector (left). Traditional agar plate (right)

The devices we describe in this report were successfully produced from low-cost materials by high school students from Harry Ainlay high school. They were tested in a high school classroom and in low-resource labs in Nairobi, Kenya, and were used by students in the Derda lab to visualize the growth of bacteria. A high school laboratory in Canada is a good test environment because it has similar resources to a developing country's medical laboratory. There is limited scientific machinery, a largely untrained workforce (in this case, high school students) and little access to expensive chemicals and other scientific materials. We also envisioned a benefit for the high school- access to an inexpensive bacterial growth device that could improve the quality of high school education. Growth of bacteria is an excellent experimental opportunity for science classes. Schools often do not have access to funding for the necessary resupply and high turnover rate of agar-filled Petri dishes.

Experimental Design

Bacterial growth requires three main components: water, nutrients, and gas exchange (oxygen in, carbon dioxide out). Additionally, once a sample has been collected and placed in a growth container, it requires some amount of protection from the environment- both contamination and evaporation of the water in the sample are major threats to the success of a bacterial culture. As our goal was to use readily available materials, the backbone of our design relies on wax paper, packing tape, and simple mixing tools (a cup and spoon from Tim Hortons, for example). Our design for this mobile Petri dish was based on previous research done by our laboratory on the ability of gel mediums to support cell culture. We aimed to replace the agar from a Petri dish setup with a nutritious growth medium that is soaked into the paper itself. As a result, the major challenge of this project became a question of how to keep a nutritious paper growth medium wet long enough for bacteria to reproduce, while still allowing for gas exchange.



Evaporation is a major concern in this type of application. A piece of paper made wet with a sample would dry out in minutes, even if stored in a humid environment. This is not long enough for bacterial replication to occur, let alone the multiple reproductive cycles required to grow a bacterial colony. The solution lies in a chemical called PDMS (Polydimethyl siloxane). A thin sheet of paper that lies under a PDMS membrane (Fig. 4a) remains moist for several hours. We also know from previous research that enough oxygen will diffuse through the PDMS membrane to support active bacterial growth.

The PDMS membrane also has the benefit of preventing contamination because the pores of the membrane are too small to allow bacteria to penetrate into the paper layer. Though PDMS is a complex chemical, it is inexpensive enough to be readily available in resource-limited environments. Finally, to hold the device layers together, we used a low-cost packing tape.

To evaluate the growth of the bacteria in our paper devices, we used a dye called PrestoBlue™. This dye reveals the presence of microorganisms, both in our paper/PDMS devices and in the environment. Whenever possible, we compared the growth of bacteria in our device with growth on traditional agar plates (Fig. 1).

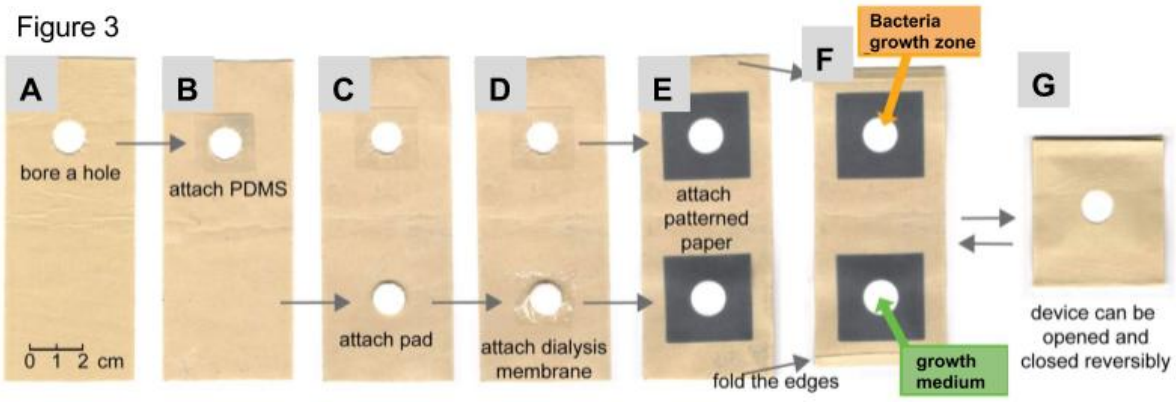
Materials and Methods

Preparation of the experimental culture device

The majority of experimental materials were purchased from nonspecific retailers- the tape, for example, was purchased from the University of Alberta bookstore. The paper was printed using a standard solid-ink Xerox Phaser printer and cooked in the oven (120 ^{O}C) for 2 minutes to permeate wax through the thickness of the paper. The PDMS solution was prepared according to the manufacturer's instructions (Dow Corning): two parts were poured in a 1:10 ratio into a paper cup (Tim Horton's) and thoroughly mixed using a plastic fork (Tim Horton's). A pre-calculated mass of the mixture was poured atop a clean, flat surface and allowed to spread to form a 1mm layer. The mixture was incubated overnight in a 60 ^{O}C oven and then cut into squares using a razor blade.

Preparation steps are documented in the figure below (Fig 3), beginning with a strip of packing tape. First, a hole is punched in the packing tape (Fig 3, step A). A PDMS membrane is stuck to the packing tape over the hole area (step B). Next, a circular paper pad is stuck on the opposite side of the tape (step C). This circular paper pad is where the growth medium was added after the device has been assembled and sterilized. A dialysis membrane is attached over top of the paper pad (step D). Patterned paper infused with wax is added over top of all of the layers on both sides of the base tape (step E). Finally, the tape is folded over on each side to provide a non-sticky lip to make opening easier (step F). Step G shows that the device can be folded over (closed) and unfolded (opened) to allow for repeated use of the device. To sterilize the completed devices, they can be placed in a beaker, covered with aluminum foil, and run through an autoclave sterilization machine. After sterilization, the devices can be stored 1-2 days prior to use.

Figure 3



Testing the experimental culture device

In the first test, we compared the growth of bacteria in our experimental device to a traditional agar plate setup (see Fig 1). Our control was developed by placing an inoculated paper pad (exposed to a concentration of *E.coli* bacteria) on an agar growth plate. The experimental device received the same *E.coli* paper pad, but this time held inside of our mobile growth device. Both samples used a paper pad that was soaked in the growth medium. Both were then incubated in conditions that are excellent for culture: a humidity-controlled chamber at 37 ^{O}C. After incubation, we determined the number of bacteria in each setup using common bacterial culture counting procedures.

The second test involved using our portable device as it is intended to be used in the field. Devices were loaded with the growth medium, sterilized, and then kept closed until the time of use. They were then loaded with 100uL of sample and cultured overnight at 37 ^{O}C to allow for bacterial growth and reproduction. To visualize the bacteria inside, the culture devices were opened and sprayed with PrestoBlue™ dye. This procedure was repeated for all surfaces tested using this method including soil, shoe soles, a bathroom door, and an elevator button.

Results

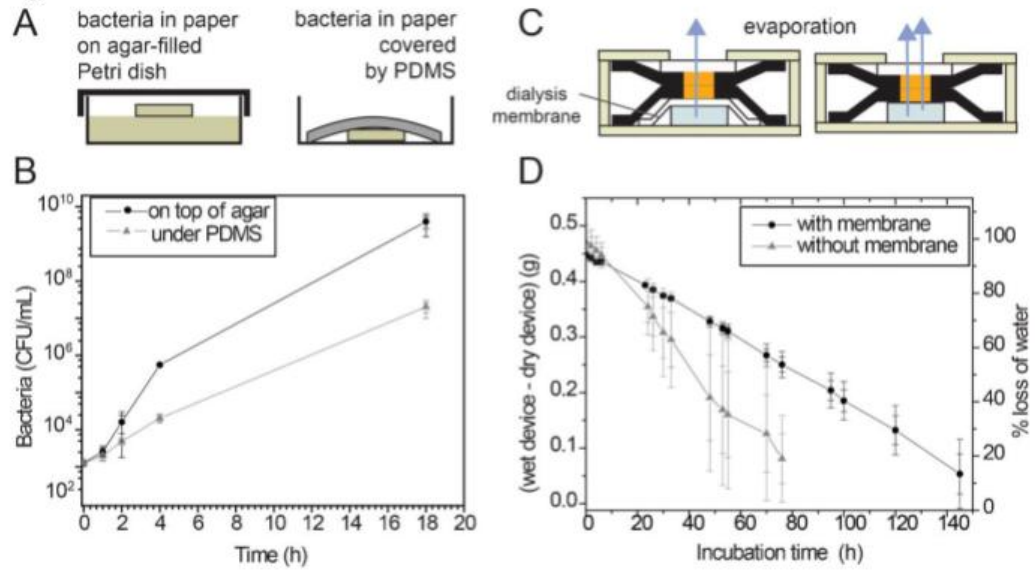
This device relies on three design elements working properly: the assembly platform based on adhesive tape, the patterned paper shaping and containing our bacterial cultures, and the PDMS sheets maintaining humidity.

Adhesive tape proved to be the simplest platform for assembly of the device. It gave us a backbone that would hold all of the device elements inside, all while allowing the device to be opened and closed repeatedly to access cultures. This was necessary to insure additional product (such as PrestoBlue™ dye) could be added as a detector at a later stage. The only issue with the adhesive tape was insuring that the paper medium attached to it remained dry at the time of adhesion. Wet paper would not adhere to the packing tape and made assembly impossible.

The patterned paper infused with wax added the necessary hydrophobic barrier to the device to prevent water from ruining the tape adhesion. This wax paper preformed its job excellently and allowed the tape to act as a durable outside layer. Although bacteria did not appear to grow on the wax-infused paper, it is known that the paper itself is non-toxic to bacterial cells.

Finally, the PDMS barrier was tested to insure it maintained humidity inside our device. To test this, a sheet of paper was soaked with a bacterial mixture in growth medium. This sheet was covered with a PDMS sheet (Fig. 4a) and showed a similar growth pattern to the traditional agar plate method (Fig. 4b). Furthermore, we tested our device using a setup with and without the semi-permeable dialysis membrane (Fig. 4c). The setup without the membrane appeared to lose water at a much more dramatic rate than the setup with dialysis membrane (Fig. 4d). Though there was a significant cost to the expensive wax-patterned paper, cheaper household materials simply didn't hold up to the assembly and use process. Usually other papers were too soft to print on, tore too easily, or deteriorated in wet conditions.

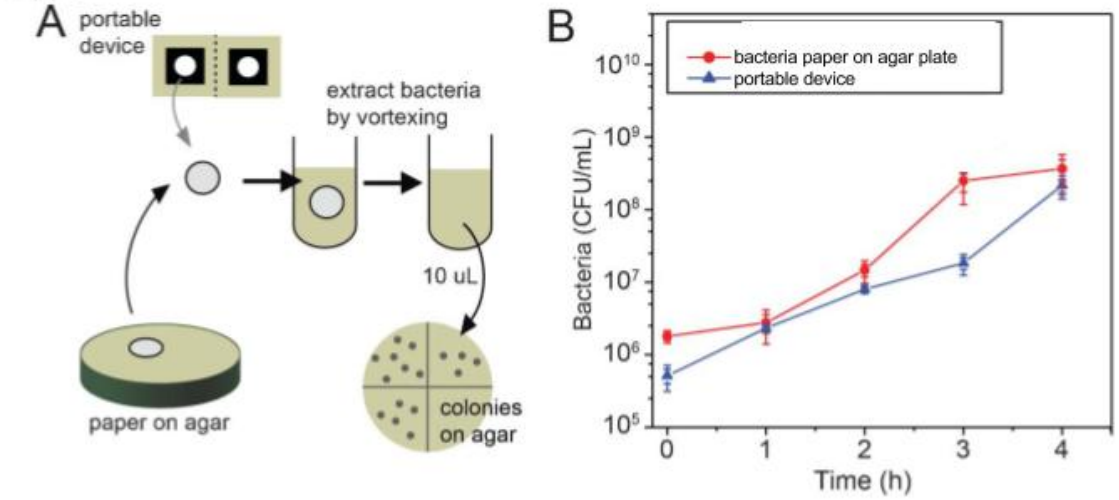
Fig. 4



Culture of bacteria in the experimental device

To test the growth of bacteria in the device, we compared the rate of bacterial growth in the experimental device to traditional Petri dish conditions. We added samples of *E. coli* bacteria in growth medium to both the experimental device and to an agar-filled Petri dish. After allowing time for the cultures to grow, we stopped the experiment and counted both cultures using a common bacterial counting method outlined below (Fig. 5a). We observed that the rate of growth of bacteria was similar in both the experimental device and in the Petri dishes, as seen after graphing the growth data from multiple tests (Fig. 5b).

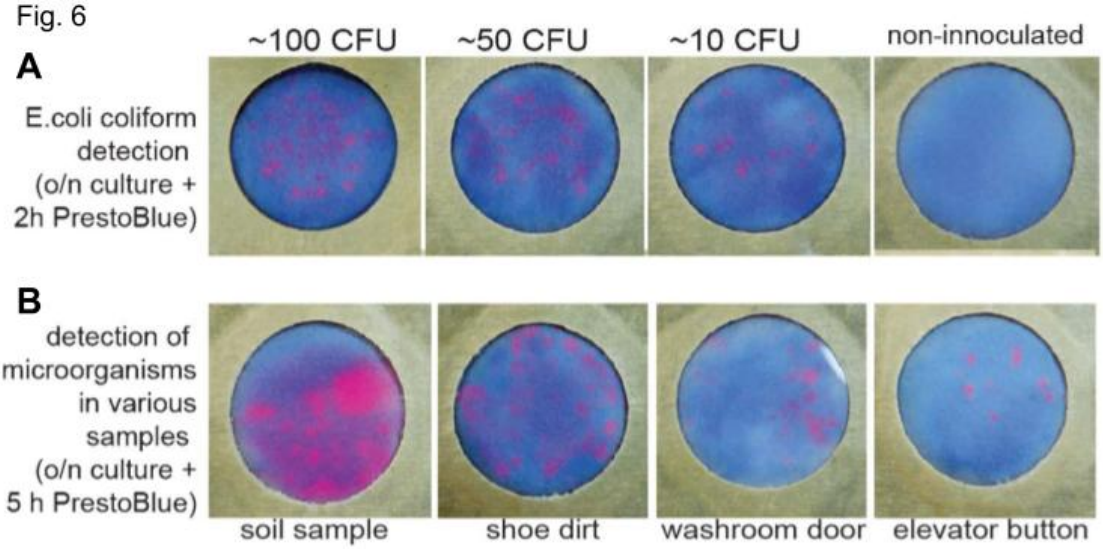
Fig. 5



Detection of *E. coli* in environmental samples

Finally, we wanted to test the ability of our device to detect environmental bacteria using the PrestoBlue™ dye. This dye goes onto a surface as a blue-colored substance. As it is absorbed by microorganisms, it is processed by them and in turn fades into a reddish color. We used a spray bottle to coat the surface of the culture zone on our device. First, we tested this dye on a known bacterial sample that was added to the device (*E. coli*) in various concentrations (Fig. 6a). This was done to insure the dye would work effectively in our device with a known quantity of bacteria. Secondly, the dye was added to samples we collected from our lab and processed by spraying with the PrestoBlue™ dye and incubating for 5 hours. Colonies (observed by the red color change) were visible as soon as 1h after the start of incubation. All of the collected samples showed varying levels of growth at the 5h mark (Fig. 6a). Initial tests in Nairobi, Kenya also showed promising levels of bacterial detection. Despite manufacturer's requirements for refrigeration of the PrestoBlue™ dye, it performed its detection job accurately after 5 days without cold storage.

Fig. 6



Conclusions

We designed a bacterial detection device that can be produced with low-cost and used in low-resource settings. These devices perform similarly to the traditional method of Petri-dish agar plates. Our devices also have the advantage of being rugged- they can be dropped, stepped on, or otherwise mis-handled without compromising their structure or their sterile environment. Though we did detect some microorganism contamination when the devices were stepped on repeatedly, this could be improved by tightening the seams of the device.

Despite their simplicity, the paper/tape-based platform could serve as a foundation for many culture-based test devices. There is also the potential to develop additional test chemicals that could be placed inside the device to survey for other environmental factors- toxins, poisons, or specific species of bacteria might be possible targets. There is also the potential for smartphone software to be used as an analysis mechanism, to be used by unskilled users that are not trained in microbiology.

Development of point-of-care diagnostic devices is a rapidly growing topic of research. Identifying alternatives to the expensive and high skill requirements of the current bacterial testing devices has not been widely explored. We found it essential to include the participation of high-school students in optimizing device production and testing of all steps described in this manuscript. This encouraged our team to develop a procedure that was easy, safe, and fast. Finally, point-of-care devices must have a low cost. Based on the retail price of materials, each device we produced cost ~10 cents and a production facility to make these devices could be established for as little as $1000-2000 dollars (mostly for the printer, sterilizer, and basic raw materials). These devices will continue to be produced and tested at an academic institution in Nairobi, Kenya. It is possible for a single, unskilled worker to produce ~500 devices per day. This could lead

to an easy, safe, and maintainable bacterial testing method to be used in low-resource countries around the world.

Acknowledgements

Dale Poon and Amanda Chrusciel (Harry Ainlay teachers) for organizing the visits of high-school students to the lab. Meriel Hughes and Rylee Mocknowed (Avanmore Junior High) and Harry Ainlay students (Bhavana, Zoey, Breanna, Tolganai) for testing the culture devices. This work was supported by Grand Challenges Canada, National Academies Keck Futures Initiative (NAKFI) in Synthetic Biology, SENTINEL Bioactive paper network, Alberta Glycomics Centre, and Canada Foundation for Innovation.