SNAP

"A System for Nucleic Acid Extraction"

for

Low-Cost Point-of-Care Medical Diagnostics

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Bryce Butcher Sunita Darbe Amit Gandhi
Mark Jeunnette Hayley Sharp Lamine Dakote

Summary

The System for Nucleic Acid Purification, or "SNAP", is a hand-held device which greatly simplifies the process of medical diagnostic testing. The SNAP System, allows DNA or RNA to be extracted from a patient's blood. This NA sample can then be analysed to check for specific diseases. Using SNAP make this process far quicker and easier to undertake than current similar diagnostic techniques.

In many rural communities, a lack of diagnostic capability, disease misdiagnosis, and poorly trained healthcare workers all contribute to this need for simple, accurate diagnosis methods. Better diagnosis allows people to receive appropriate treatment sooner, and can help to prevent the spread of epidemics. By partnering with local health centres, the SNAP System can help to bring cheap and reliable diagnostic testing to people who currently lack suitable facilities and would enable more people can be tested without having to leave their local communities. This would enable more people to benefit from medical diagnosis, and ultimately help to save lives.

CURRENT ISSUE

- Villagers must often travel 100s km to Health Centers for diagnosis

-Current testing requires pre-processing (4 hours) to extract the DNA before the diagnosis can be done

-Diagnostic testing is limited and expensive

SYSTEM FOR NUCLEIC ACID PURIFICATION

- Outputs a DNA solution from an individual's blood sample

Advantages
- Diagnostic process from DNA is more rapid and requires less skilled personnel than with blood
- Stable at room temperature (unlike blood)
- Far less regulations to adhere to when transporting DNA

use of the SNAP System could lead to better community health

COMMUNITY HEALTH

- More tests can be made at a cheaper cost, allowing for more diagnostic opportunities
- Empowerment to the local health centers
- Less lives risked transporting loved-ones to Health Centers
- Advantagous for potential epidemic situations

Contents

1. Introduction

1.1 Context

This group came together within the 2nd International Development Design Summit[1] to work on a project of importance to the developing world. We collaborated with the Klapperich Laboratory at Boston University who developed a simple method for purifying nucleic acids from samples such as blood or urine.[2] Our team's aim was to create a device that would carry out the process with as little complexity for the user as possible.

1.2 Medical Diagnostic Testing in the Developing World

Diagnostics tools allow for identification and monitoring of illnesses. In some cases, these can be very important in order to identify illnesses which do not show symptoms or to distinguish among illnesses with common symptoms. Often, rural people in the developing world do not have adequate access to diagnostic tests. More advanced health centers tend to be in urban areas where there are enough people to justify the expense of hiring trained people and buying the equipment needed for testing. For example, our teammate Guinean teammate Lamine has access to some tests only in the capitol city of Conakry, which is almost 900 km from his village. The expense of traveling to a city for testing can be great. In addition to the cost of the test itself, transportation and the work time lost in travel can also be an economic hit.

Many new diagnostic tests are being developed to address this market for rural access[3]. Easy to use tests allow untrained or poorly trained people to get accurate results from them. Quick, portable tests allow healthcare workers from urban areas to go to rural areas and quickly run necessary tests.

The System for Nucleic Acid Purification (SNAP) pictured above provides another tool to increase rural access to diagnostics. The machine simplifies sample preparation for tests based on nucleic acids**.** By partnering with local health centers and developers of nucleic acid-based diagnostic tests, SNAP can give low income and rural communities access to more sophisticated tests that are simplified enough to be easy to use in the field—enabling more people to benefit from medical diagnosis and ultimately helping to save lives.

[1] http://iddsummit.org
[2] Anal. Chem. 2006, 78, 788-792
http://nanoscience.bu.edu/papers/AnalChem2006_Bhattacharyya.pdf>
[3]http://www.rapid-diagnostics.org, Nucleic Acids Research, 2000, Vol. 28, No. 12 E63-e63 (http://nar.oxfordjournals.org/cgi/content/abstract/28/12/e63), See section III for a summary of these existing technologies

1.3. Laboratory versus Clinical tests

Diagnoses of illnesses are often made based on symptoms. This approach is reasonable if a disease has clear and distinctive symptoms or if the exact cause does not matter as much as treating the symptoms. However, diseases that do not show symptoms and those with common symptoms cannot be accurately diagnosed based on how a patient feels.

The guidelines developed by the World Health Organization (WHO) for treating suspected cases of malaria when diagnostic tools are not available are to begin drug treatment. This often leads to the use of expensive malaria drugs when it is not necessary.[4] Misdiagnosed patients are also at risk of having side effects from the drugs they are given. If adequate tools were available to confirm the identity of the illness ahead of time, resources could be better used.

Syphilis is an example of a disease that might not show symptoms in women, but if a woman with syphilis becomes pregnant, it can significantly increase the chances that the baby will be stillborn.[4]

1.4. Molecular Diagnostics

Laboratory diagnostic tools are important for identifying and monitoring illnesses. There are many different kinds of diagnostic tests. For bacterial infections, traditionally, one grows a culture and then identifies the pathogens visually using a microscope. This requires a skilled user for microscopy and equipment and infrastructure such as a microscope and fridge and a regular electrical connection. Cultures can take from several days to weeks to be effective. If people are coming from far away to get tested, they may not be able to come back again later for results.

The next generation of tests used now search for antibodies that one's body creates in response to an infection by a particular type of pathogen. Antibody tests require less skill and equipment in the lab. They can also be packaged into very easy and cheap portable tools, such as strip tests, making them a good choice for the developing world.[5] Many of this kind of simple protein based tests, however, give simple yes or no responses to the presence of pathogens, meaning that one cannot monitor the progression of an illness or distinguish between past and present infections. Additionally, antibody tests cannot distinguish among different strains of the same illness.

Molecular diagnostics are based on identification of the DNA or RNA of a specific pathogen. These tests are quantitative and can differentiate between current and past infections. They can also distinguish among different strains of same infection. With increased drug resistance, this is an important capability. More virulent, multi-drug resistant strains of illnesses can be identified straight away, so that the appropriate, intensive treatment can be given. This type of testing is not used often in rural or underdeveloped areas because traditional methods require expensive equipment and training.

The SNAP nucleic acid extraction machine, however, makes sample preparation for these tests quick and simple. The preparation can be done in the field and the purified nucleic acids can then be transported to the higher level labs. It is easier to transport nucleic acids than blood because the latter needs more temperature regulation and can be more dangerous to transport as infectious diseases can be passed through blood but not through nucleic acids. Also, if the SNAP machine can

[4] Nature Reviews: microbiology, Volume 2, March 2004, pp231
[5] http://www.rapid-diagnostics.org

be coupled with a nucleic acid amplification test (NAAT), it can allow healthcare workers with less training to carry out more sophisticated tests in the field. Research on such technological couplings is on-going. See Appendix III for a list of possible NAATs that can be used.

1.5 How the SNAP System Works

The process involves pushing a sample from the test subject, a chaotropic (cell breaking) buffer, ethanol and water through a narrow tube. The sample can be blood, urine or some other source of nucleic acids. The chaotropic buffer is used to denature the cell membrane, freeing the nucleic acids from the rest of the cell. The natural negative charge of the nucleic acids allows them to bond to the positively charged silica particles lining the interior of the tube. Ethanol flushes away the other parts of the cell which are not bond to the tube. Finally, high purity distilled water is run through to dissolve the nucleic acids into a collection chamber. The SNAP machine has a simple interface to do these steps.

This process is able to separate out and purify nucleic acids from all types of viruses, gram negative bacteria, and parasitic pathogens. In its current state, it does not work for Gram positive bacteria.

1.6 Advantages of using the SNAP System

As the needs of medical equipment for use in the field in developing countries are very different to the needs of medical equipment for use in the developed world or in laboratories, the using the SNAP System to produce purified DNA or RNA samples rather than having untreated blood or urine samples has two main advantages.

Firstly, DNA solution is easier to transport to a central laboratory rather than transporting blood samples, due to the increased robustness, shelf-life, and decreased paperwork involved.

Secondly, molecular diagnostics require the DNA or RNA to be extracted from the blood sample before the diagnostic tests can be carried out. Using a hand-held device in the field to perform this step will make the whole diagnostic process more rapid and require less skilled personnel, thereby reducing the burden of laboratory resources, allowing more tests to be performed more cheaply, and ultimately allowing more people to be diagnosed.

As the device does not perform diagnostic testing, but only prepares a sample for diagnosis, it must be used in conjunction with a laboratory which has suitable equipment for molecular diagnosis.

Issue	Blood Sample	DNA Sample
Transportation (to central laboratory)	- Requires refrigeration - Slow shelf life - There are many regulations regarding blood which have to be adhered to	-Stable at room temperature - Longer shelf life - Far less regulations to adhere to when transporting DNA (compared to blood)
Sample Analysis (in Laboratory)	- Requires pre-processing (4 hours) to extract the DNA before the diagnosis can be done	- Can be used for diagnosis easily by a relatively unskilled employee

2. Design Considerations

A number of design issues were considered throughout the design process. These issues were brought up through both personal interviews and library and online research.

2. 1 Discussion

1. Use by low-trained Personal

If this device is to be used by local volunteers, the process needs to be simple and unambiguous. Some of the people using this device may be medically trained; however, many people will be relatively untrained health volunteers. Therefore, we are aiming to have this device easy enough to use for anyone who have finished high school, or has a comparable level of education.

In many parts of the world, it is particularly important to have a simple-to-use device because many health professionals are not particularly skilled. For example, high levels of corruption in some parts of Africa means that, a person who is intellectual but doesn't have a health education can become a doctor quite easily if they have friends or parents who works in the health sector - Laminebadionkote@yahoo.fr.

2. Biohazardous Waste in Developing Countries

Waste disposal in many developing-country clinical facilities is often rudimentary – for example, any supplies such as syringes are often recycled either back into the clinic or even into the market as toys for children.

All biohazardous materials from the device must be safely and securely contained, and disposed of suitably. Lancet needles should be retractable or covered after use so as to reduce the risk of infection. Everything that is used for the patient should be used only once, to prevent spread of HIV and other diseases. The use of an incinerator at the health facility can be reasonably cheap, and is generally suitable to use for disposal of biohazardous waste.

Representative photographs to illustrate the importance of biosafety and environmental impact in medical product design for low-resource settings. Photos *a*–*c* were taken at health care facilities. (*a*) An open burning pit for medical waste in Senegal (PATH). (*b*) An incinerator overflowing with medical waste in Tanzania (PATH). (*c*) An open medical waste burner in Nigeria (PATH). (*d*) Needles and syringes in a public waste dump in India (Mark Koska). (WHO, 2008) [6].

[6] Point-of-Care Diagnostics for Global Health, Yager, 10.1146/annurev.bioeng.10.061807.160524

3. The device as part of a larger supply chain

This device needs to be considered as part of a larger supply chain. For the device to be useful and to be used, a number of criteria need to be fulfilled. There must be a reliable supply of reagent packs and other disposables available to the laboratories, as without sufficient disposable materials the device is useless. There will also need to be a network to allow the samples to be delivered to the laboratory for diagnosis, and the results then returned to the health worker in the community. The diagnosis results must be confidential, as in many cases, if a person is known to have a particular disease (such as HIV), they can become alienated by the community.

The regent packs should have a long shelf life, and be stable at room temperature. The other disposable parts (the lancet, straw, and waste and sample containers) must also be readily available. If possible, the device should be designed so that the lancet and the waste and sample containers can be purchased locally if necessary.

2.2 Design Criteria

The summarised Design Criteria are as follows:

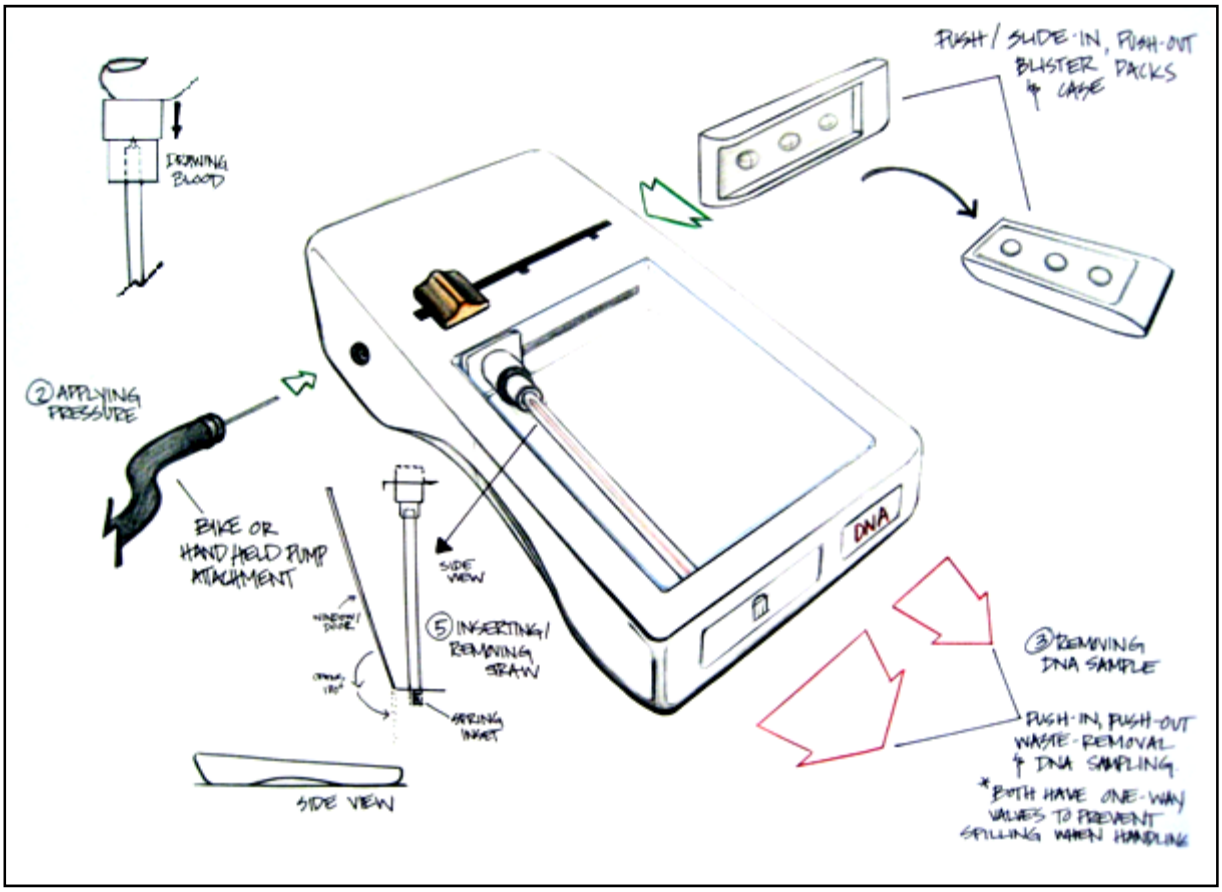
Design Criteria	Details	How to implement?
Ease of use	The device should be useable by non-medically trained personal	Minimise the number of steps required by the user. Make the device simple and obvious to use – for example, by indicating clearly where the user should press or put their hands.
Short processing time	The device should be able to process a sample within 30 minutes (and shorter processing times are beneficial)	Higher air pressures will allow shorter processing times, however higher pressures will require high-spec seals and materials.
Low cost	The device should cost around $30 USD (not including disposable parts)	
Fail-safe	If the device breaks, then all biohazardous materials must be safely and securely contained.	The parts of the device containing biohazardous materials must be strong.
Robust	The device should be suitable for use in the field.	Device should be able to withstand mild knocks, be water-resistant, and potentially be tamper-proof.
Size and Weight	The device should be suitable for carrying in a rucksack	Weight < 3kg Size < 0.3m x 0.3m X 0.2m (approx)
Adaptable	Ability to use a local bicycle pump or electrical pump if available.	The air valve should be universal or come with adapters.
Ease of sourcing disposable parts	All disposable parts (e.g. lancet, collection vials) must be easy to source and if possible, should be available locally.	Use cut-away foam so that each user can adapt the collection drawer to his type of sample collectors.
Long-shelf life and robust reagents	Any parts with a finite shelf life should have a minimum life of around one year, and the reagents should be able to withstand changes in temperature.	

3. Prototype Designs

Three main designs were considered, with the single tubular design going through to prototype stage.

3.1. Design Ideas

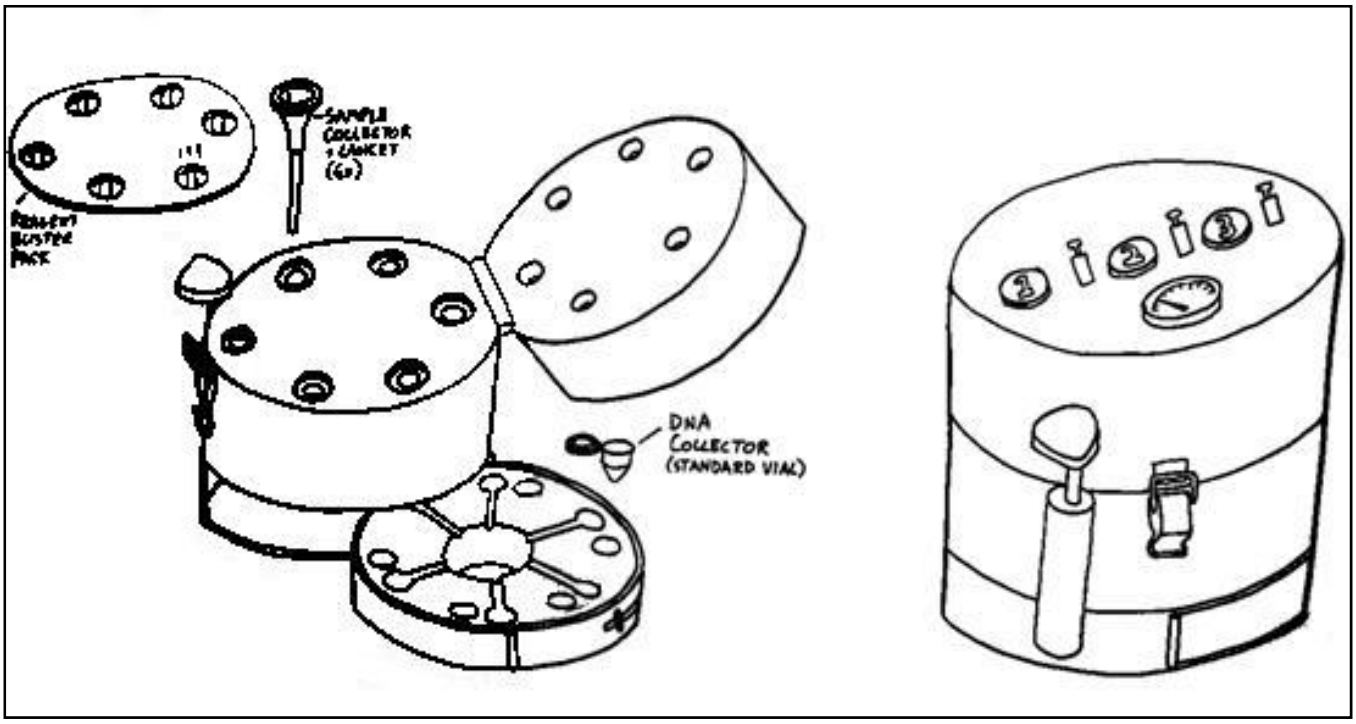
3.1.1 Design Idea: Rectangular Design



This concept uses a rectangular casing to process one sample at one time. Design features include:

- single Reagent Blister Pack per sample
- attachable or removal pump system
- single Waste Collector for all Sample Collectors
- simple operation indicated by buttons, symbols and gauge
- fail-safe waste and sample collection system

3.1.2 Design Idea: Multi-Tube Design



This concept incorporates multiple Sample Collectors into a single DNA extraction device, allowing parallel processing of the samples. Design features include:

- single Reagent Blister Pack for all 6 samples
- attached pump
- single Waste Collector for all Sample Collectors
- divots to allow the use of standard microtubes as DNA Collectors
- simple operation indicated by buttons, symbols and gauge

4. Final Prototype – Single Tube Design

4.1. The Design

4.2. How to use the SNAP System

4.3 Manufacture

Part	Manufacturing Process
Body of Device	Injection Molded, FDA approved, in India or China for specifications and costs.
Straw Collector	Extrudable thermoplastic parts; could also be made in India or China, however they need to be inserted with a silica substrate into a small internal tubing using UV cross linking, that needs to be done in the US
Seals	Still under consideration. O-rings will most likely be used to provide the airtight seal.
Blister Packs	Since blister packing is robust and available in many developing world countries, we would be likely to manufacture these in the country of use, thus eliminating shipping costs and increasing reliability

4.4. Issues Still to be Addressed

Although the prototype design has addressed many of the design considerations, there are a number of significant issues that still need to be addressed. These key issues include:

- sealing options
  - o-rings, clamp forces, and part interfaces
- reagent blister pack
  - shelf life
  - puncturing
- sample collector
  - lancets and blood collection
- air filters
- manufacturing
  - capabilities in developing countries
- packaging and "kit" assembly
- regulatory approval
- biohazard waste
  - one-way valves and technician protection
  - disposal
  - addressing the issue of reuse in certain settings
- interaction
  - user feedback upon completion of each step
  - reducing the amount of interaction required
  - design for disassembly/cleaning

5. Implementation and Dissemination

5.1. Partnership with NAAT

While continuing the technical and design research, the SNAP team is also thinking about how to disseminate this tool in the developing world once it is complete, and has a partner nucleic acid amplification test (NAAT).

The SNAP+NAAT system allows a patient to give a blood sample and get back a quantitative, accurate result in about an hour and a half making it ideal for field or laboratory use. At this point, there are two market approaches we are considering depending on the health regulations of the target country. The first is to market the tools to a local entrepreneur who can create a village diagnostic center in cooperation with the nearest health facilities. In areas with tighter regulations, we will approach intermediate and lower level labs which cannot afford and/or do not have the trained personnel for traditional NAATs. Regardless of the exact buyer, training can be provided by the employees of SNAP, who will also be points of contact for replacement of the disposable parts of the system.

For the initial pitch in a country, we will approach the more frequented clinics there with our product. We will offer use of our machines on a trial basis to get a local understanding of the strengths of the SPAN+NAAT system. With this tool in the hands of lower level labs or village level diagnostic centers, the burden on the central clinics will be reduced allowing them to attend to more skill intensive procedures. People can also give us good feedback on the training program and the device itself.

5.2. Enterprise Models

Village Diagnostics Enterprise Model

The SNAP+NAAT group can incubate the small entrepreneur with the cooperation of the closest lab. If there is someone in the villages, we need to make sure they are reliable and that the villagers will trust them. The whole process should be made as simple as possible so that one to two weeks of training are adequate for competent use of the tools.

Equipping the Health Center Model

Equipping rural labs with diagnostic capabilities allows them to do diagnostic tests closer to home instead of having to refer people to higher level laboratories which are usually farther away.

5.3. Costs and Profit Potential

Each SNAP+NAAT diagnostic test can hopefully be provided at a cost of $2 per test. Some market research has been done in Tanzania where the sales approach would be geared toward equipping health centers. There are about 3000 C level (lower capability) servicing about 10,000 to 20,000 people each. If we have 10% penetration into the market, we will sell 300 units in TZ. If we produce units at a cost of $30 and sell at $50, then there is a total of $15,000 for the machines themselves. Beyond this, marginal profits on the disposable parts from each test will provide ongoing funds to

the company. If each machine is used to do 20 tests per day 300 days of the year (i.e. 6000 tests on 10,000, to 20,000 people each year) on each of the 300 machines with a profit of 10 cents added to the disposable supplies for each test, the company will take in a total of $180,000 in gross profit annually.

5.4. Stakeholders

Stakeholder	Key Points
High level labs	Will they lose jobs or incoming by decentralizing testing or will they appreciate that the method removes some of the testing burden?
Local healthcare staff:	Have to support the local entrepreneur, and respect their results
Local entrepreneur	to have the trust of the medical establishment in the area
National Government	about untrained individuals doing health-related work
Patients/local community	need to be okay being tested by local, non-professional diagnostician; need to be okay with disposal methods
NAAT development partner	need to be on board with coupling their technology with ours.

Appendix 1:
Current Status of Diagnostic Equipment in the Developing World

Conventional laboratory diagnostics are of limited availability in the developing world. Labs are constrained because of the cost of equipment for conventional tests and lack of well-trained staff. Often diagnoses are based on symptoms which can lead to overdiagnosis of common illnesses such as malaria. Misdiagnosed patients are at risk of side effects from the drugs they are given and from worsening condition due to the illness that they do have that were not diagnosed. Additionally, drug resistances can develop when people are given inappropriate antibiotics.

Many alternatives are being considered for methods of providing point-of-care clinical diagnostic testing. Various types of rapid diagnostic tests are in use currently. First, flow-through tests involved flushing a set of reagents, starting with the sample (blood, urine, etc) to be tested through a membrane held in a cassette-type frame. This type of test does not run on its own. It can be done one or a few at a time. It requires a liquid sample. Kits are available for flow-through diagnostic tests. Secondly, there are strip tests that work like pregnancy tests by giving the user a clear two lines for yes, one line for no and no lines for a null result response. Liquid and semisolid samples can both be used, and the tests, once set up can be set aside to finish. The tester does not need to provide any additional attention. Thirdly, in solid phase tests the sample is placed on a solid substrate, which is incubated. Then, it is washed be a series of reagents which contain identifiers (EIA, protein A, sensitized latex particles). Finally, agglutination tests, which give visual confirmation of the presence of a pathogen based on the creation of an agglutinate are also available. These tests are difficult in cases of weak presence of an antigen. They can be hard to interpret in such cases. All of these types of tests search for protein matter. This means that assay search the sample for either antigens directly or for antibodies the host has produced against an antibody. This type of assay has some disadvantages. For example, one cannot distinguish between a current and past infection[7]. There is also a wide range of sensitivity [8].

Other methods include microscopy which requires having a microscope and a skilled operator. Microscopy can be used for "parasitic and mycobacterial" infections[9]. Bacteria are grown and cultured in labs for more rigorous tests. This can involve incubation of up to weeks for certain illnesses 2008) [9]. Cultures however allow drug resistance testing[9]. The downside to this type of testing is that there are strict storage requirements for the samples, there is a lot of infrastructure that needs to be in place for it to be viable (uninterrupted electricity, trained staff), and it is expensive. Nucleic acid amplification tests (NAAT) are highly specific and sensitize and are viable for multiple types of pathogen (bacteria and viruses). However, for conventional NAAT methods one needs specialized equipment and training. Avoiding contamination is a big concern with this type of test.

Our method takes only liquid samples. It only extracts DNA, after which some sort of test must still be performed on the product to give a diagnosis. We cannot store results for reference. Nucleic acid based tests allow for monitoring drug resistance of samples. While antigen/antibody tests can test for the presence of a disease, they cannot test for particular drug resistances whereas molecular tests can provide this information.

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[7] http://www.rapid-diagnostics.org
[8] www.who.int/std_diagnostics/publications/Diagnostics%20for%20the%20developing%20world.pdf
[9] FIND website said that ideal time for TB was 6 weeks.

Appendix 2: Proof-of-Concept Testing

These experiments were undertaken to prove the concept of a potentially hand-pumped nucleic acid extraction system. The test covered the potential use of a bicycle pump, the feasibility of pressure sealing, and the fluid flow through the device.

4.1 Suitability of Bicycle Pump Experiment

A water bottle was purchased, a hole drilled and a bike tire valve inserted and sealed. A hole was also drilled to insert and secure the straw.

The experimental setup consisted of:

- Basic water bottle
- Sample straw and clamp
- Bicycle pump
- Epoxy resin to secure the straw

Conclusions:

- A bicycle pump can easily be used to pressurise a container
- At 60psi, fluid was forced through the sample straw as desired.

4.2 Pressure Sealing Experiment

In the pressure sealing experiment, we tested to see if a clamping mechanism would hold air at 60 psi, and what the points of failure would be in such a setup. To easily isolate them, we scaled up our test chamber to having a 4 in OD. At a scale this large, the stress on the edges of the base would be increased by an order of magnitude causing the plastic to deform more readily. We decided to fasten the two pieces using C-clamps to see if they would be adequate.

The experimental setup consisted of:

- 4 in ID threaded PVC hub
- 4 in OD treaded PVC cap
- 4 in OD PVC pipe
- ¼ in acrylic sheet
- Rubber sheet
- Schrader valve from a bicycle inner tube
- Various C-clamps
- Teflon tape

We followed the following steps to create the apparatus:

- Drill a 8 mm hole in the center of the PVC cap and epoxy the Schrader valve through it
- Fasten the cap into the hub by screwing it in.
- Press fit the 4 in OD pipe into the non-threaded side of the hub.
- Hacksaw the 4 in OD pipe until it is flush with the base of the hub.
- Cut and glue rubber to the base of this cylinder.
- Clamp the acrylic sheet to the base of the system.

By the end of this process the apparatus should look like this:

We then pumped the chamber up as high as we could. After we detected air loss as the pressure was dropping at pressures greater than 40 psi, we filled the test chamber with water to observe where the leakage was occurring.

Conclusions:

- The seal failed in regions where it was not clamped due to the pressure deflecting the acrylic sheet. Therefore, we need to keep in mind that the base plate should be thick enough to not deform under high pressures.

- The threaded seal failed when we did not use Teflon tape. As a result, we need to use a steep thread for threaded components because we will not be able to use Teflon tape as a disposable piece.

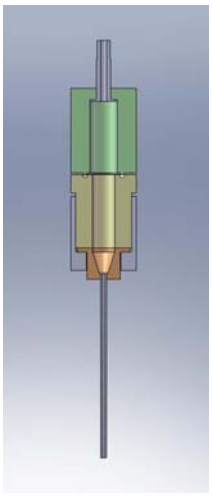
- Rubber sheeting provided an adequate air/water tight seal when fastened adequately. We will most likely default to O-rings for our final projects.

- A standard bicycle pump only requires a few pumps to pressurize a significantly larger volume to 60 psi, showing that it is feasible to make our device bicycle-pump pressurized.

We also speculate that the fluid flow experiment will give us much better insight into the specifics of dimensions we would need which would allow us to choose parts and dimensions more accurately.

4.3 Fluid Flow Experiment

The fluid flow prototype is intended to show that we can maintain the necessary seals to push the required reagents and sample through the straw reliably. Two different types of seals - hinged o-ring clamp and simple clamp ring – illustrate different options which can be used in further iterations of the device. This device illustrates the first attempt to take the DNA extraction process out of the Klapperich Laboratory, and thus is as simple as possible while retaining the use of the essential elements of the process (Reagent Blister Pack and Sample Collector).



The experimental setup consisted of:

- Schraeder bicycle valve
- inflation cap
- reagent blister pack
- main cylinder
- clamp ring
- funnel
- straw