Design and Evaluation of a Device for Trapping Hepatitis C Viral Particles at Ultra Low Concentrations

by

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ABSTRACT

A new method to quantify hepatitis C (HCV) viral particles when present in ultra low concentrations is being developed. Hepatitis C is a viral infection that affects the liver. There are 3.2 million people in the United States with an active hepatitis C infection. Untreated HCV can lead to cirrhosis, liver failure and liver cancer. HCV treatments can be very costly and physically taxing for patients; the side-effects of treatment are comparable to persistent flu-like symptoms. Physicians are looking to shorten the duration of the standard treatment, typically 24 to 48 weeks, for patients who respond quickly. Physicians must have more sensitive testing equipment to truly know when a patient has been cured and be able to successfully shorten the length of treatment. Current diagnostic tests are insufficiently sensitive when the patient begins to positively respond to treatment and the amount of the virus present in his/her blood dramatically decreases. This limitation can be overcome by employing an *in-vivo* sampling technique, where a device is placed in a vein to trap HCV viral particles present in the blood. These particles are then subsequently quantified with a commercially available test. This technique allows at least 40,000 times more blood to be sampled in 30 minutes than with a traditional blood draw, greatly increasing the effective sensitivity of the test. The approach provides significant medical benefit to the patient being treated and a strong financial incentive to the entity paying for the treatment.

Thesis Supervisor: Michael J. Cima
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Introduction

Hepatitis C (HCV) is a viral infection that causes inflammation of the liver (location of liver shown in Figure 1)[1]. The disease is usually spread through contact with the blood of an infected person. Treatments for hepatitis C range from 24 weeks to 72 weeks depending on genotype and the patient's response to treatment. Hepatitis C can be classified into two groups. Acute hepatitis C describes the virus when it is within six months of the initial infection. Chronic hepatitis C describes the virus when it progresses beyond the initial six month time window. Acute hepatitis C becomes chronic hepatitis C for 75%-85% of people infected. The remaining 15%-25% of people are able to clear the virus from their bodies without the need for treatment; it is unclear why this happens for some people and not others.

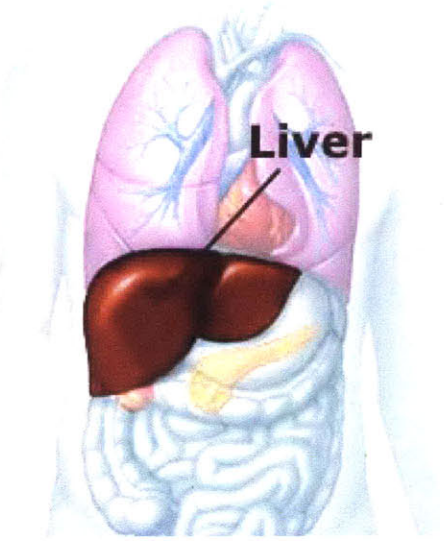


Figure 1 shows the location of the liver, the site of replication for hepatitis C.

Statistics of the Hepatitis C Infected Population

Each year about 17,000[2] to 35,000[3] people are infected with HCV; not all of these people are aware they are infected. Roughly 65,000[4] people are treated yearly for an HCV infection in the United States. This includes some new infections as well as past infections that are newly diagnosed. There are currently 4.1 million people in the United States who have HCV antibodies, as well as another 170 million worldwide. Antibodies are an indication that one currently has an active HCV infection or has had one in the past. HCV antibodies will remain even if a patient no longer has an active infection. It has been estimated that 3.2 million people in the antibody positive population have an active infection. One study has found that as many as 80%[5] of people with an active infection do not know that they are infected, presenting a serious global health problem because many people unknowingly expose others to hepatitis C. It has been suggested that the currently available HCV statistics may underestimate the problem

because many high risk populations are often not counted. The HCV infection rate has been observed to be higher than average with prisoners, intravenous drug users and the homeless. A study from 2005 estimated that 42% of homeless veterans are infected with HCV; this is much higher than the roughly 1.5% of the general population.[6] Another study shows that the prison population was observed to have an infection rate between 15% and 41%. The complications of hepatitis C can be severe if the disease goes untreated. HCV was the leading cause of liver cancer and liver transplants in the United States as of 2009.[7] The need for a liver transplant imposes two serious burdens on society. It limits the supply of healthy livers available for transplant for non-HCV related patients and costs roughly $400,000 (transplant and follow-up care)[8].

There are six genotypes of hepatitis C, numbered 1 through 6. Genotype 1 accounts for roughly 75% of all cases in the United States and 60% worldwide. Genotypes 2 and 3 account for about 10%-20% of all cases, while the remaining cases are classified as genotypes 4, 5 and 6. Most research in the United States focuses on genotypes 1 through 3 because of their prevalence in the US.

It is expected that by 2020, one million HCV patients will have cirrhosis.[9] Each year 10,000-12,000[10] deaths are attributed to hepatitis C and this number is expected to triple by 2020.[11] Projections show that the risk of cirrhosis, liver cancer and liver related deaths can be reduced by 16%, 31% and 36% respectively with the use of currently available drugs to treat patients.[12] The development of new therapies and diagnostics will only help to improve these numbers.

Transmission of Hepatitis C

There are many sources for contracting the hepatitis C virus, and it is not always possible to determine how a patient contracted the virus. However, the majority of HCV cases are a result of injection drug use (at least 60 %);[13] even if it was infrequent or well in the past, it can still be the source of the infection. Many medical procedures that are considered routine today have, in the past, exposed patients to HCV because there were not sufficient tools to screen blood transfusions and organ transplants. Patients who had blood transfusions before 1992 have an increased chance of being infected with HCV. Procedures such as receiving clotting factors, specifically prior to 1987, and hemodialysis for kidney failure can also increase a patient's

chance of contracting HCV. Birth to an infected mother remains a source of transmission, but accounts for less than 5% of total cases. Accidental needle sticks from an HCV infected person can increase one's chance of being HCV positive and pose a particular risk to healthcare workers. While they are at a higher risk for becoming infected, healthcare workers typically have a high rate of successful treatment because treatment begins almost immediately after the initial infection.

Diagnosis of Hepatitis C

There are three types of tests that are key to the treatment of hepatitis C. The first test administered to a patient who is suspected of having an HCV infection is an HCV antibody test. This is typically the first test because of its low cost compared to other diagnostic tests available (about a third to half the price). A positive antibody test is indicative of a past or present HCV infection. A follow-up test is required for anyone testing positive for HCV antibodies to determine if the infection is still active. Confirmation is done with an HCV RNA test. If a patient is positive for HCV RNA, it is an indicator of an active infection. There are two critical results obtained from an RNA test. First, the HCV genotype is determined. This helps to predict the success of treatment and dictates the duration of treatment. The second use for the HCV RNA test is to determine the concentration of virus present in a patient's blood, clinically termed viral load. The viral load prior to treatment, like the genotype, is also a strong indicator of the potential for a successful course of treatment. If the viral load is low (below 2 million viral particles/mL), the success of treatment is higher than if it is high (above 2 million viral particles/mL). The last test used is a liver biopsy. Liver biopsies are used to determine the extent of liver damage. Liver biopsies provide useful information regarding the extent of liver damage the patient has experienced. It is important to note that successful eradication of the virus does not undo existing damage to the liver. HCV RNA testing is repeated as frequently as each week during treatment to monitor a patient's response to the drug regimen.[14] It is recommended that the same HCV RNA test be used throughout treatment to avoid the inherent variations of different tests.[15]

Current RNA Testing Equipment

The table below shows the sensitivities, costs and manufacturers of current HCV tests. Because the proposed device is only used to trap the viral particles, one of the tests listed in Table 1 will need to be used to quantify the viral load. These HCV tests range between $225 and $780, depending on whether the physician is trying to quantify a high viral load or a low viral load. The first step of any testing regimen will be to use a traditional blood draw to quantify the viral load.

Manufacturer	**Description**	**Technology**	**Approximate Cost**	**Detectable Range (Viral Particles /ml)**
Abbott - *RealTime* HCV	Quantitative	PCR	$370	30 - 250,000,000
Quest Diagnostics	Quantitative	PCR	$420	108 - 173,000,000
Quest Diagnostics	Quantitative	TMA	$225	13 – 18,750
Quest Diagnostics	Quantitative and Genotyping	PCR	$420	108 - 173,000,000
LabCorp	Quantitative	PCR	$780	5 – 5,500,000
Roche - COBAS® AmpliPrep / COBAS® TaqMan®	Quantitative	PCR	$370	108 - 173,000,000
Quest Diagnostics/Siemens – Versant	Quantitative	bDNA	$420	1538 – 19,250,000

Table 1 shows the currently available HCV quantitative diagnostic tests.

Patient Classifications in Response to Treatment

Patients undergoing treatment are split into four groups based on how they respond to the treatment: patients who have a rapid virological response (RVR), those who have an early virological response (EVR), those who have an end-of-treatment response (EOTR) or non-responders. Patients who have an RVR develop an undetectable level of the HCV virus at the 4-week point of treatment. Patients with an EVR have a detectable level of the virus at the 4-week point, but develop an undetectable level of the virus by the 12-week point of treatment. End-of-treatment response is defined as patients with an undetectable level of the virus at the end of treatment (48 weeks for genotypes 1, 4, 5, and 6, and 24 weeks for genotypes 2 and 3). Non-responders are patients who have a detectable level of the virus or who have not achieved a 2-log drop in viral load at the 12-week point.

Patients, in the long term, are divided into two groups. Those who maintain their undetectable level of the virus six months after the treatment ends are defined as having a sustained virological response (SVR). Patients who test positive for HCV six months following the cessation of treatment, after having a negative result at the end of treatment, are defined as having a relapse. Patients who achieve SVR are considered "cured."

Drugs to Treat Hepatitis C and Their Side-effects

Currently hepatitis C is treated through a combination of Peginterferon and Ribavirin. Peginterferon is self-administered by the patient once per week. There are two forms of Peginterferon, alfa-2a and alfa-2b. Peginterferon alfa-2a (Genentech USA Inc.) is delivered subcutaneously in a fixed dosage of 180 micrograms (μg) per week. Peginterferon alfa-2b (Merck, previously Schering-Plough) is also delivered subcutaneously, but as a weight-based dosage. A dose of alfa-2b is 1.5 μg/kg; the weekly dosage typically ranges between 75 μg and 150 μg. Ribavirin is used as a supplement to increase the rate of successful treatment unless a patient cannot handle the side-effects.

Ribavirin, when used in combination with Peginterferon, increases the rate of sustained virological response from an average of 35% to 55%. Ribavirin is delivered orally twice a day in 200 mg capsules. Patients who weigh less than 165 lbs have a standard dosage of 1,000 mg (5

capsules); patients who weigh more than 165 lbs have a standard dosage of 1,200 mg (6 capsules).

Each of these drugs has a unique set of side-effects. Common side-effects of Peginterferon occur in more than 10% of patients and include: fatigue, nausea, vomiting, weight loss, depression and mild bone marrow suppression, as well as additional side-effects. Most of the side-effects are mild to moderate in severity. Patients describe the side-effects as flu-like symptoms. The dosage of Peginterferon is altered, in rare cases, because of the severity of the side-effects experienced by the patient. Ribavirin has additional side-effects that include anemia, itching and skin rash as well as nasal stuffiness, sinusitis and a cough. The use of Ribavirin in combination therapy can cause heart attacks and strokes in rare cases due to a drop in hemoglobin. Ribavirin is, therefore, not used in patients who are predisposed to cardiac complications.[16] These side-effects can prevent people from taking on daily tasks such as working and driving.[17]

Duration and Cost of Treatment

Treatment of hepatitis C can last between 24 and 72 weeks, depending on the genotype that the patients are infected with and their response to treatment. The standard treatment, for genotype 1, lasts 48 weeks and for genotypes 2 and 3, 24 weeks. A 48 week treatment costs roughly $30,000.[18] Currently it is recommended that treatments only be shortened if a patient cannot handle the side-effects of the drugs. Genotypes 4, 5 and 6 are relatively rare in the United States, but are treated with 48 weeks of the medication. When a patient is slow to respond, but eventually reaches an undetectable level of virus, the duration of treatment can be extended for an additional 24 weeks at the physician's discretion.[19] New HCV treatments may change the length of treatment for HCV patients, but the exact length of new combination treatments will not be known until after the end of clinical trials.

Emerging HCV Treatments

There are two competing HCV drugs that are currently undergoing clinical trials. Telaprevir, manufactured by Vertex Pharmaceuticals and Boceprevir, manufactured by Merck are both undergoing Phase III trials. Both drugs would be used in conjunction with the current standard treatment of Peginterferon and Ribavirin. These new drugs aim to increase the cure rate for

HCV and in some cases shorten the time a patient may need to receive treatment. Clinical trials show that these therapies also have the ability to successfully retreat past patients who did not respond to previous treatment regimens as well as those who relapsed. The impact of these new treatments on the use of the proposed device will be discussed in detail in a later section.

Funding for HCV Research

Research for hepatitis C, for both drugs and diagnostic tools, is heavily funded through the NIH (National Institutes of Health) and NCI (National Cancer Institute). The NCI is a division of the NIH. The NIH allotted $100 million for hepatitis C research in 2010. There was also additional funding from the ARRA (American Recovery and Reinvestment Act) in 2009 and 2010. Funding from the ARRA is about 10% of the total funding for hepatitis C research, but does not extend into 2011. The funding per year is shown below in Figure 2.

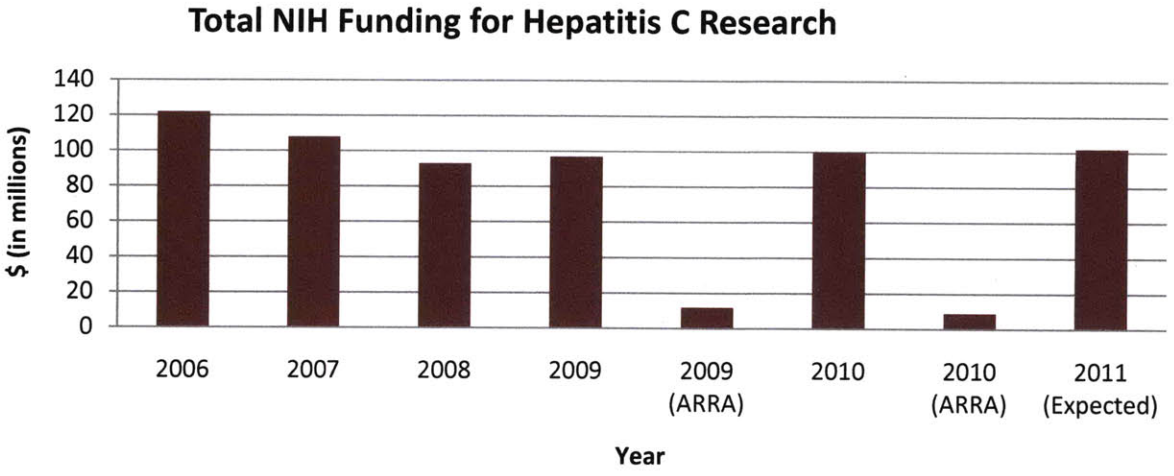


Figure 2 shows the funding for hepatitis C research from 2006 to present and the projected funding for 2011.

The Unmet Need

Proper treatment of hepatitis C (HCV) requires close monitoring of the amount of the virus present in a patient's blood, clinically referred to as the viral load. Currently, to quantify the viral load, a blood sample is taken and the number of viral particles is quantified, usually using PCR (Polymerase Chain Reaction) or TMA (Transcription Mediated Amplification) technology.

The number of viral particles is usually presented as a concentration. The concentration is determined based on the number of viral particles present and the volume of blood sampled. Initial quantification of the patient's viral load can be conducted using commercially available tests because the patient typically has a sufficiently high viral load, one well within the detectable range of the most commonly used tests. These tests have a quantification range of 30 viral particles to 250,000,000 viral particles per mL.[i] There are also more sensitive tests available (as low as 5 viral particles per mL), but they have a small range of quantification and they are only useful when a physician knows that the patient has a low viral load.

Many patients will reach a point where current methods, even the most sensitive tests, are insufficient to determine the viral load. Clinically, this point is referred to as having an undetectable level of the virus. Physicians have no way of determining if a negative test result is because the patient is cured or because the patient has a level of the virus not detectable with current methods. The latter of those two options is termed a false negative. Unless physicians are able to determine the true point of eradication, and are able to know that a negative result is in fact a true negative, they are only able to treat patients based on clinically established standards. If they can determine the true point of eradication, physicians could tailor treatments based on each patient's specific response. Continuing treatment "blindly," beyond the point at which the patient achieves a negative test result, subjects many patients to unnecessary treatment. Physicians have a strong desire to customize treatments and shorten them where possible because of the high cost of treatment, the significant side-effects associated with the treatment and the fact that some patients achieve this undetectable level only 4 weeks, or earlier, into a 48 week treatment.

The Solution

The Cima Lab is developing an *in-vivo* device to sample large volumes of blood and trap the HCV particles present. This approach overcomes the sampling limitations of current tests that work based on drawing a fixed amount of blood from a patient's arm. The proposed device will be placed in a vein and as viral particles come in contact with it, they will become trapped by antibodies lining the surface of the device. The device will then be removed from the body and

[i] Concentrations can also be listed in IU/mL. The conversion used for the tests discussed is 2.5 viral particles per IU.

the viral particles will be quantified using one of the commercially available tests. Based on the volume of blood sampled while the device is in the body, the number of viral particles will be converted to a concentration. Physicians can then determine the best course of treatment with this much more sensitive measurement. This *in-vivo* blood sampling technique allows roughly 40,000 times more blood to be sampled in a 30 minute period than with a traditional blood draw (2 mL).

Antibody/Antigen Interactions

A key component of this device is the interaction between antibodies and viral particles. An antibody is a protein that is naturally created by the body's immune system when it detects a foreign body (i.e. hepatitis viral particles). The HCV viral particles, also referred to as antigens, will bind to the antibodies when they come in contact with each other. While antibodies are produced by the body naturally, they can also be created in a lab. These manufactured antibodies can be attached to the surface of a device and used to trap viral particles. The hepatitis C antibodies used in this application are specific to the proteins (E1 and E2) expressed on the surface of the HCV viral particle. These surface proteins are referred to as surface antigens. A schematic of the HCV viral particle is shown in Figure 3.[20]

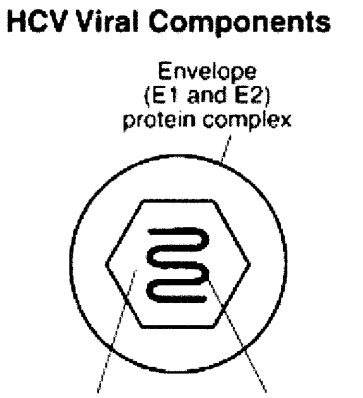


Figure 3 shows a schematic of the hepatitis C viral particle and its surface antigens.

Initial Design Concept

Initially, the focus of the device was on developing a system that could both sample large volumes of blood and quantify viral load independent of any additional testing equipment. The first concept was to use magnetic nanoparticles, coated with antibodies, to capture viral particles in the body. The antibody-coated nanoparticles would aggregate in the presence of viral particles and cause a change in the magnetic properties of the surrounding media. This change would then be detectable using either MRI (Magnetic Resonance Imaging) or NMR (Nuclear Magnetic Resonance) technology. The initial concept was that a group of the antibody functionalized nanoparticles would be placed in a polymer membrane (shown in Figure 4). This device would then be placed in a vein, and viral particles would diffuse into the device and cause an aggregation of the nanoparticles. It was hypothesized that this would allow for the sampling of a much larger volume of blood than is currently possible with a traditional blood draw. The technology, to date, has only been tested in benchtop *in-vitro* settings. It had been proven that the nanoparticle technology was capable of trapping viral particles,[21] but there were complications associated with implementing the technology in an *in-vivo* setting. The primary problem was that the group of nanoparticles would need to be encased in a polymer membrane to keep them from moving throughout the body. This created a large dichotomy between the number of viral particles that came in contact with the surface of the device and the number of viral particles that diffused through the membrane and came in contact with the nanoparticles.

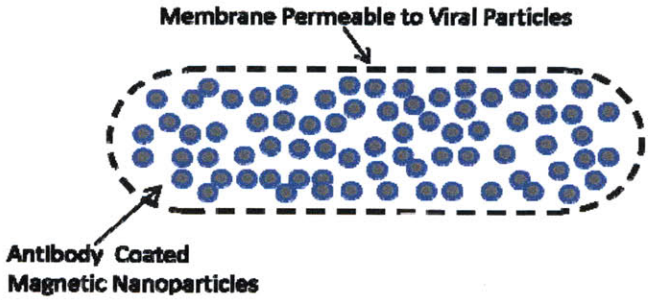


Figure 4 shows the initial design that was considered during this project. Note: image enlarged to show nanoparticles and pore size.

Based on diffusion calculations of the viral particles through the membrane, it was determined that the device would need to be placed in the vessel for a period of up to five hours to match the sampling capabilities of a current blood draw. The device would need to remain in the body for multiple days to have any competitive advantage from a sampling perspective. If the time the device is in the body is longer than one hour, it would require two procedures, one to place the

device and one to remove it. The implications of the cost associated with placement are discussed later, but avoiding a second procedure to remove the device would be advantageous. Also, the longer the device is left in the body, the more important issues such as biocompatibility and biostability become. Increasing pore size to increase the diffusion of viral particles into the device was considered, but determined to be an unsuitable solution. If the pores were made sufficiently large, the nanoparticles would escape from the device.

Another reason for the change in approach was the sensitivity of quantification using MRI or NMR technology. The lowest concentration of viral particles detected with this technique is 500 viral particles/mL. This is roughly 100 times less sensitive that the most sensitive test available on the market today and 17 times less sensitive than the most commonly used test. The device would need to be left in the body for an unsuitable length of time to overcome both the low diffusion rate through the membrane and the lower sensitivity of the quantification associated with this technique.

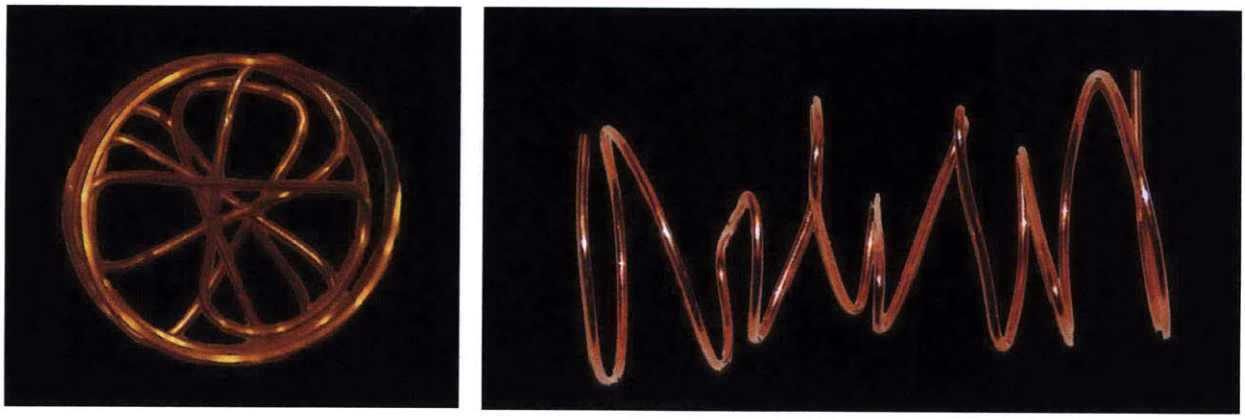
The approach has shifted from a standalone diagnostic device to a viral particle trapping device that is used in conjunction with currently available diagnostic tests. This new approach overcomes the problems of the initial design. The new approach will trap viral particles with antibodies directly on the surface of the device. Exposing the antibodies directly to viral particles (without a membrane in between) will increase the trapping capabilities of the device. Once the viral particles have been trapped, the device will be removed from the body and a more traditional technique for quantifying viral particles will be employed. This takes advantage of the most sensitive test available and the high sampling rate of an *in-vivo* device. Additionally, this gives the physician the flexibility to choose which quantification test he/she desires.

Figure 5 shows the second design that was considered for the HCV trapping device.

Initially, a straight rod design was considered. This straight rod (Figure 5) would be coated with antibodies and passed through a catheter to either the superior vena cava (SVC) or hepatic veins (implantation location will be discussed in detail in a later section). Again the goal was to keep the device in the body for no longer than 30 minutes. It was determined that four rods (3 cm in

length) would be needed to match the blood volume sampling capabilities of a 3 mL blood draw. The low sampling capabilities of this approach were attributed to the strong reliance on having particles diffuse to the surface of the device before binding could occur. It was determined that to justify the cost of placement of the device and the necessary procedure, the sampling volume would need to be higher.

The next design evaluated was the "twisted" coil. This device (a model representing the shape is shown in Figures 6a and 6b) crosses back and forth across the blood vessel, perpendicular to the direction of the blood flow. This allows the device to take advantage of the moving blood to carry the viral particles to the surface of the device. This approach does not rely solely on diffusion as in the straight rod design option, and thus samples far greater volumes of blood. Looking down the vein, one sees the device as represented in Figure 6a. It appears as though a large amount of the vein is blocked. This is not the case as can be seen in Figure 6b. Looking at the device from the side, each time the device crosses the vein it is approximately 0.5 cm from the last place the device crossed the vein. The device does not block more than 4% of the vein at any one time. This method samples over 85,000 mL of blood in a 30 minute period; this is more than 40,000 times greater than the volume sampled in a current blood draw (2 mL) when placed in the superior vena cava (SVC). When placed in the hepatic veins, the device samples 10,000 times more blood than a traditional blood draw.



Figures 6a and 6b. Figure 6a shows how the device would look if looking down the vein in the direction of flow. Figure 6b shows how the device looks from the side and its spacing. Note: Copper was used for these mockups, but a different material will be used for the actual device (discussed later).

Trapping Capabilities of "Twisted Coil" Design

While the volume of blood sampled is a critical factor when deciding on a design and implantation location, it is not sufficient to determine if a design is viable based on this criteria. The ability of the viral particles to be captured by the antibodies is equally as important. Previous research has not explored the binding of moving viral particles and stationary antibodies in blood flowing at the velocities observed in the SVC or hepatic veins. There are, however, examples from microfluidics that examine the binding efficiency of viral particles to antibodies in flowing blood. Researchers explored the ability to capture HPV (Human Papaloma Virus) viral particles with stationary antibodies while exposed to blood containing viral particles. This study explored blood flowing at 0.13 cm/s.[22] Researchers found that the binding efficiency at this velocity was determined to be in excess of 30%. This is significantly slower than the rate of blood flow in the SVC (average 22.5 cm/s), but researchers also found that the trapping efficiency plateaued at 30% as blood velocity increased.

Tables 2 and 3 below show the number of captured viral particles for multiple binding efficiencies and viral particle concentrations during a 30 minute period. The number of viral particles captured must be greater than 30 (the minimum quantifiable about with the Abbott test), to quantify the viral load using the trapping device. This is the ideal situation because the same test can be used at high viral loads without the trapping device and at low viral loads with the trapping device. If the number of particles captured is less that 30 but greater than 5, it can still be quantified, but the LabCorp test would need to be used. This still provides some useful information, but because it is a different test than the one used at the beginning of treatment there may be some inherent variations. The tables below show the great deal of variation possible in binding efficiency while still having a sufficiently sensitive device for both the SVC and the hepatic veins.

Viral Particles Captured – SVC			
Binding Efficiency	10 Viral Particles/mL	5 Viral Particles/mL	1 Viral Particles/mL
10%	85050	42525	8505
5%	42525	21263	4253
1%	8505	4253	851
0.10%	851	425	85
0.01%	85	43	9

Table 2 shows the number of viral particles that could be trapped by the device for three different viral particle concentrations and five different binding efficiencies if the device was placed in the SVC.

Viral Particles Captured -- Hepatic Veins			
Binding Efficiency	10 Viral Particles/mL	5 Viral Particles/mL	1 Viral Particles/mL
10%	21294	10647	2129
5%	10647	5324	1065
1%	2129	1065	213
0.10%	213	106	21
0.01%	21	11	2

Table 3 shows the number of viral particles that could be trapped by the device for three different viral particle concentrations and five different binding efficiencies if the device was placed in the hepatic veins.

The last column in both tables shows the number of captured particles if the concentration is 5 times less than what can currently be detected and 30 times less than what can be detected with one of the most commonly used tests. The requirement of capturing 30 viral particles can be achieved at a binding efficiency as low as 0.1% in the SVC. If the device was placed in the hepatic veins, it would still be effective at a binding efficiency of 1% if the concentration in the blood was 1 viral particle per mL. A binding efficiency of 0.1% would be sufficient if the concentration in the blood was 5 viral particles per mL. It is important to remember that the device will only be placed in the hepatic veins if there is a far greater viral concentration than present in the SVC. The number of viral particles captured, as shown in Table 3, does not assume any increased concentration. If the device was placed in the hepatic veins, the number of viral particles captured would actually be much greater than what is shown in Table 3. These calculations assume that the part of the device that crosses the vein is the only part able to trap viral particles.

Based solely on a literature review, an estimation of binding efficiency is difficult. The wide range of possible binding efficiencies makes it risky to predict the sampling capabilities of the device without conducting some *in-vitro* studies. It is clear from the above tables that success is likely because the device can still be effective at such low binding efficiencies. A technique to conduct these *in-vitro* tests, as well as *in-vivo* animal studies, is outlined later.

Implantation Location and Procedure

Implantation Location

Three locations were considered based on the following set of criteria: ease of implantation, size of the vessel and proximity to the liver/viral load. The ease of implantation refers to how easy it is to place and remove the device. The size of the vessel is important because the amount of blood that can be sampled is related to the size of the vessel. The proximity to the liver is relevant because the liver is the site of replication for the hepatitis C virus.

The initial site considered was the hepatic veins. The hepatic veins carry blood from the liver to the inferior vena cava. It has been shown that the liver tissue has a 40 times higher concentration[23] of viral RNA than peripheral blood. The comparison of blood close to the liver to that of peripheral vessels is currently being investigated and the results of this test will dictate the importance of placing the device close to the liver. While this location has the potential for a higher viral load, the implantation procedure is more costly, time consuming and taxing for the patient. There are also space constraints associated with the location. The diameter of the hepatic vein is 1.5 cm. The focus of placement location shifted to the inferior vena cava (IVC) because of these limitations in the hepatic veins.

The IVC (shown in Figure 7)[24] carries de-oxygenated blood from the lower part of the body back to the right atrium. The hepatic veins drain from the liver and join the IVC. It was hypothesized that being relatively close to the liver would still provide access to the higher viral load if it existed and, at the same time, avoid the space constraints associated with the hepatic veins. It was determined that this location had a suitable diameter (2 cm – 3 cm)[25] compared to a diameter of 1.5 cm in the hepatic veins. It was also determined that the blood velocity is 13 cm/s (mean velocity), allowing for sufficient blood sampling in a 30

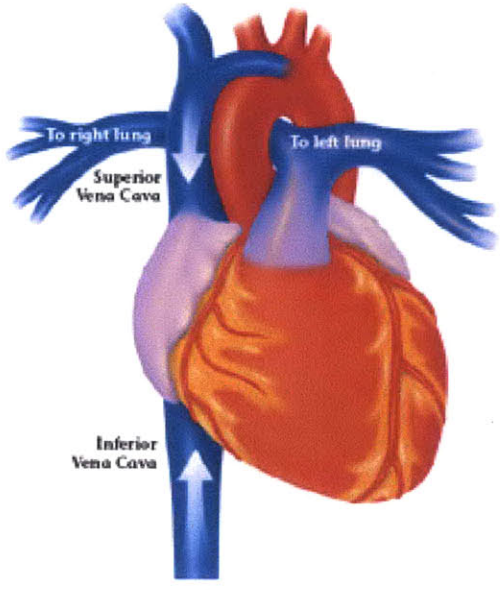


Figure 7 shows the location of the inferior vena cava and superior vena cava.

minute period. The primary problem with placing the device in the IVC is where the hepatic veins meet the IVC. There is not sufficient room in the IVC after the hepatic veins join the IVC before the IVC meets the heart. This limitation made the IVC an unsuitable placement site.

The third and final site considered was the SVC (also shown in Figure 7). The superior vena cava carries de-oxygenated blood from the upper half of the body to the right atrium. The superior vena cava has a sufficiently large diameter (3-4 cm) to place the device and a blood velocity (avg. blood velocity = 22.5 cm/s) that will allow for sufficient blood sampling capabilities. The primary drawback to this location is that the device would now be placed further from the liver. As mentioned earlier, the importance of placing the device close to the liver will be determined once clinical measurements of viral load have been taken in blood from the SVC and blood from the hepatic veins.

Implantation Procedure

The procedures to implant the device in the two locations still under consideration are different. It is more complicated to implant the device in the hepatic veins than to place the device in the SVC. The placement of the HCV trapping device in the hepatic veins will be done by an interventional radiologist. The patient's neck or leg, depending on whether the coronary vein or femoral vein is used, is cleaned and sterilized to prevent infection. The patient lies down during the procedure and a numbing agent is administered at the site of entry into the body. This will prevent the patient from feeling pain during the procedure. A plastic catheter is passed into either the leg or neck, depending on implantation location. An x-ray dye is injected into the catheter and images of the vein are taken. The radiologist then passes the HCV trapping device through the catheter to the deployment site. The catheter will be left in place during sampling (30 minutes). The device will be pulled back through the catheter after the sampling has ended, and the catheter will then be removed from the body.[26]

If the device is placed in the SVC, the procedure is much simpler. Placement in the SVC would not require an interventional radiologist. Instead, a peripherally inserted central catheter (PICC) line (shown in Figure 8)[27] would be placed by an IV therapist or certified registered nurse. The first method to insert a PICC line is to insert the catheter by feeling the vein in the arm and then confirm the location of the catheter with a chest x-ray. Alternatively, the PICC line could be placed using ultrasound equipment, followed by a chest x-ray to confirm placement. The third option is to use a combination of ultrasound and fluoroscopy. This allows for real-time imaging while placing the PICC line.[28]

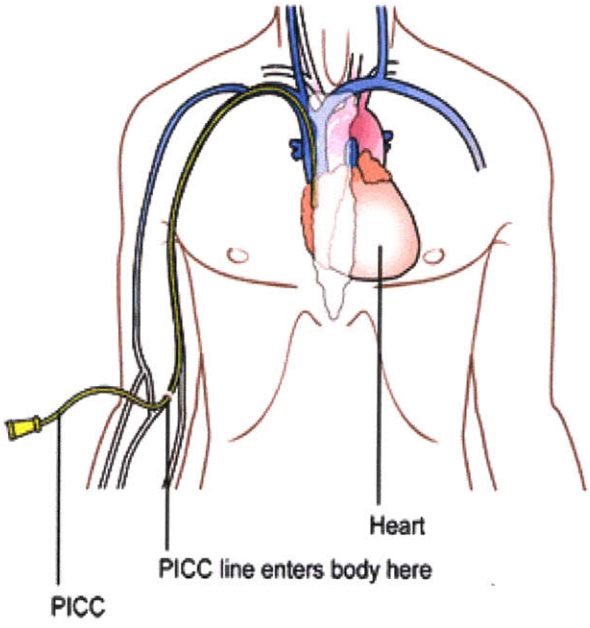


Figure 8 shows the location of the PICC line.

The procedure associated with placing the device in the SVC is significantly easier than the procedure needed to place the device in the hepatic veins. Independent of the viral load present in the two locations, the device would be placed in the SVC. Unless viral load measurements from the hepatic veins are significantly greater, there would be no strategic advantage to placing the device in the hepatic veins. Table 4 outlines the difference between the two locations.

	Superior Vena Cava	Hepatic Veins
Ease of Placement	Easier	Harder
Cost of Placement Procedure	≈$600 -$1400	≈$1,700
Personnel Required	IV Therapist	Interventional Radiologist
Proximity to Liver	Near Heart	Connected to Liver
Diameter of Vessel	3 - 4 cm	1.5 cm
Blood Velocity	10 - 35 cm/s	13 cm/s
Comparative Viral Load	Roughly Equal to Blood Draw	Unknown

Table 4 shows a summary of the benefits and drawbacks of both implantation locations being considered.

Blood Sample Preparation

Current Sample Preparation

The most common types of quantitative HCV tests are Real Time-Polymerase Chain Reaction (RT-PCR) based tests. One of the most popular tests is the COBAS® AmpliPrep/COBAS® TaqMan® HCV test manufactured by Roche. The specific blood specimen preparation technique outlined in this section is for the Roche test. This test measures viral load by quantifying the amount of RNA present in a fixed sample of blood. This system is able to process a maximum of 850 μl of serum or plasma. The HCV viral particles are lysed through incubation at high temperatures. The incubation occurs with a protease that promotes the lyses of the viral particles to release the RNA. The incubation also occurs in a buffer solution that releases nucleic acids and protects the RNA from RNAases. RNAases is the process in which the RNA from the viral particles is broken into its smaller component parts. If RNAases occurs, it would distort the true viral load.

Sample Preparation When Using Trapping Device

The preparation of samples collected using the trapping device will not be substantively different than the samples collected using a traditional blood draw. The HCV trapping device would be removed from the body and placed in blood collected from the patient (2 mL). The blood and trapping device, with bound viral particles, would then be incubated. Incubation would release the RNA contained in the viral particles. Incubation would also occur in a buffer solution to protect the RNA from RNAases as with current sample preparation. The backbone of the device will be removed from the blood sample and the sample will be processed using the same steps that are used to quantify a viral load in traditional blood draw.

Manufacturing

Materials Selection

Nitinol (NiTi) was chosen as the material for this device because it is highly elastic. The device will be shaped as a "twisted" coil, shown in Figure 6. Given the limited space provided by a catheter (2 mm diameter), the nitinol coil will not be able to reach the site of implantation without being deformed. This necessitates that the material regains its shape once it reaches the deployment site. While biocompatibility is not as important a concern (because the device is

only in the body for 30 minutes), the fact that nitinol is currently used in blood contact applications (stents and blood clot catching devices) reinforces its use in this application.

A polymer coating is applied to the backbone to promote the attachment of the antibodies to the device. The polymer chosen is PDMS (polydimethylsiloxane). PDMS is a highly biocompatible polymer; it is used in contact lenses and in a wide range of other medical applications. Another reason that PDMS was selected is because of its use in applications involving antibodies. PDMS has been used in microfluidic applications in which antibodies are bound to a PDMS surface which is then in contact with blood.

There are two forms of antibody that could be used to the trap viral particles, humanized and non-humanized antibodies. A humanized antibody is an antibody derived from another species (e.g. primate) and then modified to better match the human version of the antibody. A non-humanized antibody is derived in another species and used without altering it to match the human version. Regardless of which choice is made, the antibody will be specific to the E1 or E2 antigens present on the envelope of the HCV viral particle. Using a humanized antibody decreases immune response if the antibodies become dislodged from the device while it is in the body. It is, however, possible that a non-humanized version would work because the device is only in the body for 30 minutes.

Manufacturing of Nitinol Backbone

The manufacturing of the nitinol for this application will be a multistep process. Nitinol wire must be made into the desired shape for this device. The process of shaping nitinol is termed shape setting. The wire is set in a fixture or on a mandrel that is in the shape of the desired device, and then a heat treatment process is performed. The heat treatment process can be achieved using an air or vacuum furnace, salt bath, sand bath, heated die or other heating method. The temperature is raised to a range of 500°C to 550°C. The treatment duration must be long enough for the entire material to reach the desired temperature. The heating of smaller objects, such as the wires used in this device, typically takes less than a minute to reach the desired temperature. The nitinol is then quenched in a water bath. The specific temperature and exact heating time are determined through experimentation prior to manufacturing. The final step in the nitinol manufacturing process is electropolishing. The nitinol, on a microscopic level,

can have crevices on grain boundaries and at the edges. Electropolishing removes the crevices present after machining.[29]

Polymer Coating

The polymer coating process can be conducted by the same company that handles the shape setting of the nitinol. This is one method for coating the device with PDMS. The PDMS is mixed with a curing agent (e.g. Sylgard 184, Dow Corning) and then degassed.[30] The nitinol wire is dipped in this mixture and then removed and allowed to cure. PDMS has been shown to bind to Ti alloys through a Si – O –Ti bond.[31] It is placed in a plasma cleaner (e.g. PDC-32F, Harrick Scientific) after the curing process is completed. The plasma cleaning process serves two purposes, surface sterilization and surface preparation for bonding.[32] The preparation of the PDMS helps to promote binding of the antibodies to the nitinol backbone. The exact process may need to be adjusted based on the final design of the device.

Application of Antibodies

The application of HCV antibodies to a polymer surface has not yet been explored in a research or commercial application. There are, however, examples of other types of antibodies (cervical cancer) being bound to a polymer surface, specifically PDMS. The surface of the PDMS is treated with a 2% solution of 3-mercaptoproyltrimethosilane in toluene for a one hour period to promote the binding of the antibodies to the polymer. The surface is dried and treated with 2mM GBMS (*N*-γ-maleimidobutyry loxy succinimide ester) for one hour and then rinsed with PBS (phosphate buffered saline). A solution of antibodies is then introduced to the chemically treated PDMS for 30 minutes at room temperature to react with the GBMS. It is important that the application of the antibodies be conducted in a sterile environment because after they bind to the PDMS, the device can no longer be sterilized without damaging the function of the antibodies. The cleanliness of the lab must meet GMP (good manufacturing practice) standards. This includes sterile conditions and regularly maintained equipment to ensure consistent and uniform production. It is expected that the process for the attachment of HCV antibodies will be similar to that of the cervical cancer antibodies.[33]

Packaging and Storage

The specifics of the packaging and storage will be dictated by the antibody choice. The nitinol and the PDMS are able to be stored at room temperature without altering their functions.

However, in general, antibodies must be kept at -20°C to avoid damage. The antibodies must also not be thawed and refrozen; this can damage their function.[34] Once the device has been manufactured, it must remain sterilized until it enters the patient's body. It could be preloaded into a PICC line to avoid the physician having to handle the device. This would ensure that the device remains clean and makes the procedure easier for the physician.

Manufacturing Cost

Due to the nature of these components, there are advantages to producing at larger volumes. The production of the nitinol components requires an investment in the mandrel used to shape the nitinol wire into the component. The mandrel is a fixed cost, an investment that must be made regardless of the number of devices produced. The investment in the mandrel will be spread over a larger number of units as the volume produced increases. The cost per device of the polymer coating also decreases as production volume increases. The polymer coating process is conducted in batches of roughly 50 pieces at one time. Additionally, if multiple batches are run consecutively, the changing of tooling is minimized; this is yet another reason for decreased unit costs with increased volume. When producing at the prototype level (less than 10 units), the cost of the nitinol backbone and polymer coating is just under $57 per unit. When producing at production level (greater than 1000 units), the cost per piece decreases to just over $40 per unit. This is a 30% drop in cost per unit for the shaping of the nitinol and polymer coating.

	Unit Cost/Device (Quantity Produced)		
Manufacturing Step	**< 10**	**10-1,000**	**> 1,000**
Nitinol (Material, Finishing and Shape Setting)	$47	$37	$31
Polymer Coating (Materials and Process)	$10	$10	$9
Antibodies -- Humanized (Materials and Application Process)	$250-$400	$250-$400	$250-$400
Total	$307 - $457	$297 - $447	$290-$440

Table 5 shows the unit cost for production of the device at different quantities when using humanized antibodies. Quantities over 1,000 are considered production volume.

	Unit Cost/Device (Quantity Produced)		
Manufacturing Step	**< 10**	**10-1,000**	**> 1,000**
Nitinol (Material, Finishing and Shape Setting)	$47	$37	$31
Polymer Coating (Materials and Process)	$10	$10	$9
Antibodies -- Non-Humanized (Materials and Application Process)	$50	$50	$50
Total	$107	$97	$90

Table 6 shows the unit cost for production of the device at different quantities when using non-humanized antibodies. Quantities over 1,000 are considered production volume.

These costs, shown in Tables 5 and 6, include all equipment, tooling, technicians and associated costs. All prices shown are based on estimates from manufacturers and may change depending on the final design of the device. The cost for antibodies is the greatest current unknown. It is estimated to be between $50 and $400. This price includes the antibodies and the associated application process. This cost depends on whether humanized or non-humanized antibodies are used. The cost of antibodies can vary greatly depending on specific factors that will be determined during the development process. The most suitable and appropriate type of antibody will be determined through future testing. The total cost to manufacture the device at the prototype level is between $307 and $457 when using humanized antibodies and $107 when using non-humanized antibodies. Once at production volumes, the cost drops to between $290 and $440 when using humanized antibodies and $90 when using non-humanized antibodies. The specific packaging and storage required will be based primarily on the antibody chosen; this cost will be added once the antibody selection process has been completed. There are many companies that are capable of handling the nitinol shaping and polymer coating portions of the device. Contracting the production of the nitinol shaping and polymer coating processes to a third party avoids the need to make large capital investments (e.g. equipment and facilities). Additionally, the industry is well-established and we would be able to take advantage of the experience and expertise of these existing companies.

Supply Chain

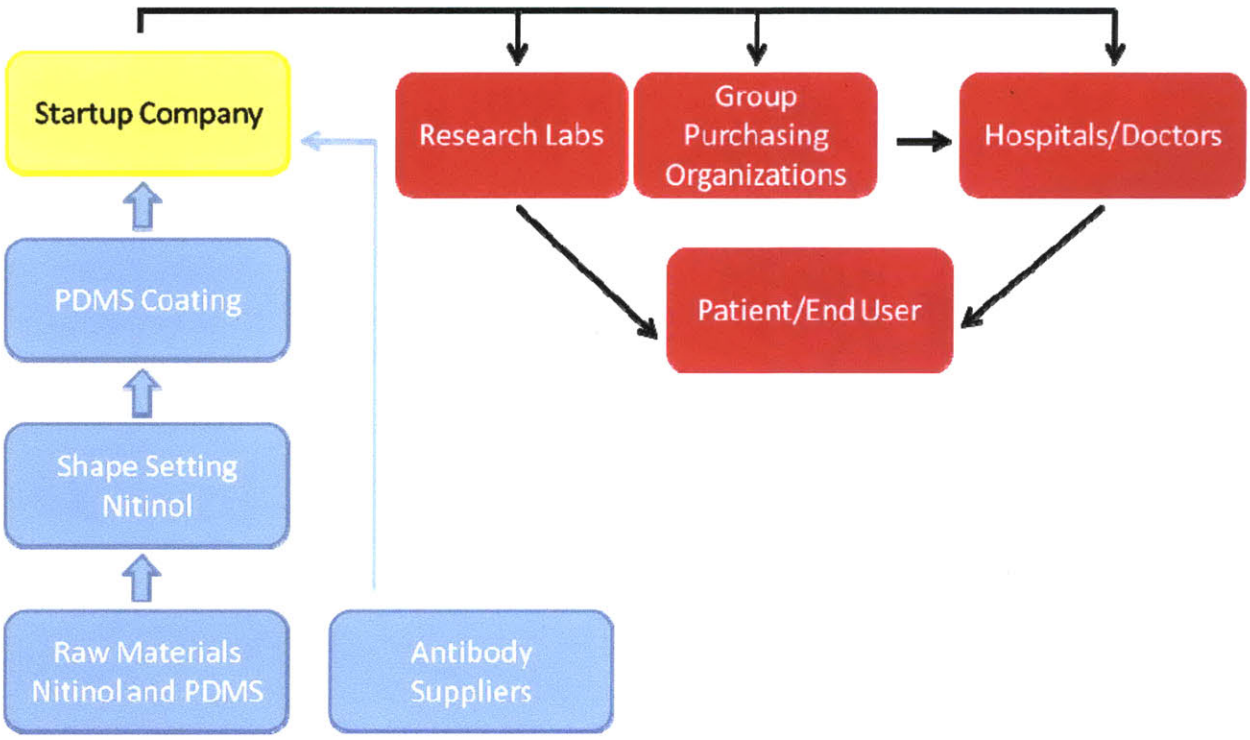


Figure 9 shows the existing supply chain , the existing distribution chain and where a startup company would operate.

This device would not be made by the same supply chain as current HCV diagnostic devices. Figure 9 shows how the supply and distribution chains will operate for the manufacturing of this device. Current HCV diagnostic tests are typically PCR or TMA based technologies and rely on chemical reagents. This technology is an antibody-based device. While antibodies are used for detection purposes in microfluidic applications, they are not used for *in-vivo* applications. The other components, the nitinol backbone and the polymer coating, are also not used for HCV diagnostic devices. This provides a key strategic advantage because the companies that will provide the antibodies, nitinol backbone and polymer are not currently involved in the HCV diagnostics or HCV drug markets. A potential startup company would purchase the nitinol components already shaped and coated and focus on the process of applying the antibodies to the device.

There are two key complementary devices that will need to be employed when using the HCV trapping device, and it is important to understand where they could be purchased. A placement catheter and a vascular access kit are both required. These two devices can be made

independently of the HCV trapping device and do not require a co-manufacturing process. The catheter will be used to place and remove the device, and the vascular access kit will be used to access the vein that the catheter will be placed in. The similarity between the implantation for this device and the placement of an IVC filter allows the same equipment to be used in both procedures. Thus, these two complementary devices can be purchased from manufacturers of IVC filters. Commercially available catheters for device placement in the IVC are $160 (Vista BRITE TIP® Guiding Catheter – Cordis) and vascular access kits are $45 (Vascular Access Kit – Cordis).[35]

Distribution Chain

Once the device is manufactured, it would be sold into the already existing distribution chain for medical devices. The medical device industry relies heavily on group purchasing organizations (GPOs). These groups pool the purchasing power of hospitals and smaller medical centers to obtain volume discounts from device manufacturers. Today, hospitals purchase about 70% of their equipment through GPOs and 96% to 98% of hospitals use GPOs as part of their purchasing structures. Hospitals are frequently members of more than one GPO; on average, a hospital is a member of 4.2 GPOs. Manufacturers present the technical capabilities and price of the device to the assessment team at each GPO as part of the process to have their device distributed. Terms regarding the agreement between the GPO and a medical device manufacturer are negotiable. Typically, selling to one GPO does not preclude one from selling to another GPO or directly to a hospital.[36] The last step of the distribution chain is for the hospitals and researchers to use the device on a patient or research subject respectively.

Insurance Companies, Medicare and Medicaid

Regardless of whether hospitals and physicians are willing to adopt and use the technology, someone must pay for it. There are three ways that medical expenses are paid for in the United States -- the patient pays out-of-pocket for the treatment or procedure, an insurance company pays for the service or the government pays for it (in the cases of Medicare and Medicaid). The goal of the entity paying for the treatment or procedure is to choose the most cost effective and efficacious treatments available. They will look at both the financial cost of the procedure and device and the benefits of conducting the test or procedure.

A device or procedure must have an associated CPT (Current Procedural Terminology) code, issued by the AMA (American Medical Association), to be covered by an insurance company. When a new medical device is introduced to the market, it must be determined whether the new device or procedure will fall under a current CPT code or if a new one will need to be created. The advantage of using a current CPT code is that there is no new CPT code application that needs to be submitted to the editorial panel at the AMA. The main drawback of using an existing CPT code is that the level of reimbursement is fixed. A new code will be necessary for this device. The procedure required would be above and beyond what is currently covered by CPT codes related to testing for HCV. These CPT codes would not provide a sufficient level of reimbursement for the placement and removal of the device and the cost of the device itself.[37]

It is also important to recognize that recently Medicare and Medicaid have frozen the amount they are willing to reimburse for clinical laboratory testing.[38] This is particularly relevant because in the next 10 years physicians are expecting a new wave of HCV infected people over age 60.[39] People in the United States enter the Medicare program at age 65. A new CPT code would keep the device from being subject to the reimbursement freeze associated with molecular diagnostics.

Potential Customers and Incentives for Adoption

The main incentive to insurance companies is the savings they would experience due to a shorter treatment length. The amount saved through use of the proposed device is discussed in a later section. The primary target customers for this type of device are the physicians. While in some instances patients may request a particular treatment, the majority of times it is the physicians who will determine which tests are most appropriate. There are, however, a growing number of online self-help forums for patients with hepatitis C. Patients discuss matters including which diagnostic tests their physicians have administered and how valuable the test results were for dictating future treatment. These forums have helped to spread the word about new diagnostic tools in the past. Regardless, the decision regarding which tests to administer still falls primarily on the physician, even with these new online forums. The physicians are primarily concerned with effectively treating their patients and receiving reimbursement for their work. Clinical trials to quantify the capabilities of the technology will be required to convince physicians of the effectiveness of this device. The procedural cost associated with this device is $600 - $1,500

(the basis for this number is discussed later). This price is the amount that the physicians would charge the patient, and already includes the physicians' profits. No additional financial incentive would be required. Patients would choose to undergo the procedure because this device could shorten their treatment, saving them the cost of drugs, physician visits, and testing. Additionally, they would avoid the hassle of self-administering medication and the problems associated with the side-effects of treatment.

Market for the HCV Trapping Device

Primary Markets

The primary use of this device is focused on genotype 1 patients who exhibit RVR. The reason for the focus on this segment of the population is the prevalence of genotype 1 (75% US and 60% worldwide) and the lengthy time these patients spend being treated after they achieve RVR. They achieve RVR at week 4, but continue with treatment for another 44 weeks. It has been shown that as many as 78%[40] of these patients can achieve SVR (considered cured for HCV) with a shortened treatment. The average savings per genotype 1 patient reaching RVR is $13,200. It is important to look at the savings per RVR patient rather than per patient of the population who is cured (the 78%) because this test would be conducted indiscriminately on all people who achieve RVR. The savings are associated with the average cost of the drug ($11,500) and the average cost of physicians' visits and tests during the second six months of treatment ($2,100). When setting the price, it is important to include the costs of all associated equipment, procedures and personnel.

Secondary Markets

The secondary markets for this device will be heavily dictated by the capabilities of the device as determined by the clinical and preclinical trials. There are a number of additional segments of the HCV infected population that could benefit from the device. The first market to focus on is the remainder of the RVR patients who are already having their viral load quantified using the trapping device. These patients, in theory, would have a detectable level of the virus using the trapping device, but not with traditional blood draw methods. Even though these patients would not be able to stop treatment at 24 weeks, they may be able to end earlier than 48 weeks depending on their viral load measurement. Clinical standards will be developed that will

outline how long treatment will need to continue based on particular viral load level at the 24 week point or at any other point where the device is used.

Another potential market is patients who respond fully at the end of treatment based on measurements using traditional techniques but not with the trapping device. Even with the current testing available and the standards for treatment length, some patients still relapse after they finish their standard course of treatment. It is highly likely that these patients relapse because they did not completely clear the virus; rather they still had a low level of the virus at the end of treatment that wasn't detectable with current tests. Patients who relapse bear the emotional burden of believing that they cleared the virus, only to find out six months later that the infection is still active. They also must deal with the complications associated with a second course of treatment. Retreatment requires undergoing the entire course of treatment again. This means that the patient will need to undergo another 48 weeks of treatment. If the virus has been exposed to treatment, but is not eradicated, it can be harder to achieve SVR during retreatment. This only reinforces the importance of fully eradicating the virus before stopping treatment. There are medical benefits to conducting viral load tests at this point, but the financial benefit is less clear.

Patients infected with genotypes 2 and 3 of hepatitis C present another secondary market. These patients are an intriguing segment of the population because their treatment is already 24 weeks. These patients, in theory, could benefit medically from the test, but the economic benefit would be minimal. The ability to capture this market will depend on data collected during clinical studies. If it is shown that it is unlikely that these patients could be successfully treated with significantly shorter treatments, it is unlikely that the trapping device will be implemented in a clinical setting for these patients.

The research and drug development settings provide an additional market for the device. Companies developing new HCV therapies rely on the same quantification techniques that are available to physicians. The goal of new drugs is to eradicate the virus more effectively than current drugs. It is difficult to evaluate the effectiveness of new treatments without diagnostic tools to objectively and definitively quantify the time point at which eradication occurs. The success of emerging therapies can be understood with much greater certainty by employing the trapping device. The necessary clinical trials for a new HCV treatment can amount to thousands

of patients and provide a significant market for the device. Vertex Pharmaceuticals is currently developing their Telaprevir drug to be used to treat HCV. Close monitoring of different subsets of the clinical trial population provides important information to companies developing HCV therapies. Vertex has included over 3,000 patients in their trials for Telaprevir. These patients were treated using various combinations of drugs and their viral loads were monitored throughout the trials. This market could become an additional source of revenue at the price point that is dictated by the genotype 1 RVR market outlined earlier.

Cost of the Device and Procedure

The primary expense is the procedural cost for implantation. This includes both the personnel and facility cost as well as the necessary profit margins for the physicians. The procedure to place a clot filter in the IVC was used as a starting point for implantation because of the similarities to the procedure needed to placed the HCV trapping device. The average cost of the placement of an IVC filter is $2,700.[41] When breaking down the cost of implantation, the cost of the filter itself should be removed. The filter costs $1,245,[42] meaning the cost of placement (including staff and equipment) is $1,455. The two other required components are the cost of the catheter ($165) to place the device and the vascular access kit ($45) to gain access to the vein. The cost of the procedure was determined to be $1,500 ($1,710 including all necessary implantation tools).

The cost of placement can be reduced if the device is placed in the SVC instead of the hepatic veins. The cost of a PICC line is between $65 and $150. The cost of the procedure to place the PICC line is between $535 and $1,250. The total cost for the procedure, if placed in the SVC, is between $600 and $1,400. It is also important to remember that the proposed device will not quantify viral load; it will only trap viral particles and augment the sensitivity of a currently available test. Thus, there will also be the cost of the HCV RNA quantification test. This cost is not included in the total price of the device because this HCV RNA test will be administered regardless of whether the proposed device is used. The use of the trapping device will enhance the effective sensitivity of the test already being administered.

It is expected that the device will be used once during treatment, ideally at the point when the physician is considering ending treatment. If a test using the device is negative, then physicians can terminate treatment with far greater confidence. One may think that if the test is positive, the

patient will need to be retested again at a point in the future. This is unlikely; depending on the viral load measured, a physician will know, based on past research, how long the patient will need to continue treatment. Alternatively, it may become standard to continue treatment for the full duration if quantification using this device provides a positive test result. The price of the device must be set so that there is still a cost advantage to the payer. The average savings per genotype 1 patient reaching RVR is $13,200. The cost to manufacture the device, purchase the implantation tools and perform the procedure is roughly $1,000 - $2,100. The retail price for the device, components and implantation has been set at $9,000. If the device is used once during treatment, the total net savings is $4,200. The savings would be $300 to $1,000 greater if the device is placed in the SVC using a PICC line. This savings could increase profit margins or be used as an added incentive for the entity paying for the treatment to adopt the new technology.

Market with New Therapies

The development of new therapies will impact the value of the HCV trapping device and the time points during treatment at which it will be used. The two treatments that will have the greatest impact on the use of the device are Telaprevir and Boceprevir. Both of these therapies have shown promising results in their respective clinical trials. Telaprevir has been able to dramatically shorten the length of treatments and Boceprevir has successfully treated a higher percentage of HCV infected patients than any other therapy.

Statistics from currently available trials of Telaprevir have shown an average SVR rate of approximately 75% for genotype 1 patients.[43] This is roughly 35% higher than the SVR rate observed for genotype 1 patients treated with the standard regimen of Peginterferon and Ribavirin (40%). The more promising results shows that there was no noticeable difference in the SVR rate between patients treated for 24 weeks and 48 weeks. Vertex also investigated a 12 week drug regimen to treat HCV during their Phase II trials. When treating patients for 12 weeks with a combination of Telaprevir, Peginterferon and Ribavirin, patients obtained an SVR 60% of the time.[44] A 12 week treatment was not possible before the development of Telaprevir. While there is a significant portion of patients cured with only 12 weeks of treatment, it would not be effective to treat the entire population of HCV infected people for only 12 weeks. Patients who would be successful with only 12 weeks of treatment must be separated from those who would require 24 or 48 weeks in order to take full advantage of the benefits of Telaprevir.

There are two ways to segment these patients: focus on patients who achieve RVR or test at 12 weeks to determine which patients have truly cleared the virus. Testing patients at the 12 week point would provide the most useful information and would be a logical point to conduct a viral load test using the trapping device. Ideally a physician would want to test every patient who has a negative test result with current methods at the 12 week point to determine if they have truly cleared the virus but this may not be necessary. Patients who have shown a fast response to treatment could be segmented and be the focus of testing with the trapping device at the 12 week point. As with current therapies, RVR is consider a good early indicator of a successful treatment and could be used as an initial filter for patients to be tested using the trapping device. When treating patients with Telaprevir in combination with Peginterferon and Ribavirin, 79% were able to reach RVR. It is likely that the vast majority of patients who can be successfully cured with a 12 week long treatment will be patients who have obtained RVR. Only testing patients who previously achieved RVR saves time and money because physicians would be focusing on those with a higher likelihood of achieving SVR with only 12 weeks of treatment. If these patients have cleared the virus, treatment could be stopped. If the patient does not have a negative test result using the HCV trapping device, then treatment could be extended by 12 weeks. The patients who undergo the extra 12 weeks of treatment could then be tested again at the end of the additional 12 weeks with the trapping device to make sure that stopping treatment makes sense for each individual patient.

Valuing the benefit to the patient of using the HCV trapping device is a multifaceted process. The goal of using the trapping device will be to shorten the length of treatment to 12 weeks for patients who have successfully cleared the virus. There are many potential benefits for patients if they employ the device and are able shorten their treatment. Patients would be able to avoid the financial cost of continued treatment, the side effects associated with the current combination of Peginterferon and Ribavirin and new therapies such a Telaprevir. These side effects can keep patients from being as productive, in their jobs and everyday activities, as they were before the treatment started.

The cost of Telaprevir or Boceprevir has been projected to be in the tens of thousands of dollars, on top of the $30,000 already paid for Peginterferon and Ribavirin. Patients are willing to pay the extra amount for treatment because of the higher success rate and shorter length of treatment.

Using the trapping device in conjunction with new therapies would shorten treatment even more. The extra $20,000 or $30,000 that people or insurance companies are willing to pay will likely cut the length of time patients are treated from 48 weeks to 24 weeks. The trapping device will likely cut the length of time a majority of patients are treated from 24 weeks to 12 weeks. By comparing the capabilities of this device with those of new therapies, the financial value of the trapping device can be estimated to be between $10,000 and $15,000. This value could change based on the final price of these new therapies. If this treatment approach was employed, roughly 38,500 patients would be tested annually using the trapping device.

The focus of this analysis has been on using Telaprevir to treat genotype 1 patients who had not been previously treated. There are also different scenarios in which the trapping device could be employed. The trapping device could be used in conjunction with the other HCV therapy being developed, Boceprevir. This drug has been shown to produce SVR rates as high as 79%, higher than any other previous treatments. Boceprevir has shown less success with shorter treatments than Telaprevir, but if approved by the FDA it would provide a legitimate alternative to Telaprevir. Using the trapping device with Boceprevir could allow more customization and the potential for shorter treatments for some patients. This would dramatically improve the chance that a physician may using Boceprevir instead of Telaprevir and potentially expand the Boceprevir market. The number of people who could benefit from using the trapping device in conjunction with Boceprevir is still unclear and will require the results of ongoing Phase III trials.

The third and final segment of the HCV infected population who could potentially benefit from this device are those being retreated for hepatitis C. People are retreated for hepatitis C for two primary reasons, either they did not respond the first time they were treated or they responded and later relapsed. Telaprevir has been shown to be particularly helpful for these patients. When used with Peginterferon and Ribavirin, patients who were previously non-responders reached SVR 40% of the time. Patients who previously relapsed now reach SVR roughly 75% of the time. When physicians are retreating a patient, they want to be certain that they have truly cured the patient before ending the treatment. This applies not only if treatment is ending early, but also if it is at the end of a full course of treatment. This necessitates the use of the HCV trapping

device at the end of treatment regardless of whether the drug regimen is ending early. Estimates show that there are roughly 300,000 patients who require retreatment with new therapies.[45]

6-Month Follow-up

It is important to only use the device at points in which it is necessary due to the cost and procedure associated with the using the HCV trapping device. One may think that the HCV trapping device should be used during the six month follow-up at the end of treatment. While it is likely that any HCV virus still present in the blood will be at low concentration, potentially below the current detectable limit, it is not necessarily advantageous to use the proposed trapping device. Current techniques are more than adequate to conduct this follow-up test. Current blood draw tests are close to 100% accurate in predicting if a patient has a true negative result. This high predictive value for current tests avoids the need for the procedure and cost associated with the trapping device at this stage of the treatment.

Current Diagnostics Market

Market Size

The tests used to detect and quantify HCV are classified as molecular diagnostic tests. The molecular diagnostics market in the United States is roughly a $3 billion market.[46] The leaders in the industry are Roche, Abbott and Siemens (acquired from Bayer in 2007); the segmentation in the market is presented in Figure 10. There are about 12,000 patients annually in the US who make up the primary target population (genotype 1 patients who attain RVR) for this device with the currently available therapeutics. The potential total annual sales are $130 million ($9,000 per device). With new therapies, the US market could expand to as many as 39,000 people and roughly $577 million ($15,000 per device) in annual revenue.

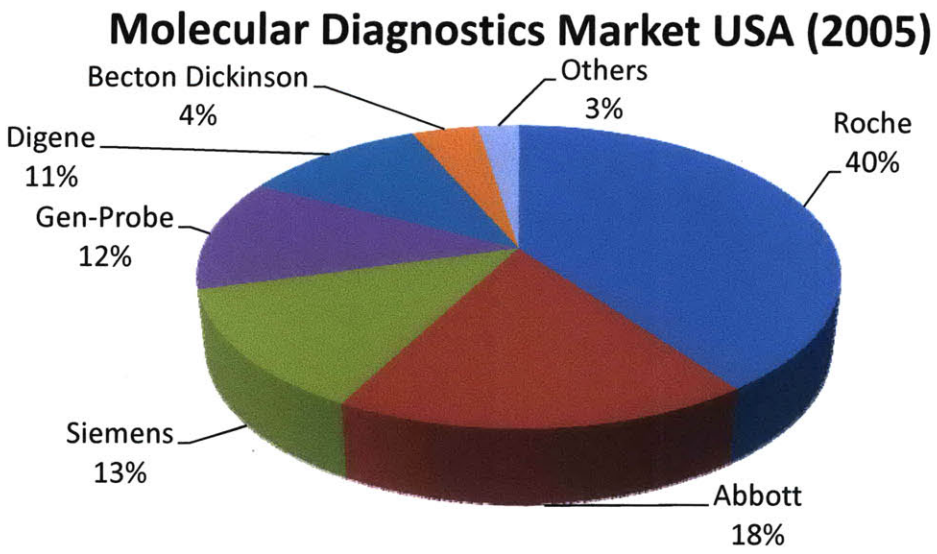


Figure 10 shows the market leaders of the molecular diagnostics market in the United States (2005).

The revenue from this device will pull market share from both the molecular diagnostics market and the antiviral therapies market. There will be a lower demand for the diagnostic tests and antiviral drugs because of the shortened length of treatments as a result of the use of this device. There may also be a shift in market share among the companies in Figure 10 because of the HCV trapping device. The most sensitive tests available today (LabCorp and Quest Diagnostics) have a small range of quantification. Their main marketing claim is how sensitive they are, but they are not very useful once outside of that range. If physicians paired the HCV trapping device with another test, one that is less sensitive but with a large dynamic range, they could end up with a test that is far more sensitive than either the LabCorp or Quest Diagnostics tests. This would greatly increase the utility of these less sensitive tests.

Profit Margin of the Current Market Leaders

While the proposed device is being used to help quantify the viral load of hepatitis C, it is not a pure molecular diagnostic device; it is still important to understand the profit structure of the industry. Roche holds 40% of the molecular diagnostics market. Molecular diagnostics account for 12% of total diagnostic sales for Roche. Roche's molecular diagnostic division (in 2009) had just over $1 billion in sales and a net profit margin of about 8.5%.[47] Abbott, the second largest company in the molecular diagnostics market, holds an 18% market share. Abbott has a profit margin of roughly 19%.[48] Siemens accounts for 13% of the total molecular diagnostics market. Siemens does not segment its diagnostic market data, but the profit margin for the entire

diagnostic department is 16.8%.[49] It is also important to understand the profit margin of nitinol based device companies because nitinol is a key component of this device. We found that Cordis (Johnson & Johnson) had a net profit margin of approximately 30%.[50] Cordis manufactures nitinol based clot catchers that are placed in the IVC.

Profit Margin of the Proposed Device

The trapping device has a gross margin of 81% to 89% depending on which implantation location is chosen. The net profit margin is between 39% and 51%, also depending on implantation location. The breakdown of the profit margins is shown in Tables 7 and 8. These profit margins are based on the estimated costs outlined earlier. The net profit margin includes the cost of ongoing research and development and a sales team as well as general administration costs. These costs are based on the percentage of revenue that current molecular diagnostics companies spend in each of these areas. Abbott spends 9% of revenue on research and development and 28% of revenue on sales and general and administrative costs.

Implantation Site	Cost of Procedure and Device	Sale Price of Device	Gross Profit Margin	% Sales and G&A	% R&D	Net Profit
SVC	$1,400	$9,000	84%	28%	10%	46%
Hepatic Veins	$2,100	$9,000	81%	28%	10%	39%

Table 7 shows the profit margins for the device if implanted in the two different locations as well as other costs associated with the development and sale of the device.

Implantation Site	Cost of Procedure and Device	Sale Price of Device	Gross Profit Margin	% Sales and G&A	% R&D	Net Profit
SVC	$1,400	$12,500	89%	28%	10%	51%
Hepatic Veins	$2,100	$12,500	83%	28%	10%	45%

Table 8 shows the profit margins for the device if implanted in the two different locations as well as other costs associated with the development and sale of the device based on markets created by new therapies.

Intellectual Property

Prior Art

There are many aspects of this technology that can be patented, but first we must understand what already exists, both in the form of patents and other public disclosures. The first area of prior art is other devices placed in blood vessels, particularly those made from nitinol. The two most prominent nitinol based devices are stents and clot catchers. Clot catchers are the more applicable form of prior art because they also trap objects present in the vein.

While the clot catchers and this device are both placed to trap things present in a blood vessel, there are two key distinctions. The first difference is that the trapping mechanism of the clot catching device is purely mechanical. Clots passing through the blood vessel become mechanically trapped and are kept from proceeding. This HCV trapping device uses the attraction between the antibodies and the viral particles, rather than a mechanical trap, to capture the virus. The second key difference about the trapping mechanism is that the clot catcher captures objects of a particular size indiscriminately. An independent claim in a patent titled "Intravascular Filter" (US Patent # 6,482,222)[51] claims "An intravascular filter for minimally invasive deployment within a vessel so as to obstruct the passage of particles of dimensions greater than a predefined value." This is different than the trapping device because the proposed device traps viral particles independent of size. Also, the HCV trapping device is specific to HCV; it does not trap other viruses or other particles present in blood even if they are of similar size to the hepatitis C viral particle. Another clot filter patent, "Retrievable Inferior Vena Cava Filter System and Method for Use Thereof" (US Patent # 5,893,869),[52] claims "A filtering system for filtering emboli from blood within a blood vessel." Emboli specifically refers to clot forming material. This HCV trapping device differs because it traps based on proteins expressed on the surface of the virus.

The second segment of relevant prior art is the use of antibodies to trap the virus. There are both published research and patents that comprise relevant prior art. One of more significant instances of prior art associated with the antibodies is nanoparticles used to the trap viral particles. A paper titled "Viral-Induced Self-Assembly of Magnetic Nanoparticles Allows the Detection of Viral Particles in Biological Media" by Perez *et al*[53] investigated the use of nanoparticles with attached antibodies to capture viral particles. A key distinction between the

technology in this paper and the current project is that this paper only discussed capturing viral particles that were in a blood sample. The authors did not discuss a technique or method for placing the antibody-coated nanoparticles in the body to sample blood flowing in a vein. The HCV trapping device is designed for use in the body and can be placed in a catheter to be passed into a blood vessel; this is not possible without altering the technique described in the Perez paper.

Another example of the use of antibodies for trapping cells and viral particles is as a diagnostic device in microfluidics. This is a particularly relevant example of prior art because, unlike the nanoparticle case, these targeted particles are moving relevant to the antibodies. Du *et al.*, in the paper titled "Microfluidic-based diagnostics for cervical cancer cells," discuss blood passed through a microfluidic channel that travels over and around antibodies that are fixed to the bottom of the channel. As the blood passes over the antibodies, the cancer cells become bound to the antibodies so that the presence of the cancer cells can be detected. A key distinction between the microfluidics example and the proposed HCV trapping device is that this microfluidics example is an *in-vitro* application. The proposed solution is an implantable *in-vivo* application. Because the microfluidics application is an *in-vitro* application, it is only capable of sampling as much blood as can be removed from the body at one time. The current technique employed with the trapping device allows blood to be sampled without removing it from the body because of the *in-vivo* approach of the HCV trapping device.

Additionally, the patents covering these technologies are specific to microfluidic applications. The patent titled "Microfluidic Devices and Methods for Separation" (US Patent # 6,444,461)[54] claims "a method of separating material in a microfluidic device." The conditions in the SVC are not close to those of the microfluidic device; particularly the scale of the SVC is much greater than a microfluidic channel. It would not pose a problem when filing a patent application for the HCV trapping device because of the specificity of what is claimed in this patent.

There is another segment of prior art that is relevant for this technology. Antibodies are being used to target specific cells or particles (i.e. tumor cells). In *"Antibody Targeted Drugs as Cancer Therapeutics"* by Schrama *et al.*, the drug is attached to antibodies and the antibodies are then released into the body. This technique allows the treatment to be focused at the site of the tumor and not affect other parts of the body. The drug bound antibodies circulate and become

bound to the antigen (cell or viral particle). There are two key distinctions between this technology and the HCV trapping device. The paper referred to above discusses antibodies used for drug delivery in which the antibodies are free to circulate throughout the body. However, for the HCV trapping device, the antibodies are in a fixed location and are attached to the device at all times. Secondly, the antibodies in the trapping device are not a delivery system as they are in the antibody/drug delivery application. Their function in the trapping device is solely to capture viral particles.

Future Patent Portfolio

When developing a patent portfolio, there are two key areas that should be patented. First, the manufacturing of the nitinol backbone is important. Because the manufacturing process used to produce the shape of the nitinol is unique to this application and dictates the function of the device, it will be patentable. Patents would specifically cover the temperature sequence used, duration at each temperature and the design of the mandrel. Another patent could cover the antibody coating process if a unique technique is employed.

It is important to file patents regarding the general function of the device. One claim would be for a device placed in a blood vessel for the purpose of capturing a virus. This could provide protection against other people filing patents in the future trying to claim variations on this embodiment. Additionally, by including this claim, it would cover a wide range of materials, including but not limited to nitinol. Dependant claims of this first independent claim would cover things like the specific shapes or design of the device. Another dependent claim would be that the trapping mechanism utilizes an antibody/viral particle interaction.

International patent applications should also be considered because of the high incidence of hepatitis C throughout the world. International patents should be focused on countries with a high rate of HCV infection and where the medical infrastructure exists to employ the technology.

FDA Approval

Approval Process

The device will be considered a Class III device because of its intended use. Therefore, it will require the PMA (Pre-Market Approval) process. The PMA process will require both preclinical and clinical trials to ensure that the device will not have any adverse effects on the human body. To lessen the burden of the FDA approval process, previously approved materials are used. The materials have already been used for *in-vivo* applications and are proven to be safe for the duration of the time they will be in the body for this application (less than 30 minutes). The greatest burden of proof will be with regard to the antibodies. While antibodies have been used for *in-vivo* applications in the past, they have not been HCV antibodies. Commercial HCV antibodies are not approved for *in-vivo* use in humans. Thus, preclinical trials will need to be conducted to determine the safety of the antibodies. Once preclinical testing has been completed, clinical tests will begin on a humanized form of the antibody. An IDE (investigational device exemption) is required to begin clinical trials. This allows a clinical trial to be conducted on humans. The costs associated with a clinical trial would be about $65 million. This includes the cost to follow 2000 patients for 12 months, all associated testing and lab costs, and the cost of treatment.[55]

Business Model

The business model for this device is a two stage process. The lack of sufficiently sensitive quantification diagnostic devices is a problem in both the clinical and research settings. While physicians require more sensitive tests to monitor a patient's response to treatment, researchers require even more sensitive tests, to monitor the effectiveness of new treatments. The research community is an ideal first market when introducing this device.

There are many advantages to first using this device in research settings. The price for research settings is less sensitive than the commercial market. When used in a research setting, while the subject may benefit from the results, the researchers conducting the study and the entire research and medical communities stand to gain useful information. The ability to charge a higher price for this device has a two-fold benefit. First, the higher revenue from sales can be used to continue research to refine the device. Secondly, the ability to charge a higher price allows the

device to be built without taking full advantage of economies of scale and to produce at a prototype level until a final version of the device is decided upon. This also provides the developers of the technology with the benefit of getting feedback from clinicians using the device for research.

Strong clinical evidence of the benefits of employing this technology will be a key second step of this business model. Previously, new HCV diagnostic techniques have been used to amplify the viral particles captured during a typical blood draw. The technique required by this device involves a lengthier sampling process. Strong clinical evidence of this device will be key for convincing physicians that the extra step is worthwhile.

Once the device reaches the point of commercialization, the best approach will be the sale of the device directly to hospitals and group purchasing organizations. Hospitals could purchase the device to augment their preferred HCV diagnostic test. Once the market for the device has become established, current molecular diagnostic companies may be interested in acquiring the technology to prevent competitors from being able to use the technology with their tests.

Licensing of this device to a molecular diagnostics company is not a viable option until a company is established to build the device from the components purchased from the current supply chain. A molecular diagnostics company currently making HCV tests may seem like a logical choice for a partner or licensee, but the production of this device deviates significantly from that of other HCV-related tests. Selling the technology to an existing HCV diagnostic company would only become a viable option once the manufacturing network has been established.

Evolution of the device design will be critical to maintain and grow the market share of the device. A PCR or TMA test will not be able to compete with the proposed solution with regard to blood sampling capabilities because of the far greater volume of blood sampled with the proposed device. Competition will come from other companies that would develop *in-vivo* sampling equipment. The design will need to continuously evolve to keep ahead of potential competitors. Changes to the device, to increase sampling volume and binding affinity of the antibodies and make the placement of the device easier and cheaper, will all be required to keep a competitive edge in the future. Ten percent of revenue is directed towards R&D annually (this

is built into the profit margins outlined earlier) because of the importance of continued research and development. Making the device easier to implant will not only help maintain market share, but it will help to grow the market share of this device. The HCV trapping device will be used to augment a greater number of traditional HCV tests once it becomes cheaper and easier to implement. It can work with the most sensitive test available at the time, allowing this device to evolve with new quantification techniques.

The revenue generated from this device would come directly from the sale of the product to hospitals and GPOs. The sale of the necessary accessories and complementary technologies could also be a source of revenue, but it would account for a relatively small portion of the net profit. It would be inefficient for a new company that manufactures the HCV trapping device to get involved with the processing of blood samples because that work is already done in either a hospital or at a well-established third party company that can take advantage of economies of scale. The focus of a company producing this device should be on manufacturing, and research and development to improve the device in the ways mentioned above.

Teaming with Companies Producing New HCV Treatments

A potential partnership can be formed with companies like Merck, Vertex Pharmaceuticals or others developing new HCV treatments. These new drugs are aimed at increasing the "cure" rate and shortening the length of the treatment. Companies that claim that their drug shortens treatment would be in a stronger position if each shortened treatment could be confirmed with an ultra sensitive testing that is facilitated by the HCV trapping device. Alternatively, if one company is unable to gain FDA approval for a shortened duration of treatment standard for all patients, it could use this device to offer shortened treatment to some patients based on test results obtained with the trapping device. There is also the scenario in which two competing therapies both lower the viral load below the currently detectable limit. In this case, the trapping device could be employed to determine which therapy helps the patient reach the lowest viral load and eventually eradicate the virus the fastest.

Future Experiments

In-Vitro Testing

Initial testing will be conducted with the rubella virus instead of hepatitis C because researchers working on the project can be vaccinated against rubella, but that is not possible for hepatitis C. The first *in-vitro* experiment will be to test the interaction of the rubella viral particles with the rubella antibodies at the conditions experienced in the SVC (i.e. blood velocity). A polystyrene channel will be coated with antibodies and blood that contains viral particles will be pumped through the channel. The rubella antibodies will be attached to the channel using the same technique that is used to prepare enzyme-linked immunosorbent assay (ELISA) tests using the sandwich ELISA technique. The channel will be coated with a biocarbonate/carbonate buffer solution containing the antibody. This channel will be sealed and the antibody-coated channel will be allowed to incubate. The channel will be washed with phosphate buffered saline (PBS) after a 12 hour incubation to remove any unbound antibody. The antibodies used to coat the channel can be obtained from Abcam Inc. (Cambridge, Massachusetts) or another antibody distributor. Bovine blood, containing the viral particles, will be pumped through the channel for 30 minutes (the time period the device would be in the body). Subsequently, a secondary antibody will be passed through this channel. The secondary antibody will be used to detect the binding of the viral particles to the antibodies attached to the channel. A binding efficiency of the viral particles will be determined based on this experiment.

The next *in-vitro* experiment will be conducted to test the design of the trapping device. The current design of the device is shown in Figure 6. This testing will be conducted to determine if the design of the device is currently optimized or requires changes to maximize the trapping capabilities of the device. This experiment will be conducted by coating a device made of nitinol that is formed into the shape shown in Figure 6. The nitinol device will be coated in PDMS to promote the attachment of the antibodies to the device. Once the device is constructed, it will be placed in a tube with the same diameter as the SVC (3 cm).

In-Vivo Animal Testing

Following the *in-vitro* tests, animal studies will be conducted. Initial *in-vivo* animal tests will be conducted for two purposes: to determine how the device will operate when put in an environment that closely mimics the human SVC and to determine the best deployment

technique. Initial animal testing will be conducted on a pig. The pig has been shown to be a good model for the human cardiovascular system. During this experiment, the device will be placed in the pig's vein and rubella viral particles will be injected into the pig. The viral particles will circulate through the pig. The viral particles will come in contact with the device and be trapped by the antibodies. Once the viral particles are trapped, the device will be removed and the captured viral particles will be compared to the total number of viral particles measured by a traditional blooding sampling technique.

The final *in-vivo* experiment for this device will be to test the device in a vein of an HCV infected animal. The only animal that can be infected with HCV is the chimpanzee. A chimpanzee will be infected with HCV and blood will then be drawn from the chimp and quantified using an existing HCV test. The HCV trapping device will then be placed in a vein in the animal and left for 30 minutes. The device will then be removed and the viral particles will be quantified using the same test that was used to quantify the blood sample from the chimp. These two viral loads will be compared to determine the accuracy of quantification using the HCV trapping device.

Funding – Next Two Years

Funding for research over the next two years is focused on *in-vitro* and *in-vivo* animal testing in pigs. The key components of funding over the next two years are the acquisition of rubella and HCV antibodies and viral particles. A detailed breakdown of funding for the next two years can be found in the Appendix.

Conclusion

There is a clear need for more sensitive HCV testing equipment. A new approach is necessary because of the limits of traditional blood draw based diagnostics tests. The proposed alternative is an *in-vivo* sampling device. The proposed device contains a nitinol backbone that is coated with PDMS and HCV antibodies. Modeling calculations were used to determine that the "twisted" coil design has far superior blood sampling capabilities when compared to current alternatives. Physicians desire to shorten the length of treatment for patients because of the high cost and the wide range of potentially significant side-effects. While studies have been conducted showing the potential success of half-length treatments, the medical community is still reluctant to recommend changes in treatment lengths. There is a sufficient incentive for all

parties involved (patients, physicians, and insurance companies) to justify the use of the proposed HCV trapping device when its use is focused on genotype 1 patients who reach RVR. This incentive becomes even greater once new therapies become available. Moving forward, the greatest concern regarding the development of this device is the use of antibodies. While there are examples of other devices that use antibodies to trap cells and viral particles from flowing blood, they have not been shown to be successful at the velocity of blood present in the IVC. This is unlikely to pose a problem because the extremely high sampling capabilities of the proposed design allow it to be an effective device even with an extremely low binding efficiency. The focus of research will be on continuing the development of the shape and design of the device and conducting tests to determine the most appropriate antibody for this application.

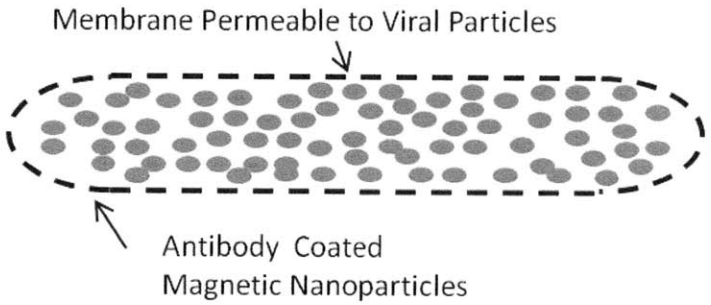
Appendix

Design #1 -- Diffusion of Viral Particles through Polymer Membrane
Cylindrical device 1-3 cm in length, 5 mm in diameter

Surface area for diffusion: ~ 160 – 500 mm^2

Membrane Permeability: 2-20 μm/min

Concentration in Blood: 5 viral particles/mL



Assuming constant concentration outside device and infinite sink inside device

$$= \frac{\text{Viral Particles}}{\text{Min}} = \text{Membrane Permeability x Surface Area x Concentration Gradient} =$$

20 μm/min x (160 mm^2) x 5 Viral Particles/ mL = .016 particles/min = 1 - 3 particles/hour

Design #2 -- Diffusion of Viral Particles in Blood

Reynolds Number

$$Re = \frac{\rho v D}{\mu} = \frac{1 \frac{g}{cm^3} \text{ x } 26 \frac{cm}{s} \text{ x } 3 \text{ cm}}{6.53 \text{ x } 10^{-3} \frac{Ns}{m^2}} = 1195$$

μ = Dynamic Viscosity of the Fluid

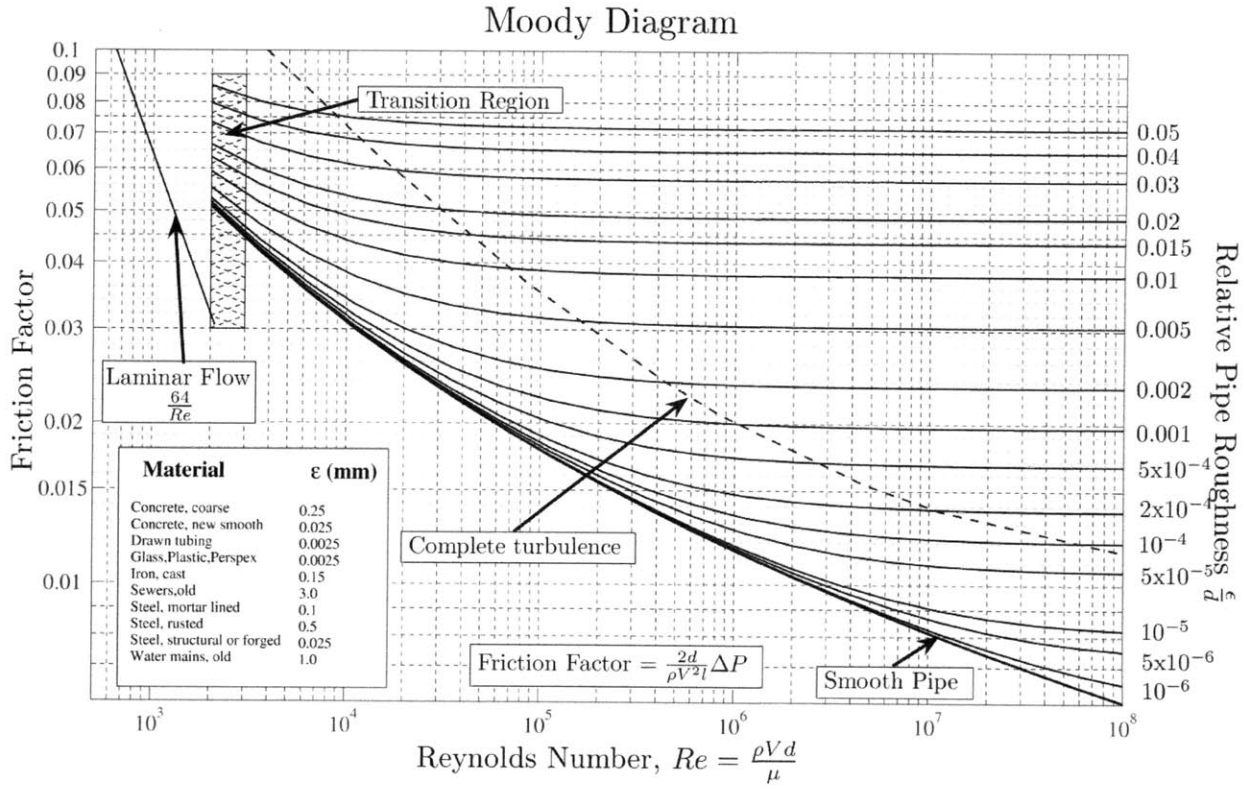
L = Characteristic Linear Dimension (Traveled Length of Fluid)

V = Mean velocity of Fluid

ρ = Density

Moody Diagram

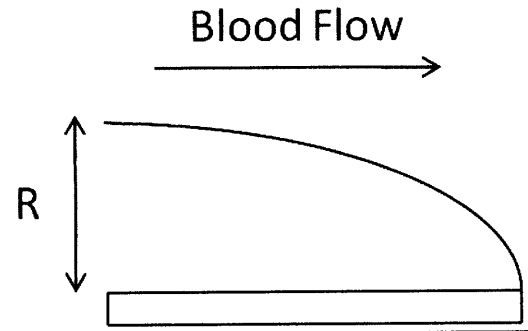
Based on the calculation above for Reynold's number and the Moody Diagram below, the conditions in the SVC are defined as laminar flow. This means that the diffusion coefficient equation used in the next step is valid.



Einstein-Stokes Equation – Diffusion of spherical particles through a liquid with low Reynold's number)

$$D = \frac{K_B T}{6\pi\eta r}$$

D = Diffusion Constant
K_B = Boltzmann's Constant
T = Absolute Temperature
η = Viscosity
r = Radius of Spherical Particle



Rod Length	1 cm	2 cm	3 cm
Diffusion Distance (R)	0.66 μm	0.93 μm	1.14 μm
Volume of Blood Sampled (30 min)	0.37 ml	0.53 ml	0.64 ml
Rods Needed to Match 2 mL Blood Draw	6	4	4

Blood Sampling Calculations – "Twisted Coil Design"

Area Available for Sampling = Cross-Sectional Area in Direction of Blood Flow

Diameter of Wire in Sampling Device = 1 mm = .1 cm, Length of Wire = 3 cm

Blood Velocity in Superior Vena Cava = 10 cm/s to 35 cm/s, Average Velocity = 22.5 cm/s

Diameter of Superior Vena Cava = 3 cm, Radius of Superior Vena Cava = 1.5 cm

Cross-Sectional Area of Superior Vena Cava = $\pi r^2 = \pi(1.5^2) = 7.07\ cm^2$

Cross-Sectional Sampling Area =
(diameter of wire) x (diameter of SVC) x (# of times the device crosses the vein) = $(.1\ cm) * (3\ cm) * (7) = 2.1\ cm^2$

Percent of Vein Used for Sampling over the Length of the Device = (2.1 cm)/(7.07 cm) = .30 => 30%

Blood Flow Rate = (Area) x (Blood Velocity) = $(7.07\ cm^2)\ x\ \left(22.5\frac{cm}{s}\right) = 159\frac{mL}{s} = 9542.6\ \frac{mL}{min} =$
286.3 L per 30 minutes

Volume Sampled in 30 minutes = (% of water sampled) x (Total Blood through Vein in 30 minutes)

= (.30) x (286.3 L per 30 minutes) = 85.1 L per 30 minutes

Two Year Research Budget

Year 1

Description	Unit Quantity	# of Units	Supplier	Unit Cost	Cost
Reagents needed to run and prepare PCR	1	480	Applied Biosystems	$12	$5,760
Use of PCR equipment onsite at MIT	96 well plate	50	MIT	$20	$1,000
Rubella virus E1 protein (ab68498)	100 ug	7	Abcam	$260	$1,820
Rubella virus E1 antibody [Ru6] (ab82464)	1 mg	7	Abcam	$319	$2,233
HCV Viral Particles	TBD	8	TBD	$260	$2,080
Antibodies - Hepatitis C Virus E1 antibody [BDI198] (ab21306)	100ug	8	Abcam	$365	$2,920
Storage of Pig in Animal Facilities	1 day	42	MIT	$35	$1,470
Purchase of Pigs	1 pig	4	TBD	$1,150	$4,600
Peristaltic Pump -- L/S® Standard Digital Drives (Product Number: EW-07523-80)	1 Pump	1	Cole Palmer	$1,710	$1,710
General Lab Supplies and Chemicals (PDMS, Buffers, Pipettes,Nitinol, etc.)	N/A	N/A	Varies	$1,350	$1,350
Animal Surgeries and Associated Equipment	1	4	MIT	$400	$1,600
				Total	**$26,543**

Year 2

Description	Unit Quantity	# of Units	Supplier	Unit Cost	Cost
Reagents needed to run and prepare PCR	1	480	Applied Biosystems	$12	$5,760
Use of PCR equipment onsite at MIT	96 well plate	50	MIT	$20	$1,000
Rubella virus E1 protein (ab68498)	100 ug	7	Abcam	$260	$1,820
Rubella virus E1 antibody [Ru6] (ab82464)	1 lmg	7	Abcam	$319	$2,233
HCV Viral Particles	TBD	8	TBD	$260	$2,080
Antibodies - Hepatitis C Virus E1 antibody [BDI198] (ab21306)	100ug	8	Abcam	$365	$2,920
Storage of Pig in Animal Facilities	1 day	42	MIT	$35	$1,470
Purchase of Pigs	1 pig	4	TBD	$1,150	$4,600
General Lab Supplies and Chemicals (PDMS, Buffers, Pipettes, Nitinol, etc.)	N/A	N/A	Varies	$1,350	$1,350
Animal Surgeries and Associated Equipment	1	4	MIT	$400	$1,600
				Total	**$24,833**

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