Microfluidic Device for the Detection of *V. cholerae* in Drinking Water

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Since August of 2004, Cholera Microbusters, Inc. has been developing a credit-card-sized microfluidic device for the detection of *Vibrio cholerae*, the bacterium that causes cholera, in drinking water. The device is intended to provide an inexpensive and reliable means for tourists and local health officials in the developing world to determine if their water supply is contaminated with deadly cholera bacteria. This summary briefly outlines the final status of the design project.

The device design relies on the accuracy of an immunometric assay to detect *V. cholerae* cells. Antibodies specific to *V. cholerae* will be immobilized on the detection surface of the device. When a collected sample is passed over these antibodies, any *V. cholerae* cells present in the sample will be captured. Afterwards, antibodies labeled with gold particles will be passed over the detection surface and will bind to these captured cells. Because of the gold colloids, a contaminated sample would result in a reddish color on the detection surface. However, the design also utilizes the recently developed technique of silver enhancement of the gold colloids, resulting in a strongly amplified and more definitive signal. None of the reagents required for these processes has been found to pose an environmental or safety hazard.

Finger-actuated micropumps consisting of elastic silicone-rubber chambers are used to collect the sample and dispense reagents to the detection chamber. Theoretical and experimental analyses of finger-actuated pumps were performed to determine the pump dimensions that would minimize the unused, or "dead," pump volumes. From these analyses, it was concluded that percent dead volume decreases with increasing pump diameter for the reagent dispensers, increases with increasing pump diameter for the sample collector, and is independent of height for both chamber types. Furthermore, for a set chamber height, a reagent dispenser with a little more than half of the diameter of a sample collector yielded the same effective volume. Because each reagent volume dispensed should fully replace the fluid present in the detection chamber before its release, it was assumed that equivalent effective volumes of reagent and sample are desired. Ultimately, to maximize the effective volume, a height of 0.0625 inches, a reagent-dispenser diameter of 0.625 inches, and a sample-collector diameter of 1.0 inch were selected.

The desired width of the channels from the micropumps to the detection chamber was also determined using the experimental data. Optimally, the device operator should have to press a finger-actuated micropump for a length of time on the order of only one second. After evaluation of the relationship between the required pumping time and channel width, a channel width of 1.0 millimeter was found to meet this criterion.

The micropumps and channels were arranged on the device so as to maximize convenience for the user. The sample collector and detection chamber are situated in opposite corners of the device to facilitate sample aspiration, and the reagent dispensers are arranged in the order in which they are to be pressed to minimize confusion during operation. A prototype demonstrating the effectiveness of this arrangement, including the optimized micropumps and channel width, has been created and is available for inspection. Three-dimensional schematics have also been generated and are included in the final report.

This final report presents a design in which most of the technical aspects have been analyzed. With a total estimated materials cost of $2.16 per device, this device has major potential to forge a new market of inexpensive, point-of-care diagnostic tools of which tourists, residents, and local health officials can take advantage. To complete development of this design, experimentation must be performed to research the specific effects of device operation on *V. cholerae* cells and to study the appropriateness of the recommended manufacturing techniques.

Introduction

Over the past several decades, specifically the last thirteen years, multiple cholera epidemics have occurred in Latin America, southeastern Asia, and Africa[1]. During the 1990s, the number of cases worldwide ranged between two hundred thousand and six hundred thousand per year[2]. Although cholera is a disease that can often be treated with simple rehydration, inexpensive and effective detection devices could help local health services identify the risk of an outbreak as soon as possible and help travelers and residents avoid the consumption of contaminated water[3,4]. Products are on the market for diagnosing cholera or determining if the bacteria that cause cholera are in a water sample, but they require trained operators and supplemental laboratory equipment[5,6]. This report presents the design of an accurate and inexpensive microfluidic device, capable of performing both sample preparation and analysis, for the detection of cholera-causing bacteria in drinking water.

According to Nair (2004), the Center of Disease Control (CDC) (2003), and Todar (2001), cholera is caused by the gram-negative bacterium *Vibrio cholerae*. The various strains of *V. cholerae* are divided into 206 serogroups based on the differences of their O antigens. The serious cases of cholera are caused by O1 and O139 serotypes. The O1 serotype is further divided into classical and El Tor biotypes. Classical biotypes produce more severe illness than the El Tor biotypes. The O1 serotype is responsible for epidemics in Latin America and Africa, and the 0139 serotype is responsible for outbreaks in southeastern Asia[7,8,9].

In order to develop cholera, a normal person must ingest one hundred million *V. cholerae* cells[10]. However, those taking antacids, such as bicarbonate, are at a higher risk because of the neutralizing effect these agents have on stomach acid. Symptoms of *V. cholerae* infection can manifest in as little as one day and can last up to seven days. *V. cholerae* infection begins with the ingestion of contaminated food or water and proceeds with the colonization of the epithelium of the small intestine. The bacteria produce a toxin that causes large quantities of chloride ions to be pumped into the small intestine. This electrolyte imbalance results in vomiting, diarrhea, and dehydration[11]. If not treated promptly, cholera can be fatal[12, 13,14].

V. cholerae and the toxin it produces can both be detected with immunometric assays, which rely on the natural specificity of antibodies to antigens. According to Wild (2001), a

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[1] Nair, G. B. 2004. p. 123-125.
[2] *WHO*. 2001. p. 41.
[3] Cholera Technical Information. 2003. p. 1.
[4] Lowenhaupt *et al*. 1998. p. 1.
[5] Lowenhaupt *et al*. 1998. p. 1.
[6] Hasan *et al*.. 1994. p. 249
[7] Nair, G. B. 2004. p. 123
[8] *Technical Guidelines-Cholera*. n.d. p. 1.
[9] Todar. 2001. p. 4.
[10] Madigan *et al*. 2003.
[11] Nair, G. B. 2004. p. 121
[12] Nair, G. B. 2004. p. 124
[13] *Technical Guidelines-Cholera*. n.d. p. 1.
[14] Todar. 2001. p. 2.

sample suspected of containing the bacteria and/or cholera toxin is passed over the appropriate antibody immobilized on a plastic surface (**Figure 1a**), typically polystyrene. Any analyte present in the sample binds to the antibody in a process known as capturing (**Figure 1b**). The toxin itself functions as an antigen while surface sugars and proteins function as the antigen on the bacterium. Next, labeled antibodies, called tracers, pass over the surface and bind to the captured analyte (**Figure 1c**). The antibodies can be labeled with fluorescent tags, in which case the fluorescence intensity of the treated sample is an indication of analyte concentration[15]. Alternatively, Sia *et al* have investigated labeling these antibodies with gold colloids to produce a visible response. Gold-colloid labeling can be coupled with silver enhancement, a technique that builds a silver film around the colloids to amplify the visual signal. The degree of opacity of the resulting film can be correlated to analyte concentration[16]. Whatever the detection technique, removal of unbound tracer from the assay is crucial to ensure accurate concentration measurement[17].

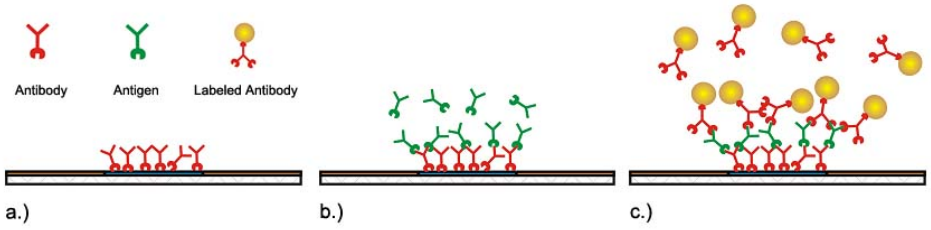

Figure 1: Immunometric Assay. **a.)** Antibody immobilized on substrate. **b.)** Introduction of antigen and attachment to antibody. **c.)** Introduction of labeled antibody and attachment to antigen[18].

Ahn-Yoon *et al.* (2003) have developed a highly sensitive immunometric assay to detect cholera toxin (CT) through the use of surface-bound antibodies and labeled ganglioside-incorporated liposomes (GM-1). Although gangliosides are used in place of labeled antibodies, they still have a strong affinity specific to the toxin. Labeled GM-1 first captures the CT in solution; then the resulting complexes bind to the surface-bound antibodies, forming a dark band. Although this method proves to be effective, rapid, and cost effective, it cannot detect the presence of dormant *V. cholerae* cells, which do not release CT[19].

V. cholerae can remain dormant but viable in aquatic environments for extended periods of time[20,21]. The agent responsible for activating the dormant bacteria is not known[22]. At least two products are on the market for *V. cholerae* O1 cell detection: The Cholera Direct

[15] Wild. 2001. p. 3-4.
[16] Sia *et al*. 2004. p. 499.
[17] Madigan *et al*. 2003.
[18] Shalini Gupta, NCSU chemical engineering graduate student.
[19] Ahn-Yoon *et al*. 2003. p. 2256.
[20] Nair, G. B. 2004. p. 129
[21] Lowenhaupt *et al*. 1998. p. 1.
[22] Lowenhaupt *et al*. 1998. p. 1.

Fluorescent Antibody (DFA) by New Horizons Diagnostic Corporation and the Sensitive Membrane Antigen Rapid Test (SMART) by New Horizons Diagnostic Corporation (Columbia Maryland, USA) (http://www.nhdiag.com/cholera01.shtml)[23]. The DFA uses monoclonal antibody probes with fluorescent tags and requires the preparation of sample slides. The limited mobility and extensive preparation of this device make it unsuitable for on-site testing. The SMART uses monoclonal and polyclonal antibodies with gold-colloid labels and has also been evaluated using monoclonal antibodies only. Simple yes/no answers as to the presence of cells are provided by the appearance or absence of a red dot resulting from the aggregation of the gold colloids. The sensitivity of this assay is six million cells per mL and is too high for the device to be used as a preventative tool; it is only intended to serve for diagnostic purposes. However, if its sensitivity were increased by silver enhancement, it is possible that it could be used to assess the contamination of drinking water instead of stool only. Furthermore, although this assay provides accurate results in five to ten minutes, sample preparation must take place outside the device and requires buffer, a reaction vial, eyedroppers, and swabs[24].

The design of the device described in this report uses immunoassay techniques similar to those of the SMART, but the detection method is improved by incorporating silver enhancement and microfluidic technology. Researchers continue to find microfluidic alternatives to conventional "bench-top" processes, furthering the technology known as "lab-on-a-chip"[25]. Microfluidics involves the manipulation of small volumes of liquid on the nano- to picoliter scale [26], usually with devices containing solid channels (**Figure 2**). This small volume requirement means only small quantities of reagents are needed, reducing costs and waste. Yield can also be increased since efficiencies are higher when working on a smaller scale[27]. Moreover, recent advances in the implementation of pumping and valving on microfluidic devices have made sample preparation on such a small scale viable[28]. The compact nature of these systems provides the potential for easy portability, which is convenient for on-site testing.

[23] Lowenhaupt *et al*. 1998. p. 1.
[24] Hasan *et al*..1994. p. 250.
[25] Koch *et al*. 2000. p. 1.
[26] Nguyen *et al*. 2002. p. 4.
[27] Koch *et al*. 2000. p. 282.
[28] Ahn *et al*. 2004.

Figure 2: Examples of previously developed microfluidic devices currently on the market for handling biological materials[29].

According to Frost & Sullivan (2004), portable, disposable microfluidic devices for the detection of infectious agents have the potential to stimulate the global clinical diagnostics market[30]. This US$22 billion market experiences an annual growth rate of only 5%, while its US$4.7 billion home and self-testing sector has a much larger annual growth rate of 11%. Consumers and companies would be receptive to the device described in this report because it is for on-site testing of *V. cholerae* cells, an infectious agent. The consulting and market analysis firm, Frost & Sullivan, has identified infectious disease diagnostics as another significant source of growth for the clinical diagnostics market[31].

The market for microfluidic devices with uses in environmental monitoring, biotechnology, pharmaceuticals, DNA diagnostics, and cell analysis[32,33] is very strong because of the applications in the booming biotechnology and analytical/clinical chemistry industries[34]. The world market for microsystems is growing at a rate of 20% annually and had a total worth of US$25 billion in the year 2000, with lab-on-a-chip *in vitro* diagnostics and inkjet print heads making up a large portion. The fastest amount of growth within the microsystems industry is occurring in the biomedical and information technology fields[35].

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[29] Dr. Orlin Velev, professor of Chemical Engineering at NCSU.
[30] Frost & Sullivan. 2004. p. 1.
[31] Frost & Sullivan. 2004. p. 1.
[32] Nguyen *et al*. 2002. p. 4.
[33] Tay, F.E. 2002. p. xi.
[34] Koch *et al*. 2000. p. 283.
[35] SEMICON Europa. 2001. p. 1.

Some of the major manufacturers of microfluidic devices are Micronics, Inc.[36], MicroFluidic Systems, Inc.[37], and Fluidigm Corporation[38]. Micronics, Inc. develops "lab cards" for use in medical detection. They have recently started to develop disposable microfluidic devices for the detection of biologically threatening agents[39]. MicroFluidic Systems, Inc. develops dual-use products for the government and for commercial use, especially in the area of biodefense[40]. This company is currently working with the FBI to develop microfluidic devices to automatically handle DNA from forensic samples[41]. Fluidigm Corporation works with extremely complex microfluidic systems known as integrated fluidic circuits. Their current products focus on the crystallization of proteins, and the company at the beginning of 2004 raised US$21 million in financing[42].

Aside from the apparent readiness of the market and industry for the entry of a microfluidic device to detect *V. cholerae*, the device would serve a humanitarian purpose, the value of which would be difficult to express in dollars. In 2004, the World Heath Organization's Global Task Force on Cholera Control released a document detailing recommendations for local health officials coping with cholera outbreaks[43]. In the document, titled *Cholera Outbreak, Assessing the Outbreak Response and Improving Preparedness*, the organization stresses the importance of including water and sanitation experts as part of a strong preparedness plan. After an outbreak has occurred, the duty of these experts would be to infer from environmental clues what the source of the outbreak is and to eliminate the hazard it poses for residents.

The tasks for these workers would no doubt be simpler if they had an accurate, inexpensive, and portable device to test the water sources they investigate for *V. cholerae* directly. Better still, such a device, like the one described in this report, could help identify contaminated water sources before they even have a chance to cause an outbreak; the identification process as currently suggested by the World Health Organization is first set in motion only after a person ill with cholera has been diagnosed. This dependency on diagnosis is a result of the lack of commercially available *preventative* tools for *V. cholerae* detection on the market.

Recent developments in microfluidic and immunoassay technology have provided new design solutions that should exploited to lessen the impact of cholera. The inexpensive microfluidic device capable of detecting the bacteria that cause this disease, proposed herein, would help save many people from infection and would be attractive to travelers, residents, and health workers in affected areas alike.

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[36] Micronics. n.d. p. 1.
[37] Microfluidic Systems Inc. n.d. p. 1.
[38] Fluidigm. n.d. p. 1.
[39] Micronics, Centrex Developing Biothreat Lab-on-a-Chip. June 4, 2003. p. 1.
[40] Silber, J. 2003. p. 1-2.
[41] FBI Taps Microfluidic Systems for DNA Analyzer. May 28, 2004. p. 1.
[42] Fluidigm Drinks up $21 Million. January 6, 2004. p. 1.
[43] World Health Organization. 2004. p. 10-22

Technical Background

This section provides an overview of the knowledge necessary to complete and understand the proposed design. Cholera and its global impact are addressed first, followed by relevant biological and biochemical aspects of the disease. The discussion then focuses on the development of the proposed device design, including issues concerning detection, fabrication, and transport phenomena.

Cholera

Cholera is a disease that has affected humanity on a worldwide scale for almost two centuries. There is a general consensus in the literature that the first pandemic occurred in India from 1817 to 1823, the second from 1829 to 1851, the third from 1852 to 1859, the fourth from 1863 to 1879, the fifth from 1881 to 1896, the sixth from 1899 to 1923, and the seventh from 1961 to the present[44,45]. However, the number of reports from the days of Hippocrates and Lord Buddha describing diseases with cholera-like symptoms suggests that cholera probably existed well before 1817[46, ,47,48]. The durations of the pandemics are indistinct because cholera usually affects multiple countries at once, and the bacteria that cause the disease can be dormant for extended periods of time in the natural environment before reemerging[49].

The current pandemic has lasted for over forty years and affects communities in Africa, Asia, and Latin America[50]. **Figure 3** illustrates the rapid spread of this latest pandemic in its early stages between 1961 and 1971. Cholera did not reach Peru until 1991, but then it quickly expanded into the rest of Latin America and even the US Gulf Coast[51,52]. By 1999 over 250,000 cases and 9,000 deaths had been officially reported to the World Health Organization by Latin American countries, though some researchers suspect the number of fatalities is closer to an astonishing 120,000 because of a lack of resources and intentional underreporting[53]. Africa experienced one of its highest cholera mortality rates (12%) during the height of the Rwandan refugee crisis in 1994[54]. Cholera mortality rates can reach 50% in local areas where treatment is not available[55].

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[44] Barua *et al*. 1992. p. 1-2.
[45] Nevondo *et al*. 2001. p. 1.
[46] Todar. 2001. p. 2.
[47] Barua *et al*. 1992. p. 1.
[48] Wachsmuth *et al*. 1994.
[49] Barua *et al*. 1992. p. 2.
[50] Nair, G. B. 2004. p. 120, 123-125.
[51] Nair, G. B. 2004. p. 124-125.
[52] Nevondo *et al*. 2001. p. 1.
[53] Nevondo *et al*. 2001. p. 1.
[54] Nair, G. B. 2004. p. 124.
[55] *Technical Guidelines-Cholera*. n.d. p. 1.

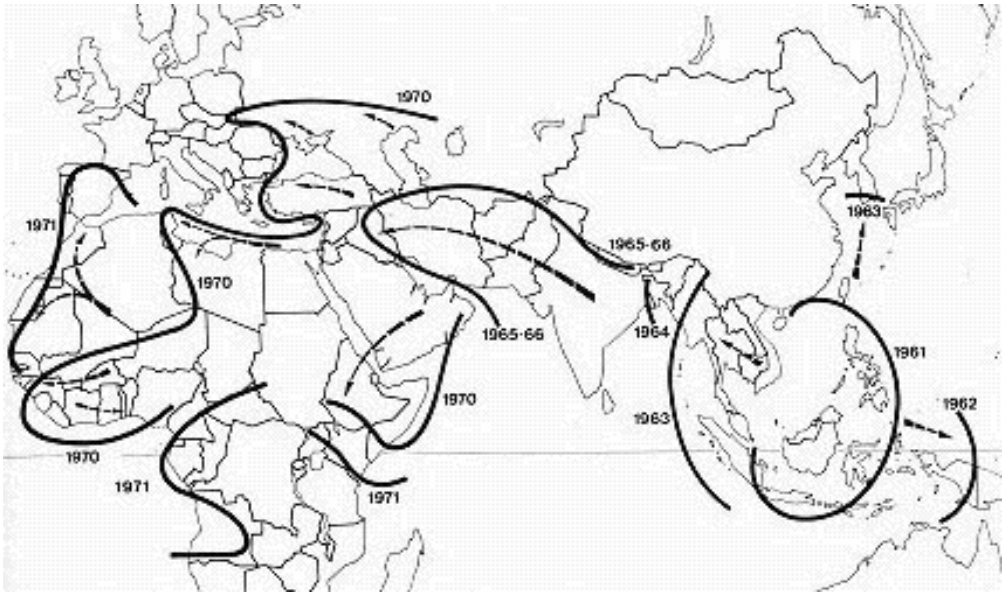

Figure 3: The Spread of Cholera From 1961-1971[56].

Cholera is caused by the rod-shaped gram-negative bacterium *Vibrio cholerae*, pictured in **Figure 4**[57,58]. Most *Vibrio* species that are pathogenic cause gastroenteritis, or inflammation of the stomach and small intestines; other species infect wounds and cause blood poisoning[59]. They are usually motile in aqueous environments by monotrichous and lophotrichous flagella, and although some have been observed in the laboratory to move in solid environments with the use of peritrichous flagella, these are chiefly aquatic organisms[60,61]. In addition to having a peptidoglycan layer, gram-negative bacteria have an outer membrane layer made up of lipopolysaccharide[62,63]. Members of this genus are usually facultative fermentative anaerobes and contain oxidases, which is a trait that distinguishes *Vibrios* from other enteric bacteria[64,65]. *Vibrios* also are chemoorganotrophic and therefore must obtain their carbon and energy sources from organic substrates[66].

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[56] Todar. 2001. p. 3.
[57] Hasan *et al*.. 1994. p. 249
[58] Todar. 2001. p. 3.
[59] Wachsmuth *et al*. 1994. p. 103.
[60] Todar. 2001. p. 1.
[61] Barua *et al*. 1992. p. 38.
[62] Madigan *et al*. 2003.
[63] Wachsmuth *et al*. 1994. p. 150.
[64] Todar. 2001. p. 1.
[65] Barua *et al*. 1992. p. 38.
[66] Barua *et al*. 1992. p. 38.

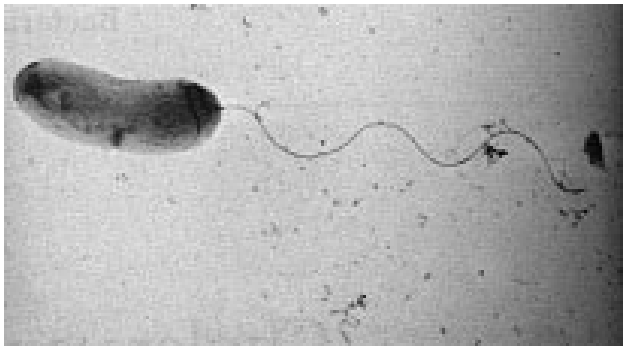

Figure 4: Micrograph of *Vibrio cholerae*[67].

In 1883, Robert Koch proved that cholera is caused specifically by the bacterium now known as *Vibrio cholerae*[68,69]. This single species has 206 different serotypes, but only the O1 and O139 serotypes produce the toxin that causes cholera[70,71]. The O1 serotypes can be further classified as one of three biotypes: Ogawa, Inaba, or Hikojima, which can be expressed phenotypically as either classical or El Tor[72,73]. As described by Todar (2001), the classical O1 biotype was responsible for the first six pandemics, and the El Tor biotype caused an epidemic in the Philippines in 1961, which catalyzed the seventh pandemic. The El Tor biotype is more easily spread than the classical 01 biotype because infected people appear healthy for a longer period of time before onset of illness and more people are carriers for the strain. The El Tor biotype had become the primary cause of cholera in India by 1974 and is suspected to be responsible for all cholera cases in Latin America. The second cholera serotype, O139 "Bengal," is believed to have evolved from the El Tor biotype. It first appeared in 1992 in Bangladesh and has spread to many other countries in Southeast Asia[74].

The serotypes of *V. cholerae* are differentiated by the particular type of O antigen (a type of thermostable polysaccharide) present on their lipopolysaccharide membranes[75,76]. The O antigen for the Bengal strain is particularly unique, and individuals previously infected with other strains have no residual immunity against it[77]. The discovery of these serotypes has lead to the development of an alternative to the categorization of the cholera pandemics by time, which,

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[67] Todar. 2001. p. 2.
[68] Todar. 2001. p. 3.
[69] Wachsmuth *et al*. 1994. p. 293.
[70] Nair, G. B. 2004. p. 119.
[71] *Technical Guidelines-Cholera*. n.d. p. 3.
[72] Todar. 2001. p. 4.
[73] Wachsmuth *et al*. 1994. p. 77.
[74] Todar. 2001. p. 4.
[75] Todar. 2001. p. 4.
[76] Wachsmuth *et al*. 1994. p. 9.
[77] Todar. 2001. p. 4.

as stated previously, is difficult[78]. This second theory suggests that there have only been three pandemics: one caused by classical O1 *V. cholerae,* another by El Tor *V. cholerae*, and a third by O139 *V. cholerae*[79].

People can be infected by *V. cholerae* by drinking untreated water or eating contaminated food[80]. Infected persons pass cells in their feces, and surface water sources are easily contaminated by fecal seepage[81]. A study in Bangladesh discussed by Barua *et al*. (1992) between 1968 and 1977 found that people living along small canals were at higher risk for contracting cholera than those living along main rivers, perhaps because less water is available in canals to dilute infected waste. A study conducted in the Philippines showed that sound environmental sanitation and improved toilet facilities decreased the number of cholera cases by as much as 73%. Other researchers have argued that the availability of clean water is often not sufficient to prevent disease as people will continue to bathe in or drink untreated water for cultural reasons or personal preferences[82]. Educating the population in conjunction with improved sanitation of high-risk areas is thus essential to any attempt at cholera prevention[83].

Barua *et al*. (1992) also discuss that in endemic areas, *V. cholerae* can survive in aquatic environments for extended periods of time by undergoing a starvation response. Cells can continue to divide with no gain in net mass, resulting in dormant "ultramicroorganisms". In this state, *V. cholerae* is both difficult to filter out and culture. Fish and shellfish from contaminated waters and raw vegetables from fields irrigated with contaminated water have caused cases of cholera in the past. Food prepared with contaminated water also poses a significant risk[84].

V. cholera cannot tolerate highly acidic environments[85]. Consuming food or antacids with the bacteria has been proven to decrease the number of cells required to cause infection from 100 million to 1000 as both protect the cells from stomach acid[86,87]. After ingestion, the bacteria colonize the host's intestines during an incubation period as short as fourteen hours or as long as five days[88]. Typical incubation periods last one to two days[89]. Then the bacteria begin to produce toxin, which, as mentioned in the introduction, can cause severe diarrhea[90].

The *V. cholerae* cholera toxin (CT) is a protein encoded by the *ctx*A and *ctx*B genes, both of which are controlled by one operon, the toxR gene[91,92]. As **Figure 5** illustrates, CT is

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[78] Wachsmuth *et al*. 1994. p. 9.
[79] Wachsmuth *et al*. 1994. p. 9.
[80] Barua *et al*. 1992. p. 140.
[81] Wachsmuth *et al*. 1994. p. 28.
[82] Barua *et al*. 1992. p. 140-144.
[83] Nevondo *et al*. 2001. p. 3.
[84] Barua *et al*. 1992. p. 144.
[85] Barua *et al*. 1992. p. 140.
[86] Madigan *et al*. 2003.
[87] Barua *et al*. 1992. p. 140.
[88] Barua *et al*. 1992. p. 140.
[89] Barua *et al*. 1992. p. 141.
[90] Todar. 2001. p. 4-5.
[91] Todar. 2001. p. 5.
[92] Madigan *et al*. 2003.

composed of an A subunit and five B subunits[93]. The A subunit is then further processed into two components, A1 and A2[94]. During infection, it is hypothesized that the B subunits form a pore for the A1 subunit to pass into host cells and interrupt metabolism of the membrane enzyme, adenylate cyclase[95].

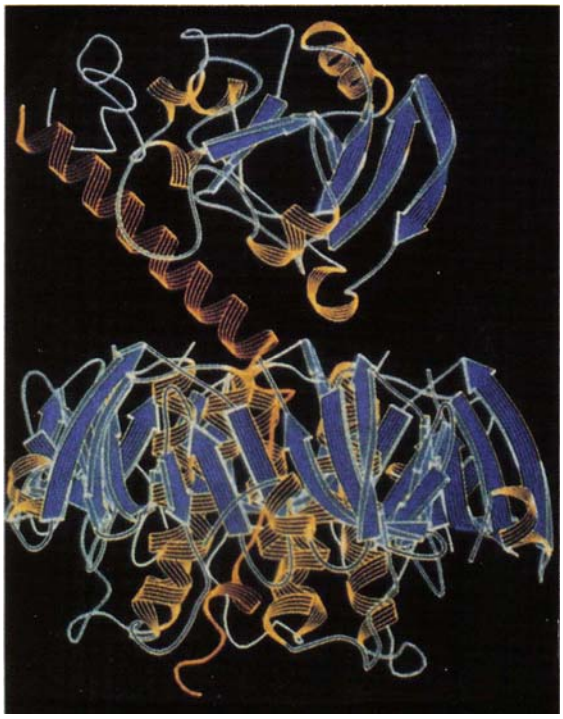

Figure 5: Visualization of CT protein structure. The A unit is on top and the B pentamer is on the bottom[96].

Todar (2001), Wachsmuth *et al*. (1994), and Madigan *et al*. (2003) discuss the effects CT has on humans. Normally, adenylate cyclase catalyzes the conversion of adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP). Usually regulated by G proteins, adenylate cyclase acts as an intracellular messenger in many cell-signaling pathways. In a normal cell, adenylate cyclase is activated briefly by the protein G_s and guanidine triphosphate (GTP) and then turned off through a GTP hydrolysis reaction catalyzed by the G_i protein. However, in a cholera infection, CT binds to the regulatory proteins, preventing GTP hydrolysis and regulation of cAMP production. cAMP stimulates cells in the small intestine to pump out large amounts of chloride ions. In addition to increasing cAMP production, CT also acts to

[93] Madigan *et al*. 2003.
[94] Wachsmuth *et al*. 1994. p. 150.
[95] Todar. 2001. p. 5.
[96] Wachsmuth *et al*. 1994. p. 147.

inhibit sodium absorption and increases bicarbonate excretion. Large quantities of water are excreted to reestablish the osmotic balance, causing severe diarrhea[97, 98].

The most effective course of treatment remains rehydration and restoration of electrolytes, as mentioned in the introduction. Attempts have been made to protect high-risk populations from cholera infection by vaccination, but these have not been very effective[99,100]. The protection from vaccinations is usually short-lived, and those who are protected are older children and adults who typically already have some degree of immunity. Vaccinations also do very little to prevent the transmission of asymptomatic infections, which contribute significantly to the spread of the disease[101]. Treatment does not usually include antibiotics, as antibiotic resistance in *V. cholerae* is a problem. *V. cholerae* has shown increased resistance to antimicrobial agents such as tetracycline and chloramphenicol. Over a period of five months in 1978 over half of *V. cholerae* O1 samples tested during a Kenyan epidemic were recorded as developing partial resistance to either one or both agents[102].

Prevention is of the utmost importance, and the need for quick and cheap detection of contamination has motivated the development of several assays. Some assays detect CT, but more comprehensive tests can confirm the presence or absence of viable *and* dormant cells, which do not produce CT. Currently, New Horizons manufactures two kits for detection of *V. cholerae* O1 cells[103,104]. Neither are microfluidic, but both employ detection mechanisms that take advantage of the natural specificity of antibodies to the cells.

Immunology

Murray *et al*. (2000) describes antibodies as one of the primary components of immune responses in humans to bacterial infection[105]. An antibody is a protein or glycoprotein molecule comprised of two "light chains" and two "heavy chains", forming the shape of a "Y" as pictured in **Figure 6**[106]. These chains are polypeptides connected by disulfide bridges. Antibodies are capable of recognizing specific antigens, which, from an immunological standpoint, can be proteins or sugars expressed on the surface of an infectious agent. Within each antigen exists a specific molecular sequence, called an epitope, that the antibody is capable of recognizing. Recognition occurs at one of the ends of the two branches as seen in **Figure 6** and results in the binding of the antibody to the antigen[107].

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[97] Todar. 2001. p. 4.
[98] Madigan *et al*. 2003.
[99] Barua *et al*. 1992. p. 43.
[100] Wachsmuth *et al*. 1994. p. 305, 395.
[101] Wachsmuth *et al*. 1994. p. 305, 395.
[102] Wachsmuth *et al*. 1994. p. 305.
[103] Lowenhaupt *et al*. 1998.
[104] Hasan *et al*.. 1994. p. 249-252.
[105] Murray *et al*. 2000. p. 102.
[106] University of Arizona (n.d.). p. 1.
[107] Murray *et al*. 2000. p. 102.

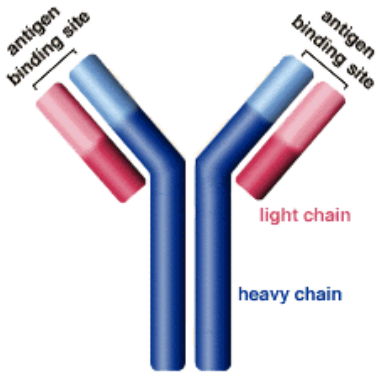

Figure 6: Space filling molecular view of a typical antibody[106].

Antibodies can be divided into different subclasses based on traits in their heavy chains and their function. The nomenclature follows the form IgX, where Ig stands for immunoglobulin (another word for antibody) and X is a letter that signifies the type of antibody. A summary of different types of antibodies can be found in **Table 1**. IgG, IgM, and IgA will be explained in more detail later. IgD exists in the membranes of B-cells and upon recognition of an antibody, stimulates B-cell growth to produce an immunological response. IgE are anchored on mast cells and serves as protection against parasites. This antibody is also responsible for allergic reactions[108].

	IgG	IgM	IgA	IgD	IgE
Percent of Ig in Humans	85	5-10	5-15	<1	<1
Molecular Mass (kDa)	154	900	160	185	190
Half-life (days)	23	5	6	2-3	2-3
Function	Secondary Infection Response	Primary Infection Response	Mucous Membrane Protection	B-cell Activation	Anaphylaxis

Table 1: Summary of immunoglobulin classes[109].

IgG, IgM, and IgA exist as free-floating immunoglobulin. IgG and IgM are present inside the body, while IgA exists in mucous secretions. The binding of these antibodies to antigens has several consequences, but the main response to binding is a process known as complementing, in which complement proteins (designated C1 through C9) attach to the antibody/antigen complex to form an activation unit. The activation unit then performs two

[108] Murray *et al*. 2000. p. 105-106.
[109] Murray *et al*. 2000. p. 104.

major functions: It produces 1.) phagocytic promoters and 2.) a membrane attack complex. The phagocytic promoters consist of C3a and C5a, which, in conjunction with surrounding mammalian cells, increase vascular permeability and act as chemotactic factors to attract neutrophils and macrophages to the infection site. Neutrophils and macrophages are phagocytic cells that can kill the bacterium associated with the target antigen. Additionally, the promoter C3b binds to the surface of the bacterium and increases the affinity of phagocytic cells to the bacterium. Alternatively, the complement membrane attack complex can kill the bacterium. This complex is formed by complement proteins C5-C9 and works by forming a hole in the side of the bacterium and causing the lysis of the cell[110].

Types of Micro Detection Devices

Antibody technology is the basis for immunoassay detection methods. In recent years, much work has been done in the development of micro immunoassay detection devices[111]. There are many advantages to having immunoassay tests contained on a single chip. One advantage is that the entire analysis can be carried out with a single device instead of using a wide range of equipment in a laboratory. In addition, the use of these devices requires little or no training, whereas skilled technicians are necessary to carry out traditional tests in laboratories. Another advantage of micro immunoassay devices is that they require a small sample size, allowing for low reagent and material costs[112].

Yakovleva *et al*. (2002) have developed a microfluidic enzyme-linked immunosorbant assay (ELISA) for the detection of atrazine, a toxic herbicide[113]. This device utilizes a competitive immunoassay, where the immobilized antibody is the only antibody present. **Figure 7** illustrates how this competitive immunoassay works.

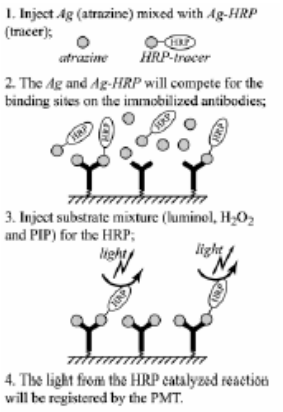

Figure 7: Competitive ELISA for the detection of toxic herbicide[114].

[110] Murray *et al*. 2000. p. 105-106.
[111] Price *et al*. 1997. p. 1.
[112] Price *et al*. 1997. p. 1.
[113] Yakovleva *et al*. 2002. p. 2994-3004
[114] Yakovleva *et al*. 2002. p. 2998.

For competitive immunoassays, labeled analyte, known as the tracer, is added at the same time as the analyte. The proportion of the tracer bound to the immobilized antibody is indirectly proportional to the concentration of the analyte[115]. The label for the immunoassay of Yakovleva *et al*. (2002) was horseradish peroxidase (HRP). To determine the concentration of the analyte present in the sample, a mixture of luminol, PIP, and hydrogen peroxide is added to the system. These substances follow a light producing reaction that is catalyzed by the HRP labels. Finally, a photomultiplier tube (PMT) measures the amount of light emitted. From this information, the concentration of atrazine can be calculated. This method of detection is known as chemiluminescence (CL) and is very useful in micro scale applications because it does not require an external light source[116].

Lin *et al*. (2004) developed a microfluidic ELISA device for the detection of *Helicobacter pylori*, which can lead to gastric cancers, by sensing visual color changes[117]. This device employs immobilized lysate antigens on a PDMS rubber substrate. After sample injection, goat anti-human IgG antibody labeled with HRP is added to detect human *H. pylori* anitbodies. Finally, a peroxidase substrate is added, which will change color upon an antigen-antibody reaction. A digital camera is used to monitor color in the microfluidic channels. These images are then analyzed using software that measures color differences. If the color difference between the microfluidic channel and the "background" color (which is calculated by the software) is greater than two standard deviations above the average of the color difference of the known negative samples, the test result is positive[118].

Sia *et al*. (2004) developed a portable and cost-effective ("POCKET") immunoassay device for the detection of infectious diseases[119]. This group aimed to develop an alternative to enzyme-linked immunosorbant assays (ELISA) for use in a microfluidic device. Although ELISA is one of the most effective types of bench-top assays, they are not entirely practical for use in microfluidic devices for two reasons. First, since enzymes generate diffusible products, detection is difficult under continuous flow. Second, the sensitivity of assays using optical detection is lowered because of the small cross-sectional area of the microfluidic channels. In addition, ELISAs require large, expensive equipment for optical detection, making them impractical for a completely portable device[120]. The immunoassay used in the POCKET device can be seen in **Figure 8** below.

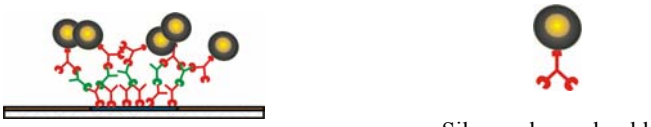

Figure 8: Immunoassay with Gold Labeling and Silver-Enhancement[18].

[115] Wild. 2001. p. 4.
[116] Yakovleva *et al*. 2002. p. 2995.
[117] Lin *et al*. 2004. p. 823-826
[118] Lin *et al*. 2004. p. 823-824.
[119] Sia *et al*. 2004. p. 498-502
[120] Sia *et al*. 2004. p. 498.

Gold-labeled antibodies are used in place of enzyme labeling on the device of Sia *et al*. (2004)[121]. After these labeled antibodies are added to the analyte, a solution containing silver nitrate and hydroquinone is added. The gold catalyzes a reaction through which silver ions are reduced to atomic silver, creating a silver film that is attached to the surface of the device. This process is known as silver enhancement. The opacity of this film is related to the concentration of the analyte present in the sample. Using a low-cost, battery-powered, reusable detector, the opacity can be easily detected and related to the concentration of the analyte[122]. Silver enhancement could also be used for simple visual assessment of contamination.

Quality of Devices

When evaluating the overall quality of microfluidic detection devices, there are several parameters that need to be considered. The speed of detection greatly affects the quality of the device. Obviously, the faster the device can detect a toxic substance, the better it is. In addition, it is imperative that devices have a limit of detection lower than the *V. cholerae* cell concentrations considered to be dangerous. The following is a detailed discussion pertaining to the issue of limits of detection as applied to the immunoassay devices described in the previous section.

The sensitivity is very important to the overall quality of the assay, as discussed by Wild (2001)[123]. High sensitivity is desirable for a number of reasons. One reason is that high sensitivity can allow much smaller samples to be analyzed. A common way to determine the sensitivity is to assay a zero standard several times. Then, the limit of sensitivity can be defined as the concentration that corresponds to about three standard deviations greater than the mean. This sensitivity measurement is known as both the analytical sensitivity and the limit of detection (LOD). The analytical sensitivity, however, is not always a reliable indicator of the true sensitivity of the assay. Another value, known as the functional sensitivity, is a much better indicator of the true sensitivity. The functional sensitivity is the lowest concentration in which the coefficient of variation is less than an arbitrary value. Functional sensitivity is determined using a precision profile, which involves measuring how the precision changes over the range of the assay. In many cases, the functional sensitivity is greater than the analytical sensitivity[124]. Many researchers use different methods to determine the LOD and/or sensitivity. Most good researchers do, however, explain what method was employed to calculate these parameters.

Yakovleva *et al.* (2002) calculated the LOD for their device by multiplying the standard deviation of the zero analyte doses by three. Their atrazine detection devices all had an LOD of less than 0.1 μg/L, which is the limit for atrazine concentration in drinking water in the European Union. They attribute high device sensitivity to the high antibody affinity, the high sensitivity of chemiluminescence detection, and the direct assay format. Their microfluidic device is more sensitive than microtiter-plate ELISAs developed by other groups. In addition to finding the LOD, they also studied how stable the device is over time. To do this, they measured the signal for several different concentrations of atrazine at several different points in time. They found

[121] Sia *et al*. 2004. p. 499.
[122] Sia *et al*. 2004. p. 499.
[123] Wild. 2001. p. 78.
[124] Wild. 2001. p. 78.

that the signal intensities varied over time at constant analyte concentrations. However, after the signal values were normalized, the results were found to be precise[125].

Lin *et al*. (2004) measured ten known *H. pylori* negative samples and ten *H. pylori* positive samples. They had one false positive result and no false negative results. They defined sensitivity as the percent of known positives accurately detected and selectivity as the percent of known negatives accurately detected. Therefore, the sensitivity was 100% and the selectivity was 90%. They also calculated the percent of tests that yielded the correct result and found it to be 95%[126].

Sia *et al*. (2004) calculated the sensitivity and LOD for their device and for several standard bench-top immunoassay methods. To calculate sensitivity, a plot was made of the normalized signal vs. the concentration of rabbit IgG for each method (**Figure 9)**.

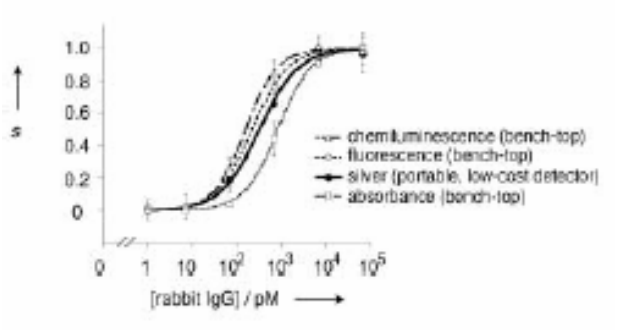

Figure 9: Comparison of POCKET with Bench-top Method[127].

They defined the sensitivity as the midpoint of the linear range of detection. Their "POCKET" device was found to be almost as sensitive as traditional bench-top ELISA methods. The limit of detection for the POCKET device was 89 pM, compared to 22 pM, 55 pM, and 163 pM for chemiluminescence, fluorescence, and absorbance, respectively. The POCKET device also provided reproducible results. The standard deviation of several measurements at the same concentration was 7%, which is in the same range as the standard deviation for traditional ELISA techniques[128].

Device Materials and Fabrication Methods

The complexity of designing an immunoassay is compounded for this project by the challenges of microfabrication. Standard pumps, valves, and materials are not appropriate for device construction on the micro scale. The following paragraphs outline the information that will be used to select the optimal materials and fabrication methods for the planned device.

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[125] Yakovleva *et al*. 2002. p. 3002-3003.
[126] Lin *et al*. 2004. p. 826
[127] Sia et al. 2004. p. 500.
[128] Sia *et al*. 2004. p. 500.

The Detection Chamber

The substrate of an immunometric assay detection chamber is one of the assay's most crucial elements. The quality of the solid-phase antibody layer, the layer to which the analyte binds, is very dependent upon the quality of the substrate used.

Because proteins naturally adsorb to hydrophobic surfaces, glass and plastic are ideal materials[129]. Polystyrene is one of the most widely used plastics for detection substrates because it is inexpensive, easily molded, and after molding naturally has some oxygen atoms adsorbed on its surface[130]. Researchers have found that this scattering of oxygen atoms can improve antibody-binding conditions[131]. Polydimethysiloxane (PDMS) rubber and cylic olefin copolymer (COC) are also advantageous in immunoassay applications[132,133]. These polymers can be treated with oxygen plasma, UV light, or corona discharges to oxidize their surfaces[134,135].

To coat a polymer surface with antibodies, the polymer would have to be exposed for several minutes to an aqueous solution of the antibody, which typically would have a concentration within the range of 10-100 μg/mL[136]. The hydrophobic force is effective only within a distance of several molecules from the surface, so no more than a thin coating is required, and the solution can be spotted on the surface[137]. Higher concentrations may cause antibody aggregation (a monolayer of surface-bound antibodies is desired), while concentrations that are too low can cause binding of too many of the hydrophobic sites of an antibody to the plastic[138]. The resulting contortions of the antibody can greatly reduce binding affinity to the analyte[139]. After a few minutes of coating solution exposure, the solution is typically aspirated and the surface dried in air or nitrogen gas[140,141]. Kai *et al.* found that rat IgG adsorbed well to COC with a contact surface angle to water droplet of 70 to 95 degrees[142]. Untreated COC has a native contact angle of 93 degrees, and untreated PDMS rubber has a contact angle of 108 degrees[143,144].

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[129] Wild. 2001. p. 151.
[130] Wild. 2001. p. 151.
[131] Madigan *et al*. 2003.
[132] Makamba *et al*. 2003. p. 3607.
[133] Kai *et al*. 2002. p. 419.
[134] Ahn *et al*. 2004. 154-160.
[135] Makamba *et al*. 2003.
[136] Wild. 2001. p. 151.
[137] Wild. 2001. p. 152
[138] Wild. 2001. p. 152.
[139] Wild. 2001. p. 152.
[140] Wild. 2001. p. 152.
[141] Kai *et al*. 2002. p. 420.
[142] Kai *et al*. 2002. p. 421.
[143] Kai *et al*. 2002. p. 419.
[144] Duffy *et al*. 1998. p. 4978.

Covalent-binding can also be used to secure antibodies to the surface[145,146]. As reported by Makamba *et al*. (2003), many polymers have mostly carbon-hydrogen bonds on their surfaces, and PDMS in particular is essentially inert. One particularly successful covalent-binding technique involves chemical vapor deposition (CVD) of poly[para-xylylene carboxylic acid pentafluorophenol-ester-co-paraxylylene] (PPX-PPF) onto PDMS rubber, followed by substitution of the PPF with amino-terminated biotin ligands. These steps create a biotin monolayer on the surface held in place with stable amide bonds to PPX. After removal of excess reagents and incubation in a 10 mM solution of streptavidin for an hour, the streptavidin binds to the biotin. Biotin-conjugated antibody can then be bound to the streptavidin layer. These layers, shown in **Figure 10**, can remain stable in dry air for several weeks, which is significant as such durability is key to the development of a device that is portable and accessible to the average consumer[147].

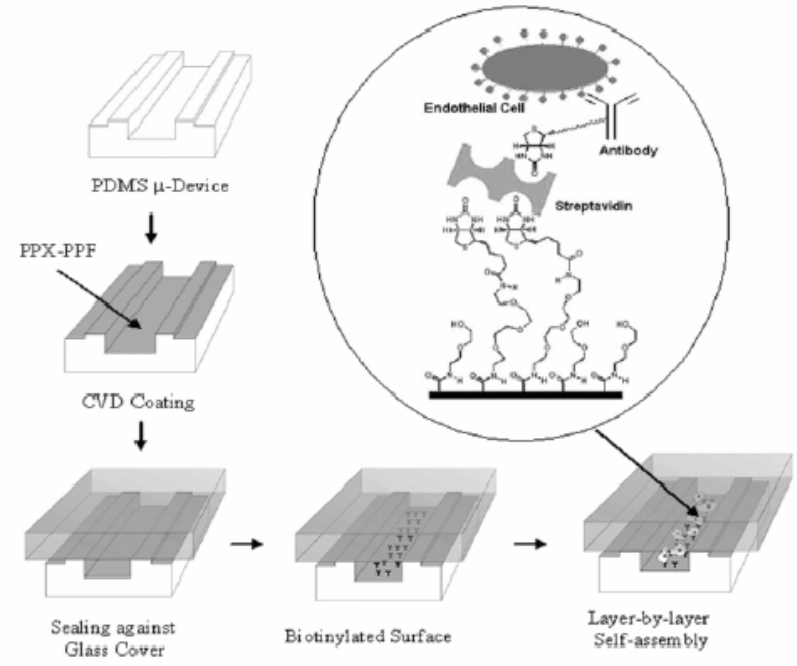

Figure 10: PDMS surface modification for covalent antibody securing[148].

Valves and Pumps

The mobilization and manipulation of fluids in our device is essential to its operation. Many novel valving and pumping mechanisms independent of moving parts have been

[145] Wild. 2001. p. 153
[146] Makamba *et al*. 2003. p. 3611-3613.
[147] Makamba *et al*. 2003. p. 3611-3613
[148] Makamba *et al*. 2003. p. 3614.

developed for use in microfluidic devices. Several types of these passive valves and pumps have been examined, but because low cost and independence from electricity requirements are important to the success of the project, the options are somewhat limited.

Liu *et al.* (2002) integrated multiple-use paraffin valves into microfluidic devices[149]. Paraffin blocked the microchannels in the closed position, and resistive heaters were used to melt this paraffin to allow fluid flow. Liu *et al.* designed their channels to be wider after the valve so that the paraffin would not clog the channel as it cooled. Melting the paraffin again and blowing it back to the channel section of smaller diameter with air pumps effectively resealed the channel[150]. Similar phase-change microvalves have been successfully implemented on devices capable of preparing and amplifying DNA from whole *E. coli* cells, but thermally actuated valves would not be appropriate for this project because of the dependence on electricity for heating[151]. In addition, the design-project microfluidic device is not intended for multiple uses.

Ahn *et al*. (2004) have studied the alternative of passive capillary valving extensively[152]. Capillary valves are regions in the microchannels with diameters small enough that fluid cannot pass through unless pressure is applied. Ahn *et al.* also hydrophobized the entrances to the valves via plasma treatment to further reduce flow with surface tension. They used COC as their channel material and hydrophobized it with simultaneous exposure to oxygen plasma and tetrafluoromethane plasma. It is suspected that the oxygen plasma breaks the surface's carbon-hydrogen bonds, and the tetrafluoromethane plasma replaces them with carbon-fluorine bonds. Using this plasma treatment, Ahn *et al.* were able to increase the contact angle of a water droplet on COC from 92 degrees to 136 degrees. Because Ahn *et al.* did not create these valves for an immunoassay, the effect this surface treatment would have on antibody solutions is not known. Still, Ahn *et al.* found that hydrophobicity is crucial for the proper functioning of passive valves, and we have already mentioned the potential importance of substrate hydrophobicity in the detection chamber for strong hydrophobic bonds between a polymer and the first layer of antibody[153]. **Figure 11** demonstrates the meniscus formation at the entrance to the valve. The valve geometry is also significant, as rounded or tapered valve openings, as seen in **Figure 12**, have been shown to avoid the trapping of residual fluid before abrupt valves[154].

[149] Liu, R. H. *et al*. 2002.
[150] Liu, R. H. *et al*. 2002.
[151] Liu, Y. *et al*. 2002.
[152] Ahn *et al*. 2004. p. 154-173.
[153] Ahn *et al*. 2004. p. 158.
[154] Ahn *et al*. 2004. p. 161.

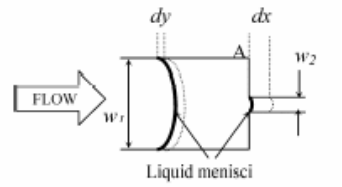

Figure 11: Hydrophobic Capillary Valve[155].

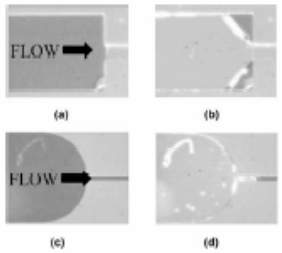

Figure 12: Abrupt valve a) before and b) after air detonation. Rounded valve c) before and d) after detonation[156].

However, the senior design microfluidic device could not use the same pumping technique Ahn *et al*. employed to move fluid through these capillary valves because they also relied upon melting paraffin valves[157]. They designed novel air detonators to serve as pumps. The paraffin valves sealed off chambers of pressurized air. When the valves were melted to open, the air expanded, overcoming the surface tension and abrupt diameter reduction to push the fluid through the thin capillaries. The movement of the fluid towards tapered and rounded valves is depicted in **Figure 13**; the menisci invert because of the hydrophobic valve opening. Because the air will not recompress, the fluid will not move backwards through the valve[158].

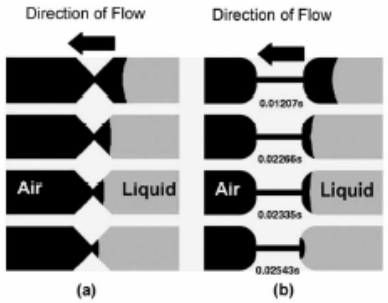

Figure 13: Depiction of fluid approach in a) a tapered valve and b) a rounded valve[159].

Grover *et al.* (2002) created accurate and reliable valves by sandwiching PDMS rubber membranes between top and bottom glass plates[160]. Applying a pressure increase or vacuum to the displacement chambers of the membranes caused the membranes to expand into the channels to close them or to contract to open them, respectively. Grover *et al.* even constructed pumps

[155] Ahn *et al*. 2004. p. 161.
[156] Ahn *et al*. 2004. p. 161.
[157] Ahn *et al*. 2004. p. 159.
[158] Ahn *et al*. 2004. p. 155.
[159] Ahn *et al*. 2004. p 162.
[160] Grover *et al*. 2002.

from configurations of multiple membranes and displacement chambers. Although their technique is inappropriate for our purposes because of the vacuum and power requirements, their use of PDMS rubber membranes is encouraging as our pumping method will rely on the same material[161].

The current design calls for aspirating sample and pump reagents to the detection chamber using simple finger-sctuated micropumps consisting of squeezable chambers[162]. PDMS rubber is a natural choice for the micropump material because it is an elastomer; the elongation at break can vary from 100% to 800%, while many plastics like polymethyl methacrylate (PMMA) and PS have values around a mere 2.5%[163]. Two options are under consideration for the pump and valve design. The first scenario, which would be an adaptation of the Ahn *et al.* technique[164], would be to press a chamber of pressurized air sealed with paraffin to break this paraffin seal, allowing the air to expand and push stored reagent through a hydrophobized capillary valve. In the second, the chamber of stored reagent would be sealed with paraffin and squeezed directly to break the seal and expel the reagent. The first option would prevent the possibility of reagent returning to the storage chambers after the squeeze bulb is released, but it would also consume more space on the device and involve more fabrication steps than the second option. In either case, tapered or rounded valve openings will be used.

The device could have been constructed almost entirely out of PDMS rubber, as it has been proven to be a viable option for immunoassay microchannel and detection-chamber material[165]. PDMS rubber, a type of silicone rubber, is made by vulcanizing PDMS gum[166]. Microfeatures can be created in PDMS by molding or laser engraving[167, 168,169]. In a typical molding process, a photolithographically defined master containing the negative of the microfeatures is set in PDMS gum or a prepolymer solution[170,171 ,172]. The PDMS gum is then cured by exposure to elevated temperatures and pressures and small amounts of benzoyl peroxide in a process known as heat vulcanization[173]. Silicone rubber can be created with a wide range of elasticity because the degree to which silicone rubber is elastic is a direct result of the degree to which it is vulcanized[174]. Unfortunately, photolithographically defined molds can be very expensive, resulting in a total fabrication cost of over $50,000 per device, but the technique of direct laser engraving into PDMS rubber sheets has been found to be less

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[161] Grover *et al*. 2002.
[162] Dr. Orlin Velev, professor of Chemical Engineering at NCSU.
[163] Calister, W. D. 2000.
[164] Ahn *et al*. 2004. p. 155, 160.
[165] Makamba *et al*. 2003. p. 3607.
[166] Ellis, B. 2000. p. 1-6.
[167] Makamba *et al*. 2003. p. 3607
[168] Duffy *et al*. 1998. p. 4977.
[169] Chudy *et al*. 2002. p. 392.
[170] Makamba *et al*. 2003. p. 3607-3608
[171] Duffy *et al*. 1998. p. 4977.
[172] Lee *et al*. 2002. p. 668.
[173] Roberts *et al*. 1997. p. 2037.
[174] Lee *et al*. 2002. p. 668.

expensive[175]. Researchers have even sent their sheets to common laser service centers for engraving of microfeatures[176]. In both the cases of molding and engraving, the PDMS rubber would be formed into a top and bottom sheet for the device, either of which could contain the microfeatures.

These sheets must be sealed to create a device. A relatively hydrophilic surface is important for sealing of PDMS rubber, which can be made more hydrophilic by treatment with oxygen plasma[177]. Conformal contact of PDMS rubber sheets immediately after oxidation by plasma can create a seal[178, 179]. Laminating the bottom PDMS rubber sheet with a 35 mm PET/PE film instead of top sheet is also an option[180]. Even the use of special clamps to seal two PDMS rubber sheets with spring force has been successful[181].

Makamba *et al*. (2003) report that PDMS rubber is naturally very hydrophobic and chemically inert; so multiple surface treatments (like the PPX-PPF CVD/streptavidin treatment discussed previously) would be required for it to meet the project's needs. If unaltered PDMS rubber were used as the channel material, labeled antibodies would bind to the channels as they travel from the reagent chamber to the detection chamber. Multiple coating techniques have been developed to prevent such non-specific binding. The microchannels would have to be hydrophilized before these treatments to enable passage of the aqueous coating solutions through them[182]. As mentioned earlier, Duffy *et al*. (1998) along with Makamba et al. (2003) say PDMS rubber can be made hydrophilic through oxygen plasma exposure, which oxidizes the surface to a silica-like layer and decreases the water droplet contact angle to 30 degrees. The increased hydrophilic nature of the channels could perhaps be used alone to enable flow of reagent solutions during device operation, but, unfortunately, the increased hydrophilicity has been shown to last for only forty-five minutes in air and four days in water[183,184].

Hybridization

The device could be created, for example, with a COC platform and PDMS rubber squeeze bulbs. The squeeze bulbs would probably have to be membranes sandwiched in between the COC sheets, similar to the Grover *et al.* technique, as PDMS rubber does not bond well with polycarbonate (PC) or polymethyl methacrylate, and its binding affinity to COC could not be found in the literature[185,186]. PDMS rubber does bind well to PS, glass, and silicon after

[175] Chudy *et al*. 2002. p. 392.
[176] Chudy *et al*. 2002. p. 392.
[177] Makamba *et al*. 2003. p. 3608.
[178] Makamba *et al*. 2003. p. 3608.
[179] Lee *et al*. 2002. p. 668.
[180] Roberts *et al*. 1997. p. 2027.
[181] Hasegawa *et al*. 2002. p. 694.
[182] Makamba *et al*. 2003. p. 3609.
[183] Makamba *et al*. 2003. p. 3609.
[184] Duffy *et al*. 1998. p. 4978.
[185] Duffy *et al*. 1998. p. 4977-4978.
[186] Grover *et al*. 2002. p. 136-137.

oxidation and immediate conformal contact, but glass and silicon would be inappropriate for the planned device because of their fragility[187].

Microstructures have been successfully micromachined into PC with CO_2 and UV lasers, PMMA with CO_2 lasers, and PS with UV lasers[188,189,190,191]. Ahn *et al*. (2004) developed an improved injection molding for COC sheets that employs rapid thermal annealing of a replaceable mold disk to insure optimal flow properties of the molten plastic in the mold[192]. Thermoplastic fusion bonding, which employs a combination of moderate temperature and pressure increase, is an excellent option for COC as it prevents the deformation of microstructures associated with high temperature annealing. High-pressure thermal bonding has also been explored for untreated PC[193]. Despite the availability of these methods to perform hybridization, such a design could greatly complicate device fabrication.

[187] Duffy *et al*. 1998. p. 4978.
[188] Liu, R. H. *et al*. 2002. p. 163.
[189] Roberts *et al*. 1997. p. 3687.
[190] Cheng *et al*. (1). 2002. p. 407.
[191] Cheng *et al*. (2). 2002. p. 458.
[192] Ahn *et al*. 2004. p. 156.
[193] Rosenberger *et al*. 2002. p. 4.

Technical Results

This section of the report describes the development of the microfluidic immunoassay design. First, theoretical and experimental analyses of the micropumps, which are used as energy sources for fluid transport on the device, are presented. A design of the of device layout and data from the micropump experiments were used to determine the optimal width of the channels extending from the micropumps to the detection chamber, and the methods employed to determine the channel width are described in detail. A design for a valve to prevent backflow of dispensed reagents is also included. The final device layout is discussed next, and a three-dimensional schematic and prototype of the design are presented. Possible methods for assembling the device, silver enhancement, and an outline of the operation of the device are also addressed.

Micropump Analysis

A rigorous analysis of the finger-actuated micropumps, which are the primary means of manipulating fluid flow on the device, was critical to the successful development of the device design. There are two types of micropumps employed in the design. The first is the sample collector, which is pressed, dipped into the water to be sampled, and released to aspirate sample into the detection chamber. The second type is the reagent dispenser. The reagent dispenser is initially filled with reagents required for immunoassay or silver enhancement. Once a reagent dispenser is pressed, the fluid contained in it is released and replaces all of the fluid previously in the detection chamber. The basic structure of either type of micropump is the same and is depicted in **Figure 14**.

The sample collector had to be sized so that it would be able to aspirate an adequate sample volume, while the reagent dispensers were sized to enable dispensing of an adequate amount of reagent and to minimize the amount of residual reagent waste. Conserving space on the device was an important consideration with respect to both micropump types.

The micropump analysis is particularly significant because it provided the basis for sizing all other components of the microfluidic device, including the channels and the detection chamber. More specifically, once the optimal micropump sizes and their pumping abilities were known, the iterative process of determining the most appropriate channel dimensions, detection-chamber dimensions, reaction times, and reagent concentrations could begin.

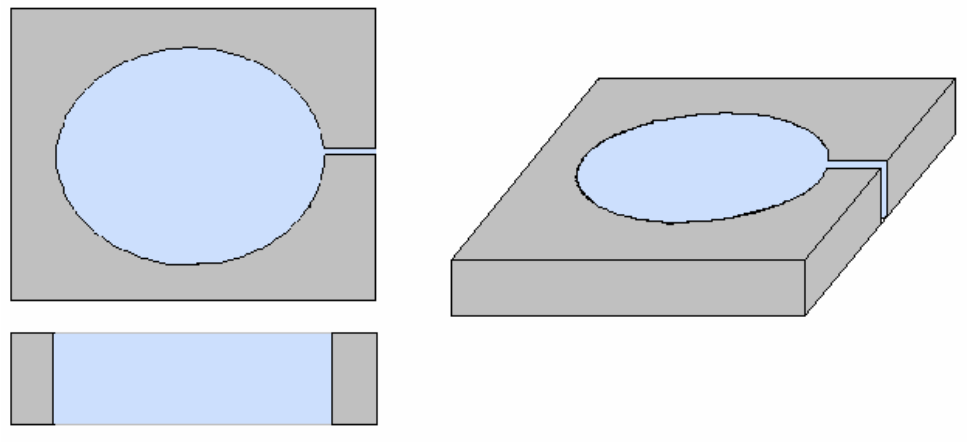
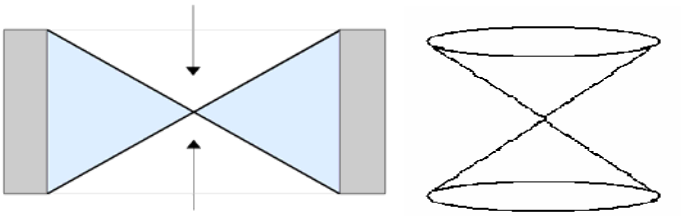


Figure 14: Top, side, and 3-dimensional view of a finger-actuated micropump. The areas shaded in gray represent the chamber walls, while the areas shaded in blue represent the chamber and microchannel. The top and bottom layers of silicone rubber that are pressed to employ the micropump are not shown.

Optimization of the finger-actuated micropumps consisted of theoretical and experimental analyses of the dependence of pumping effectiveness on chamber diameter and height. When a sample collector is pressed, not all of the air in the chamber is expelled. Only the volume that is expelled can be used to aspirate sample. The volume that does not contribute to aspiration is "dead" volume. For a reagent chamber, the dead volume is the volume of fluid not expelled after the chamber is pressed. For both micropump types, the dead volume is the portion that cannot be squeezed. Percent dead volume is one way to measure the effectiveness of such a pump. It is also important to determine how much fluid a sample collector of a given size can aspirate and how much fluid a reagent chamber of a given size can dispense.

Theoretical Finger-Actuated Micropump Analysis

The same theoretical analysis was conducted for both the sample collector and the reagent chamber. It was assumed that even though the functions of the two types of micropumps are different, pressing a single chamber for either purpose would displace the same volume. To theoretically calculate the dead volume of the chamber, a model visualized in **Figure 15** was considered.



a.) b.)

Figure 15: a.) Finger-actuated micropump schematic with a single point of contact. The dead volume is blue, and the walls of the chamber are gray. The displaced volume is white. b.) 3-D representation of the theoretical displaced volume.

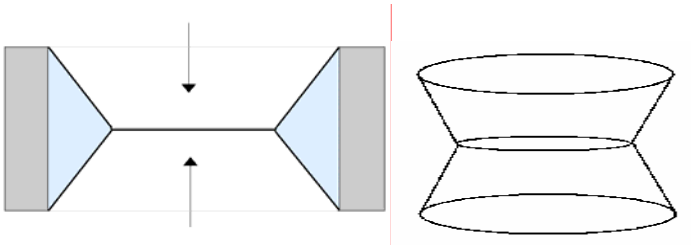
The arrows illustrate the force supplied by the fingers of the operator to the flexible top and bottom silicone-rubber layers. This scenario assumes that the layers of silicone rubber meet at a single point, forming two cones of equal volume, as shown in the diagram on the right. The white area is the theoretical displaced volume, while the area shaded in blue is the theoretical dead volume. The dead volume can be approximated as follows

$$V_d = V_{tot} - V_{dis}$$
$$V_d = \pi R^2 h - 2\left[\frac{1}{3}\pi R^2 \frac{h}{2}\right] \qquad \textbf{Eq. 1}$$
$$V_d = \frac{2}{3}\pi R^2 h$$

where V_d is the dead volume, V_{tot} is the total volume of the chamber, V_{dis} is the displaced volume, R is the chamber radius, and h is the chamber height.

The results of this analysis suggested that the percent dead volume was the same regardless of chamber diameter and height. Because, intuitively, this result does not make sense, a second analysis was performed using a different assumption.

Instead of assuming the top and bottom layers of silicone rubber would meet at a single point, a second dead volume approximation was formulated assuming the silicone sheets would make a contact area in the shape of a circle. This new model is depicted in **Figure 16**.



a.) b.)

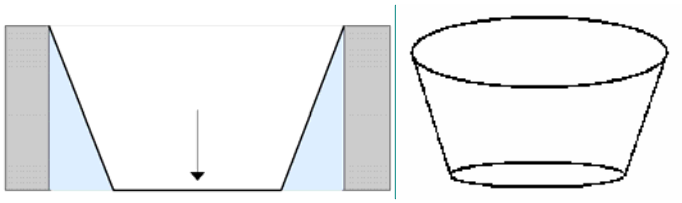
Figure 16: a.) Finger-actuated micropump schematic with circular contact. The dead volume is blue, and the chamber walls are gray. The displaced volume is white. b.) 3-D representation of the theoretical displaced volume.

The theoretical dead volume can be found by subtracting the volume of two tapered cylinders from the total chamber volume and is mathematically described in the following equations

$$V_d = V_{tot} - V_{dis}$$
$$V_d = \pi R^2 h - 2\pi\left(\frac{R^2 + rR + r^2}{3}\right)\frac{h}{2} \qquad \textbf{(Eq. 2)}$$
$$V_d = \pi h\left[R^2 - \left(\frac{R^2 + rR + r^2}{3}\right)\right]$$

where V_d is the dead volume, V_{tot} is the total volume of the chamber, V_{dis} is the displaced volume, R is the chamber radius, r is the contact radius, and h is the chamber height. The contact radius was estimated based on the radius of the chamber, with larger chambers having larger contact radii, and on the typical contact surface of a finger tip.

The previous two cases examined chambers with flexible silicone rubber on the top and bottom. Since the middle layer is also constructed of silicone rubber, the entire device is flexible, which may make it less durable. This could be avoided by constructing the device with a rigid plastic as the bottom layer. If this were to be done, the micropump would operate as shown in **Figure 17** below.



a.) b.)

Comment [JRM1]: Same

Figure 17: a.) Finger-actuated micropump schematic with circular contact and rigid bottom layer. The dead volume is blue, and the walls of the chamber are gray. The displaced volume is white. b.) 3-D representation of the theoretical displaced volume.

With this chamber configuration, only the top layer is pressed. The theoretical dead volume for this design can be calculated as follows.

$$V_d = V_{tot} - V_{dis}$$
$$V_d = \pi R^2 - \pi h\left(\frac{R^2 + rR + r^2}{3}\right) \qquad \textbf{(Eq. 3)}$$
$$V_d = \pi h\left[R^2 - \left(\frac{R^2 + rR + r^2}{3}\right)\right]$$

Analysis of both configurations with circular contact yielded the same results. The results of the calculations can be seen below in **Figure 18** for a micropump with a chamber height of 0.0625 inches.

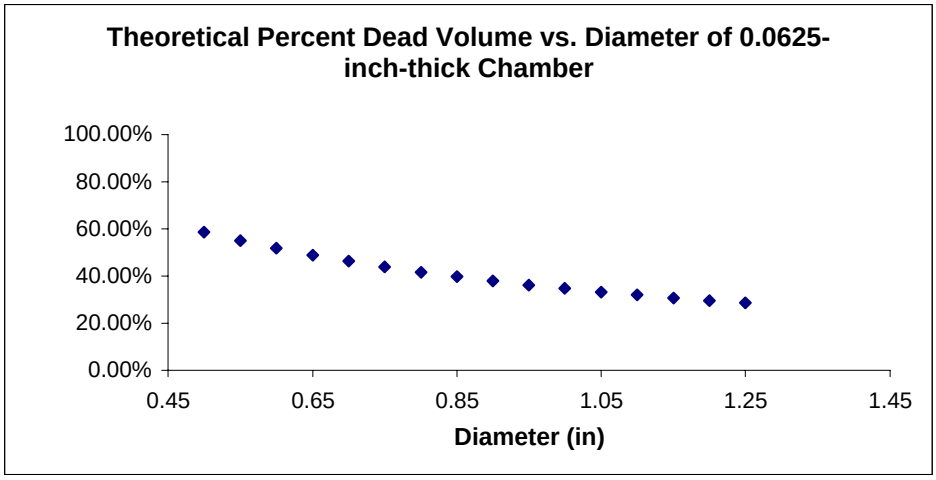


Figure 18: Theoretical percent dead volume versus chamber diameter for a chamber height of 0.0625 inches.

This graph indicates that large-diameter chambers would have a smaller percent dead volume than chambers with smaller diameters. It is also important to note that the percent dead volume did not vary with height for chambers of the same diameter. The complete results of this analysis can be seen in **Appendix 4**.

Experimental Finger-Actuated Micropump Analysis

An experimental analysis was performed to further illuminate the trends that were used in the selection of the most effective chamber dimensions. For the experimental work, the sample collector and reagent dispenser were considered separately because of their different functions. The sample collector is intended to be squeezed while empty, dipped into the sample water, and then released to create a pressure drop in the chamber that would aspirate the sample. Thus the dead volume of a sample-collector chamber is the total volume of the chamber minus the volume of the water drawn in. Conversely, the reagent dispenser is intended to initially be filled with reagent and then squeezed to push out the reagent. Thus the reagent-chamber dead volume is the total volume of the chamber minus the volume of reagent remaining after squeezing.

Materials

Five chamber diameters (3/8", 1/2", 5/8", 3/4", and 1") and three chamber heights (1/50", 1/32", 1/16") were examined. The diameters selected ranged between the minimum diameter that could reasonably be squeezed between a typical index finger and thumb and the maximum reasonable diameter for a chamber on a credit-card-sized device. The heights were chosen based on the desire for a thin device and availability of materials.

A total of fifteen devices with one chamber each were created to test all possible combinations of diameters and heights. The devices were 3" long and 1.5" wide and each had a 1.5" long and 1/16" wide channel extending from the chamber to the edge of the device. This layout is visualized in **Figure 19**.

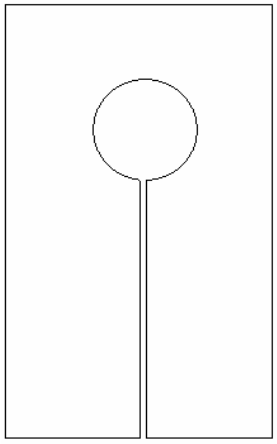


Figure 19: Basic layout of middle layer in experimental apparatus. The main features include one chamber and one channel.

The devices consisted of three layers of silicone-rubber sheets purchased from McMaster-Carr. The features of the micropump and the channel were cut into the middle layer using a sharp knife, and the chamber and channel height was the thickness of this middle layer. The top and bottom silicone-rubber layers each had a thickness of 1/50". These two layers contained the fluid inside the middle layer and were the surfaces pushed together inside the chamber to displace the fluid. Tap water was used to mimic reagents and sample.

Adhesives were used to bind the three layers of silicone rubber together. The silicone rubber sheets were already coated on one side with an adhesive as purchased. The bond between the bottom and middle sheets was adhesive-on-adhesive and essentially permanent. The bond between the middle and top layers was formed by adhesive from only the top sheet and could be broken and easily resealed. Circles of cellophane were used to cover the adhesive in the chambers and prevent sticking. No cellophane was used in the channels.

Procedure

The same fifteen devices were used for both scenarios.

Sample-Collector Scenario

Each device was first weighed empty using a digital balance. Next, the bulb was pressed between the index finger and thumb of the operator, and the channel end was dipped in water while the device was held vertically. Upon releasing the bulb, the water would quickly move up into the device. After the water flow appeared to cease, excess water was dabbed away with a paper towel, and the device was weighed again. Finally, the top sheet of the device was removed, the remaining water inside the device dabbed away, and the device resealed. Measurements were repeated three times for each device.

Reagent-Dispenser Scenario

After weighing each device empty again, a syringe was used to fill the chamber and the channel. It was necessary to fill the channel, too, because water had to be pushed through the channel to exit the device. If only the chamber were filled, much of the water pushed out of the chamber would not have exited the channel, and thus would have been measured as dead volume. The filled device was then weighed, squeezed while vertical, and weighed again. Finally, the top sheet of the device was removed, the remaining water inside dabbed away, and the device resealed. Measurements were repeated three times for each device.

Calculations

The volumes were not measured directly; the masses of water were converted to volumes assuming a specific gravity of unity.

Sample Collector

$$V_{ave} = \frac{\sum_{i=1}^{3}\left(Mass_{i,final} - Mass_{i,initial}\right)}{3} \qquad \textbf{(Eq. 4)}$$

$$V_{tot} = \pi r^2 h \qquad \textbf{(Eq. 5)}$$

$$V_d = V_{tot} - V_{ave} \qquad \textbf{(Eq. 6)}$$

Reagent Chamber

$$V_d = \frac{\sum_{i=1}^{3}\left(Mass_{i,final} - Mass_{i,empty}\right)}{3} \qquad \textbf{(Eq. 7)}$$

$$V_{dispensed} = \frac{\sum_{i=1}^{3}\left(Mass_{i,initial} - Mass_{i,final}\right)}{3} \qquad \textbf{(Eq. 8)}$$

Results

Sample Collector

The percent dead volume increased very clearly with increasing diameter for the sample collector, but its behavior with respect to increasing chamber height was not as straightforward. For the 1" and 3/4" diameters, there was an obvious decrease in percent dead volume with increasing height. For the 5/8" and 1/2" diameters, there was neither a clear increase nor decrease; the trend appeared to be that the height did not affect the percent dead volume. In fact, the percent dead volumes for all of the 1/2" diameter sample collectors were 17.1%. However,

Comment [JRM2]: Kate, I think this section would be a lot stronger if you showed graphs of your trends. Try doing this section in the following order: Intro. Sentence, show graph of data, point out the obvious trend, what does this say about %Vd. I don't have the mindset to change it myself right now

the percent dead volume decreased with increasing height for the 3/8" diameter. Unfortunately, there were too few data points to determine accurately whether or not any of these functions should have been linear or curved. Data and graphs can be found in **Appendix 5**.

The average volume intake was more dependent upon the height of the chamber than the diameter. The plots of average volume intake versus diameter appear to begin to plateau the larger the diameter becomes. For the 1/32" height plot in particular, there was not much gain in average volume intake from the 0.625" diameter to the 1" diameter, and the graph for the 1/50" height was almost flat relative to that for the 1/16".

Reagent Dispenser

The percent dead volume decreased very clearly with increasing diameter for the reagent dispenser, but, as for the sample-collector scenario, the trends for the percent dead volume's dependence on height were less clear. For the 1", 5/8", and 3/8" diameters, there was an obvious decrease in percent dead volume with increasing height, but for both the 3/4" and 1/2", there was no apparent increase or decrease. Again, there were too few data points to determine from the data alone whether or not theses functions are linear or curved. The average volume dispensed appeared to be more dependent upon the diameter than the height. Data and graphs can be found in **Appendix 5**.

Discussions and Conclusions

As predicted by the theoretical analysis, the percent dead volume decreased very clearly with increasing diameter for the reagent dispenser. However, the percent dead volume increased with increasing diameter for the sample collector, as seen in **Figure 20**. This discrepancy indicated a fault in the theoretical analysis: The theoretical analysis considered incompressible fluids only. Thus, as the reagent dispenser was filled first with water (an incompressible fluid) and then squeezed, it behaved as predicted by the theoretical model. Conversely, the sample collector was filled first with air, which is compressible, and then squeezed. Consequently, some of the air that was expected to leave the sample collector remained in the chamber compressed and simply expanded upon release.

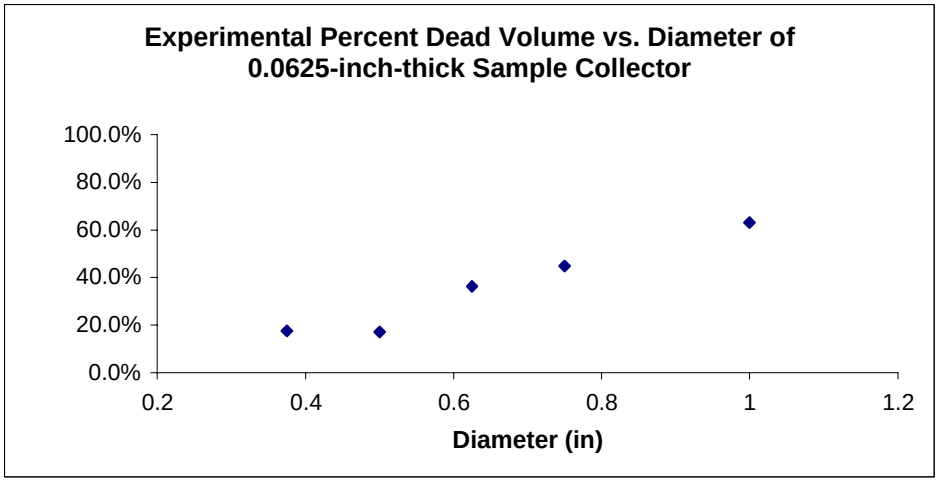


Figure 20: Experimental percent dead volume versus sample-collector diameter for a chamber height of 0.0625 inches.

Micropump Conclusions

Sizing for both types of micropumps was accomplished using the experimental data. The dependence of percent dead volume on height was unclear for both types. However, a large height appears to be more important than a large diameter for the sample collector to have a high average volume intake. The apparent difficulty in increasing the average volume dispensed from a reagent chamber with a small height by increasing the diameter further solidifies the argument for a large height. Because the height of the pumps was found to have no clear effect on percent dead volume, a height of 0.0625 inches, which was the largest height examined, was chosen to maximize effective volume.

The most appropriate dimensions for minimizing the limit of detection cannot be selected without costly immunoassay analysis, but it was assumed that the largest amount of sample would provide the best capability for detecting *V. cholerae*. Hence, the largest diameter studied, 1.0 inch, was selected to maximize the amount of sample to be collected. The reagent dispenser diameter was chosen to be 0.625 inches because this diameter allows the reagent dispenser to have approximately the same effective volume as the 1.0-inch-diameter sample collector. Both of these micropumps are capable of displacing 268μL of fluid. The equality of the effective volumes is crucial because the volumes of reagents dispensed must displace any unbound material inside the detection chamber.

The reagent dispenser requires a smaller diameter than the sample collector because it has a lower dead volume at that size. This is advantageous because costly reagents are stored inside of them. Moreover, the smaller reagent-dispenser diameter allows for a smaller device size, especially considering the design has four reagent dispensers.

Because the sample collected and reagents dispensed must fill the detection chamber, the detection chamber was sized to have a volume equal to the effective volumes of the micropumps.

Because the detection chamber is not pressed, it was assumed that there is no dead volume associated with its operation.

Channel Width Analysis

Before the device layout could be finalized, the width of the channels extending from the micropumps to the detection chamber had to be determined. Some limitations on the range of appropriate widths were already known: The channels needed to be wide enough such that frictional forces would not prevent fluid from flowing at a reasonable rate but thin enough such that they would fit on the device. For user convenience, the channel width was constrained further by limiting the time required for fluid to flow from the micropumps and fill up the detection chamber to approximately one second. A series of two mechanical energy balances was used to determine the dependence of this fill-up time on the channel width. The theory behind the used equations is discussed in **Appendix 3**.

The first mechanical energy balance (**Eq. 9**) was used to approximate the pressure gradient the micropumps were capable of supplying to drive fluid flow. This pressure gradient was calculated using a rough approximation of the time required to collect sample and dispense reagent as observed during the experimental work on micropumps. This time was about 0.1 seconds, and the nature of its determination restricts the channel width determined by this analysis to an order-of-magnitude estimate only. The mechanical energy balance is as follows,

$$\frac{1}{2\alpha}\left(v_2^2 - v_1^2\right) + g\left(z_2 - z_1\right) + \frac{p_2 - p_1}{\rho} + \Sigma F + W_s = 0 \qquad \textbf{(Eq. 9)}$$

where v is velocity, g is the acceleration of gravity, z is height, p is pressure, ρ is density, W_s is work and $\alpha = 0.5$ for laminar flow. The term ΣF accounts for all frictional losses, including those from the straight channel, sudden enlargements, sudden contractions, and bends. It can be expressed mathematically by the following equation,

$$\Sigma F = \underbrace{\frac{12\mu v L}{h^2 \rho}}_{(1)} + \underbrace{0.55\left(1 - \frac{A_2}{A_1}\right)\frac{v^2}{2}}_{(2)} + \underbrace{\left(1 - \frac{A_1}{A_2}\right)\frac{v^2}{2}}_{(3)} + \underbrace{K_f \frac{v^2}{2}}_{(4)} \qquad \textbf{(Eq. 10)}$$

where μ is viscosity, L is channel length, h is channel height, A is cross sectional area, and K_f is the friction loss factor associated with a bend or valve. Term (1) is the friction loss due to the straight rectangular microchannel[194], term (2) is the friction loss due to a sudden contraction, term (3) is the friction loss due to a sudden expansion, and term (4) is the friction loss due to a bend.

Figure 21 below illustrates the device used to collect experimental data.

[194] Ahn *et al*. 2004.

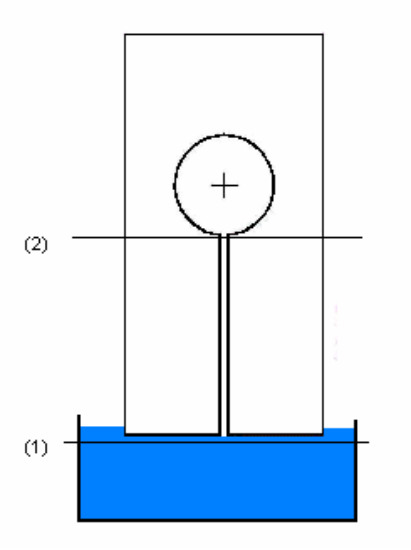


Figure 21: Mechanical energy balance boundaries for experimental device.

Within the boundaries of the balance, there is one sudden contraction. Since $A_1 >> A_2$ (the cross-sectional area of the bulb is much greater than that of the channel), the friction loss for the experimental device can be written

$$\Sigma F = \frac{12\mu v_2 L}{h^2 \rho} + 0.55\frac{v_2^2}{2} \qquad \textbf{(Eq. 11)}$$

(1) (2)

Substituting this into the mechanical energy balance and solving for $p_1 - p_2 = \Delta p$

$$\Delta p = \rho\left[\frac{v_2^2}{2\alpha} + g(z_2 - z_1) + \frac{12\mu v_2 L}{h^2 \rho} + 0.55\frac{v_2^2}{2}\right]$$

It is important to note that since the micropump is located outside the boundaries of the energy balance, W_s is equal to zero. The analysis estimated that $\Delta p = 655$ Pa.

Next, a second mechanical energy balance was derived to determine the dependence of required fill-up time on channel width using this pressure gradient for the micropumps. The balance is written between points (1) and (2), which are based on the physical dimensions of the device described in the final device layout section of this report, as shown in the figure below.

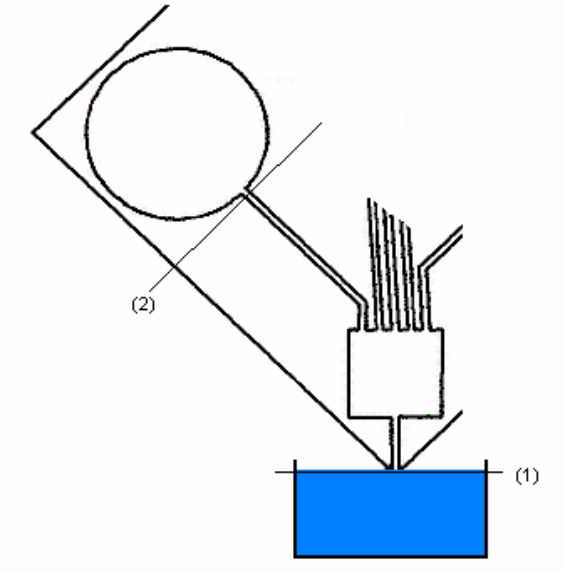


Figure 22: Mechanical energy balance boundaries for the device.

As can be seen from the diagram, there are two sudden contractions, one sudden expansion, and one bend. Therefore,

$$\Sigma F = \frac{12\mu v_2 L}{h^2 \rho} + 2(0.55)\frac{v_2^{\,2}}{2} + \frac{v_2^{\,2}}{2} + K_f \frac{v_2^{\,2}}{2} \qquad \textbf{(Eq. 12)}$$

where K_f = 8.5 for a 45 degree bend. In this equation, L represents the total channel length. After the design of the device layout, which is discussed in a following section, the length of the channel from the sample collector to the detection chamber was known. This length was used as the length required to perform the second mechanical energy balance.

Because the velocity through the reaction chamber is much less than the velocity through the channels, friction loss due to the length of the reaction chamber is negligible. Substituting this into the mechanical energy and solving for v yields

$$v_2 = \sqrt{2\alpha\left[-g(z_2 - z_1) + \frac{\Delta p}{\rho} - \frac{12\mu v_2 L}{h^2 \rho} - 10.6\frac{v_2^2}{2}\right]} \qquad \textbf{(Eq. 13)}$$

The velocity was found through iteration and has a value of approximately 0.25 m/s. Then, for several possible channel widths, the volumetric flow rate was calculated using this velocity. Finally, the volume of the sample collection chamber was divided by the volumetric flow rate to get a chamber fill-up time for each possible channel width, as seen in **Figure 23**.

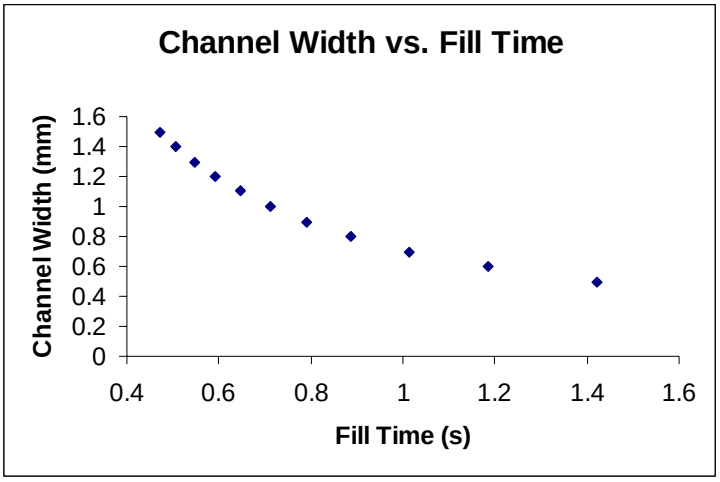


Figure 23: Calculated channel widths as a function of fill-up time.

A channel width of 1.0 mm corresponds to a fill-up time of approximately 0.7 seconds. This channel width is ideal because it provides a fast fill-up time while still fitting well on such a small device. Also, 1.0 mm is a large enough channel width to be produced without costly nanofabrication techniques. A table containing all values used in the channel width analysis can be found in **Appendix 6.**

Valving

Finger-actuated micropumps manipulate liquid on the microfluidic device through displacement. However, once the device operator releases a reagent dispenser, a vacuum is created which draws fluid back into the chamber. Additionally, any liquid stored inside a reagent dispenser must remain stored until released by the operator.

These two technical issues can be overcome with the implementation of a one-way valve. Several one-way valves have been reported and discussed in the background section of this paper (p. 40), but the best valving system for this design is the flap valve.

The flap valve only allows fluid to flow in one direction and does not open unless the micropump is activated. **Figure 24** depicts this system. In **Figure 24a**, liquid (blue) is stored in the micropump reagent dispenser. The flap valve (black) prevents the liquid from leaving and has a small amount of paraffin to keep it shut. In **Figure 24b**, pressing the micropump breaks the paraffin seal, making the flap swing open and allowing the liquid to flow out of the chamber. In **Figure 24c**, the operator has released the micropump, which creates a vacuum, but fluid does not reenter the chamber because the flap moves backward before the fluid and is stopped by the abutment, sealing the channel.

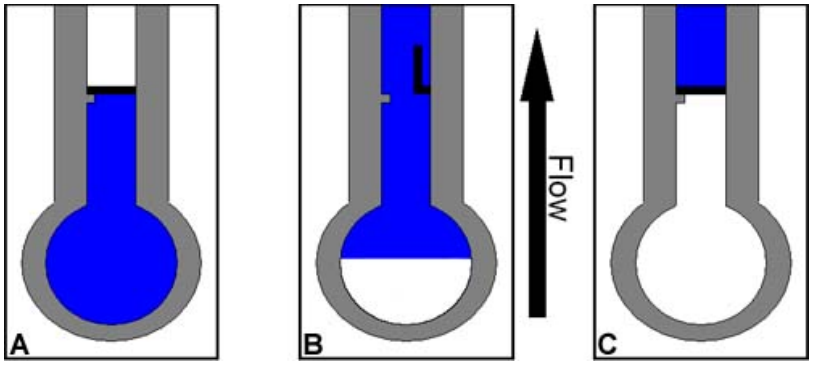


Figure 24: Visual depiction of flap valve. **a**.) Liquid (blue) is stored in the micropump reagent dispenser. **b**.) The micropump has been pushed, causing the flap valve (black) to open up and the liquid to flow out. **c**.) The micropump is released, and the resulting vacuum closes the flap valve, preventing backflow.

This mechanism of storing liquid in reagent dispensers until user operation and preventing backflow is optimal for the microfluidic immunoassay. It does not require the hydrophobicity treatment of passive valving (p. 23) nor the complex wiring of air-bursting detonators (p. 24). Furthermore, because of the large dimensions of this structure relative to other microfeatures, it may be feasible to manufacture the flaps via stamping, as described later.

Final Device Layout

Using the conclusions from the previously described analyses, the final device layout was designed. These previous conclusions determine that the device would employ a sample collector with a diameter of 1.0 inch (25.4 mm), four reagent dispensers with a diameter of 0.625 inches (15.88 mm), and a detection chamber with a volume that would correspond to the effective volumes of the micropumps. This section details other considerations in device layout.

The micropumps, detection chamber, and channels were arranged to minimize the lengths of the channels and to maximize the angles of bends in the channels. Both of these considerations were taken in hopes of reducing the frictional forces the fluids will experience. Furthermore, the channels were designed to extend from the micropumps in such a way that they will take full advantage of the curvature of the chambers. The channels remain separate until reaching the detection chamber, at which point they are parallel to one another and perpendicular to the square detection-chamber wall. A square detection chamber was chosen to allow enough room along the wall to keep the five channels separate. Furthermore, a rectangular detection chamber would have had to have been long in the direction perpendicular to flow to accommodate the effective-volume requirements; this would have encouraged the flow to bypass the extremes of the detection chamber. Finally, the layout was designed to accommodate the user. The sample collector and detection-chamber inlet were each placed in corners, and the reagent dispensers were situated in the order in which they are to be pressed.

The final device, with the optimized channel width of 1.0 millimeter, is shown in the figures below. The valves are not included.

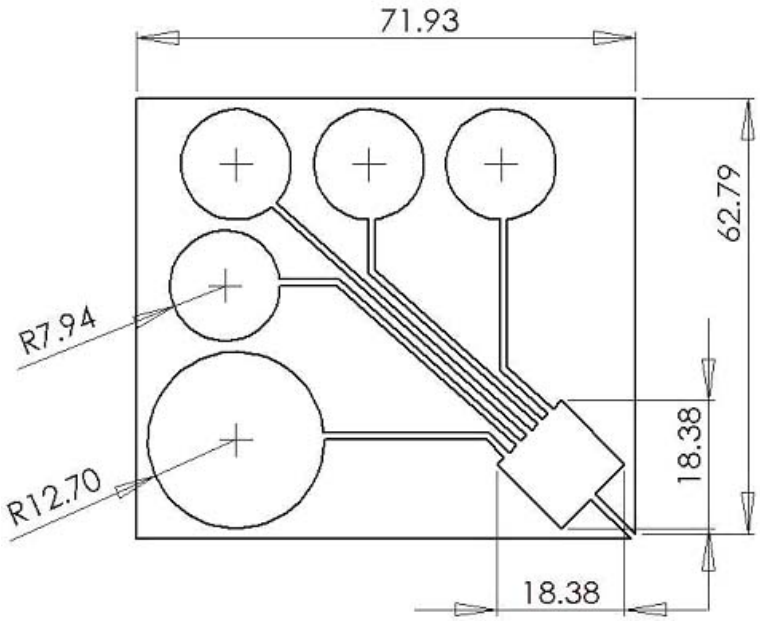
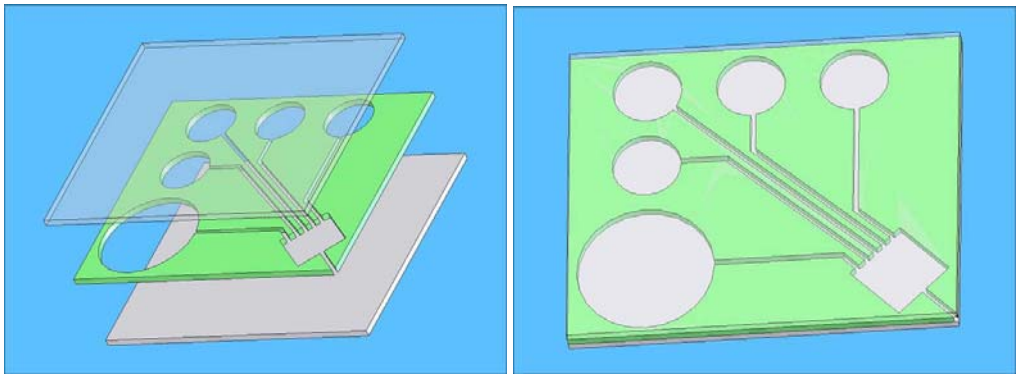


Figure 25: Top view of final device layout. All channels are 1.0 millimeter in width. All numbers are in units of millimeter.



a.) **b.)**

Figure 26: a.) Three-dimensional expanded view of final device. **b.)** Three-dimensional assembled view of final device.

As can be seen in the figures above, all points of the design criteria have been met. The final design is approximately 2.5 by 2.8 inches (63 by 72 mm), which is very compact. The

micropumps are arranged in a logical order. The user will start the assay with the sample collection pump and continue with the reagent dispensers, which are placed in the order in which they are to be pressed.

Device Prototype

A preliminary prototype was created to assess the effectiveness of the micropump and detection-chamber sizing. It was fabricated in much the same way as the devices created for the micropump experimental analysis (p. 32), except punchers were used to stamp the chambers into the middle layer, and Parafilm was used in place of cellophane to prevent adhesion of the chamber tops and bottoms. Although translucent, the Parafilm did not wrinkle like the cellophane and could also be stamped with the punchers. The channel width of one millimeter was adhered to as strictly as possible.

The prototype proved the sizing to have been fairly accurate because the sample collector is capable of aspirating enough sample to fill the detection chamber. **Figure 27**, seen below, is a picture of the prototype in use.

Figure 27: Preliminary prototype demonstrating the effectiveness of the sample collector. When this picture was taken, the sample collector was being released, and sample (tap water and blue food coloring) was being aspirated to fill the detection chamber. The company logo can be seen in the top-right corner of the prototype, as it would appear on the final device.

Device Assembly

This device is designed to be available for the people who need it most: local health officials, tourists, and residents in developing countries. Therefore, the final manufacturing

methods must be simple and realistic. Potential manufacturing techniques are discussed in this section.

Defining the Device Features

Initially, polydimethylsiloxane rubber (PDMS) was considered to be the best material from which to construct the device because of its elasticity and potential as an excellent substrate for the solid-phase antibody layer discussed earlier (p. 21). However, fabrication of a PDMS device is too complex to be considered. Because a supplier for PDMS sheets could not be found, the PDMS parts of the device would have had to have been constructed in-house from a prepolymer solution via cast molding.

Cast molding is complex but, by far, the most common method for defining polymer microfeatures in the literature. The process as described is similar to that found in Li *et al.* (2005). It begins with the creation of a photolithographically defined master containing the negative of the features to be molded into the PDMS. The master is usually made from a silicon wafer. After the wafer is made, it is cleaned with piranha solution (a highly acidic aqueous solution) and dried with gaseous nitrogen. The silicon wafer is then coated with a thin layer of carbon via reactive ion etching (RIE) to allow for easier removal of the PDMS rubber after curing[195].

The curing process is not any simpler. A 1:10 weight ratio mixture of curing agent to PDMS prepolymer solution is prepared, and the mixture is degassed in a 20-25 mm Hg vacuum chamber for one hour to remove bubbles and ensure adequate mixing. The solution would be poured into the mold, excess solution would be removed, and the solution would be cured for an hour, probably on a hot plate. The resulting sheet would be analogous to the bottom and middle layers of the prototype. A second mold would have to be created for the top layer[196].

Because the microfeatures of the microfluidic design are much larger than those required by this technique, a simpler alternative has been selected: stamping of the features into purchased silicone-rubber sheets available from McMaster-Carr. Additionally, many of the surface treatments developed for PDMS are too complicated to be considered realistically for a device for which cost is an issue. Because the only place requiring a solid-phase antibody layer on the device is the detection chamber, and the detection chamber does not need to be pressed, a thin square of polystyrene (PS), a rigid plastic, has been selected as the substrate for the solid-phase antibody layer. Polystyrene, as mentioned previously (p. 21), is an excellent substrate for solid-phase antibody layers. The PS square is designed to have the same area as the detection chamber and, after treatment to deposit an antibody layer on its surface, to adhere to the bottom of the detection chamber. The deposition technique of this layer onto the PS will be described later.

The comprehensive assembly of the device is as follows: First, the features of the micropumps, valves, channels, and detection chamber will be stamped into a 1/16”-thick sheet of silicone rubber with a single puncher. This and other silicone-rubber sheets mentioned will have already been cut to have the area of the final device. Epoxy adhesive from McMaster-Carr (McMaster.com), which is more appropriate for bonding rubber than acrylic or urethane adhesives, is spread across the bottom of this middle layer with a roller. The middle layer is then

[195] Li *et al.* 2005. p. 3.
[196] Li *et al*. 2005. p. 3.

set upon the 1/50”-thick bottom layer. Next, epoxy adhesive is applied to the bottom of the detection chamber, and the treated PS square is inserted. Paraffin wax is then applied to close the flap valves. Another layer of epoxy resin is applied, and the 1/50”-thick top silicon-rubber layer is added. Once the adhesive has set fully, the reagents are injected with a syringe. As this syringe is injecting a reagent, a second syringe is used to remove air from the chamber to prevent bursting of the flap valve. The silicone rubber will self-heal upon removal of the syringes, provided that they are twenty-three gauge or smaller. Finally, the micropumps will be labeled one through five to assist in user operation.

Preparation of the Polystyrene Insert

The squares of polystyrene will be cut from a 1/32”-thick, 40”-wide, 6’-long sheet available from McMaster-Carr. After cleaning, deposition of the solid-phase antibody layer will occur as follows.

Because a positive control in the detection chamber is desired, a microprinting technique must be used to selectively deposit the solid-phase antibody layer. The microprinting technique that will be implemented is similar to that used by Howell *et al*. (2002) in the fabrication of their patterned protein microarrays[197]. The technique requires two PDMS stamps, the preparation of which will not be considered here. The first stamp, molded in the shape of the circular detection surface, is used to transfer anti-cholera antibodies. The second stamp, molded in the shape of the bottom of the detection chamber minus the circular detection surface, is used to transfer anti-cholera antibodies bound to dead cholera cells. Gold-colloid-labeled antibodies will bind to this second area of the detection chamber irrespective of sample contamination, and these gold colloids will be amplified via silver enhancement. Thus, this area serves as a positive control by indicating whether or not the silver enhancer has indeed worked properly. The two types of antibodies are each initially in phosphate buffer saline (PBS) solutions with a pH of 7.4. Because of the limitations of this design project, the concentration of antibodies in these solutions has yet to be determined experimentally.

In preparation for the transfer of antibodies onto the PDMS stamps, the PDMS stamps are hydrophilized in oxygen plasma to assist in collection of the solution. After treatment, the stamps are set in the antibody solution for twenty minutes and then dried with nitrogen gas. Next, the circular stamp is locked with the larger stamp, forming a composite stamp. To transfer the antibodies onto the PDMS substrate, the composite PDMS stamp is pressed onto the PS substrate for one minute. After the stamps are removed, the substrate is immersed in a solution of 0.05% Tween-20 and 1% Bovine Serum Albumin (BSA) in Phosphate Buffer Solution (PBS) (Sia et al. 2001) to protect sites where antibodies fail to bind from nonspecific binding of gold-colloid-labeled antibody and subsequent silver enhancement background. The substrate is then rinsed with deionized water, dried, and adhered to the bottom of the detection chamber as describe previously.

Reagents

The reagents contained in the four reagent dispensers are essential to the immunoassay and silver enhancement. The first reagent dispenser contains a solution of gold-colloid-labeled antibodies in a buffered solution like the ones used to treat the PS substrate. These labeled

[197] Howell *et al*. 2002. p. 4.

antibodies bind to any captured *V. cholerae* cells in the detection chamber. The second reagent dispenser contains deionized water to remove any unbound gold-colloid-labeled antibodies to reduce the risk of a false positive. The water needs to be deionized so that ions are not unnecessarily introduced into the system. Such species could interfere with silver enhancement. The third reagent dispenser contains the silver salt solution. This solution contains the silver cations that are reduced by the agent released from the final reagent dispenser to form a silver film around the colloids. The reagents required for silver enhancement are addressed in more detail in the following section.

Silver Enhancement

Silver enhancement will be used to amplify the visual signal provided by the aggregation of gold-colloid labeled antibodies in the detection chamber. This method has been implemented previously on microfluidic devices[198] and has been shown to increase the limit of detection by 50,000%[199]. In the absence of published material and experimental data concerning silver enhancement of biological cells, the following results are based on other available published material and specific constraints of our device.

The micropump analysis has shown that 314 μL of reducing and silver agents are necessary for each reagent dispenser. Sia *et al*. in 2001 reported that using a 1:1 ratio of reducing and silver agents from the silver enhancer kit available from Sigma-Aldrich (St. Louis, Missouri, USA) was an excellent choice for their microfluidic bioassay[200]. While Sigma-Aldrich will not release specifics of their product, the 1996 review article by Lackie says a typical silver solution contains 0.2% w/v silver acetate and the reducing buffer contains 0.5% w/v hydroquinone in citrate buffer (24 volumes of 2.55% w/v citric acid, 22 volumes of 2.35% trisodium citrate, and 50 volumes of distilled water)[201]. Without additional information, the Sigma-Aldrich silver enhancer kit is currently the best option for silver enhancement of the immunoassay.

This analysis provides a good starting point for future investigation into silver enhancement for this device. Due to the lack of literature describing the utilization of silver enhancement in the detection of biological cells, the above recommendations could only be based on silver enhancement usage on microfluidic devices detecting macromolecules. For any realistic application of this immunoassay, in-depth laboratory research of silver enhancement with biological cells must be conducted.

Device Operation

The following list describes the nine steps an operator of the microfluidic immunoassay would have to follow in order to properly use the device. Waiting times between operations were adopted from the 2004 Sia *et al*. paper[202]. This procedure allows ample time for sample collection, gold labeling of captured *V. cholerae* cells, and silver enhancement. A wash is

[198] Sia *et al*. 2004
[199] Shyu *et al*. 2001. p. 257
[200] Sia et al. Supporting Information. 2004. p. 3.
[201] Lackie. 1996. p. 13.
[202] Sia et al. Supporting Information. 2004. p. 5-6

included to remove unbound gold-labeled antibodies, which will reduce the likelihood of false positives. Instructions on result interpretation are also provided.

1. Press the sample collector. This is the circular bulb labeled 1. It is larger than the other chambers. While still pressing on the sample collector, dip the device inlet (the corner where a channel exits the device) into the water you would like to test. Release the sample collector gently to draw the water into the detection chamber.
2. Wait 10 minutes
3. Press the first reagent dispenser for one full second. This dispenser is the circular bulb labeled 2. Some fluid will come out of the device inlet because the reagent just released will expel the water previously collected. This will happen each time a reagent dispenser is pressed. This step introduces gold-colloid-labeled antibodies into the detection chamber.
4. Wait 10 minutes
5. Press the second reagent dispenser for one full second. This is the circular bulb labeled 3. This step rinses the detection chamber with deionized water to remove unbound gold-colloid-labeled antibody.
6. Press the third reagent dispenser for one full second. This is the circular bulb labeled 4. This step releases the silver salt solution.
7. Press the fourth reagent dispenser for one full second. This is the circular bulb labeled 5. This step releases the silver-cation reducing agent.
8. Wait 13.5 minutes
9. Observe the square detection chamber.

If the bottom of the detection chamber is completely silver in color, the sample is contaminated. If there is a circle in the middle of the detection chamber that remains as it was before, the sample is not contaminated. If no silvering occurs anywhere is the chamber, the test has failed.

Technical Results Summary

The information presented in this section thoroughly discusses the technical results of the Cholera Microbusters, Inc. senior design project in the spring of 2005. A finger-actuated micropump height of 0.0625 inches, a sample-collector diameter of 1.0 inch, and a reagent-dispenser diameter of 0.625 inches were found to be optimal for this device. Provisions for valves to prevent backflow into the reagent dispensers have been made. Considering fluid fill-up time, a channel width of 1.0 millimeters was chosen, and an ergonomic device layout was developed. The device will be made from silicone-rubber sheets with features defined by stamping. A thin polystyrene insert will serve as the substrate for the solid-phase antibody layer in the detection chamber. Micro-contact printing will be employed to deposit this antibody layer. Finally, estimated quantities of silver enhancer and recommended user instructions were presented.

Environmental and regulatory analysis

Over the years, the federal government has imposed increasingly stringent regulations on industry in an attempt to reduce damage to both human health and the environment. The finger-actuated microdispenser will be used in the field and must comply with the government regulations where it will be used. Although the device will be used in developing third-world countries where cholera still poses a significant health concern, health and environmental regulations in the United States serve as a more stringent standard for permissible chemical usage. A comprehensive literature search yielded no regulations on silver enhancer in the United States. However, a search on the components of silver enhancer showed that the U.S. government regulated silver nitrate and hydroquinone under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Citric acid and trisodium citrate were found to be unregulated.

CERCLA, otherwise known as Superfund, was enacted in 1980 to control and clean up hazardous waste sites (EPA CERCLA). The act imposed a tax on the chemical and petroleum industries over a five-year period to fund a trust that was used for waste cleanup when no party could be found liable. CERCLA also revised the National Contingency Plan (NCP), a listing of protocols that was to be followed in response to hazardous waste spills. CERCLA is concerned with the control and cleanup of major spills. The small quantity of chemicals used in the finger-actuated microdispenser will be of little concern to CERCLA regulations[203]. As a precaution, a Material Safety Data Sheet (MSDS) of each chemical used in silver enhancement is included in **Appendix 7** to address any hazard the user should be made aware of when operating the device. In addition, **Table 2**. briefly summarizes chemicals of interest on the device and whether or not they are regulated in various legal acts.

Chemical	CERCLA	RCRA	TSCA	SARA 313
citric acid	No	No	No	No
trisodium citrate	No	No	No	No
silver nitrate	Yes	No	No	No
hydroquinone	Yes	No	No	Yes

RCRA=Resource Conservation and Recovery Act
TSCA=Toxic Substance Control Act
SARA=Superfund Amendments and Reauthorization Act
CERCLA=Comprehensive Environmental Response, Compensation, and Liability Act

Table 2: Summary of chemicals and which regulations mention them.

[203] CERCLA. 2005.

Economic Analysis

The following outlines the amount of material necessary per device, current market prices, and cost per device.

Material	Amount per device	Price per amount	Price per unit
Silicone Rubber (1/50" thickness)	Two 7 inches^2 Sheets	\$0.0833/inch^2	\$1.17
Silicone Rubber (1/16" thickness)	One 7 inches^2 Sheet	\$0.0736/inch^2	\$0.52
Antibody	5.2 ng	\$3.0/μg	\$0.02
Tween-20	0.268 mL	\$0.0209/mL	~\$0.00
Bovine Serum Albumin	0.268 mL	\$3.820/mL	\$0.01
Gold-labeled Antibody	1.046 mL	\$110.497/mL	\$0.12
Silver Enhancer	314 μg	\$0.554/mL	\$0.17
Epoxy Adhesive	0.305 mL	\$0.24/mL	\$0.15
Polystyrene Square	13x13x0.79mm	\$0.014/m^2	\$0.001

Table 3: List of amount, unit price, and cost for each material/reagent per each device.

Total Material Cost per Device = \$2.16

Some of these prices are more reliable than others based on availability. Tween-20, Bovine Serum Albumin, and silver enhancer were all found directly from Sigma-Aldrich. The silicon rubber, polystyrene, and epoxy adhesive can be found from McMaster-Carr. Antibodies specific to the surface of our target bacteria were not available. Xiangming Fang, M.D. Ph.D. of Genway Bio, Inc on April 11th, 2005 suggested, for affinity-purified antibodies, the above price per amount. Since antibodies specific to the surface of our target bacteria were not available, gold-labeled versions were not. The given price is for the gold-labeled antibodies used in the Sia *et al.* 2004 paper (from Sigma-Aldrich also)[204].

Compared to the SMART device, this analysis is extremely favorable. The quoted price for the SMART is \$368.75 for 25 uses, or \$14.75 per use[205]. Compared against the materials of construction, the SMART, which is the most similar device to the one described in this report on the market, is approximately seven times more expensive. Furthermore, an operator has to carry a small suitcase of pouches, buffers and reagents, tubes, filters, and droppers along with the SMART.

[204] Sia *et al*. Supporting Information. 2004. p. 2-3
[205] Cholera SMART II Pricing Information.

Discussion

This senior design project has been highly successful in generating significant accomplishments. Optimal sizing and placement the device features were determined. The resulting design has completed the goals planned for this project. The overall size of the device makes it easily portable and discrete. Additionally, the sizing optimizations have resulted in low reagent requirements, which allowed for the low cost of materials per device of $2.16. This device also has a tolerance factor built into it; even if the optimized sizing is somewhat incorrect, the device will still operate properly. This is because the detection chamber does not have to be completely filled to operate, and if too much fluid has been placed inside the detection chamber, it will simply drain out the device inlet. This device also has the capacity to comply with stringent US environmental, health, and safety regulations. Having minimal amounts of reagent, with most of the reagents benign enough to not require any regulation what so ever, frees this design of any restrictions that would otherwise result.

The greatest weakness of this design is the amount of manipulations the operator has to perform. To maximize convenience during the procedure, special attention was paid to the device layout. The sample collector is placed such that the angle achieved when aspirating sample is comfortable for the user. Additionally, the reagent dispensers have been situated in a clear and logical order. These factors, in conjunction with low fluid fill-up times, mitigate the negative effects for the user caused by the long operating procedure. Also, because of the restrictions native to the nature of this design project, including time and budget concerns, the research that is necessary to confirm and determine many of the precise design details could not be performed.

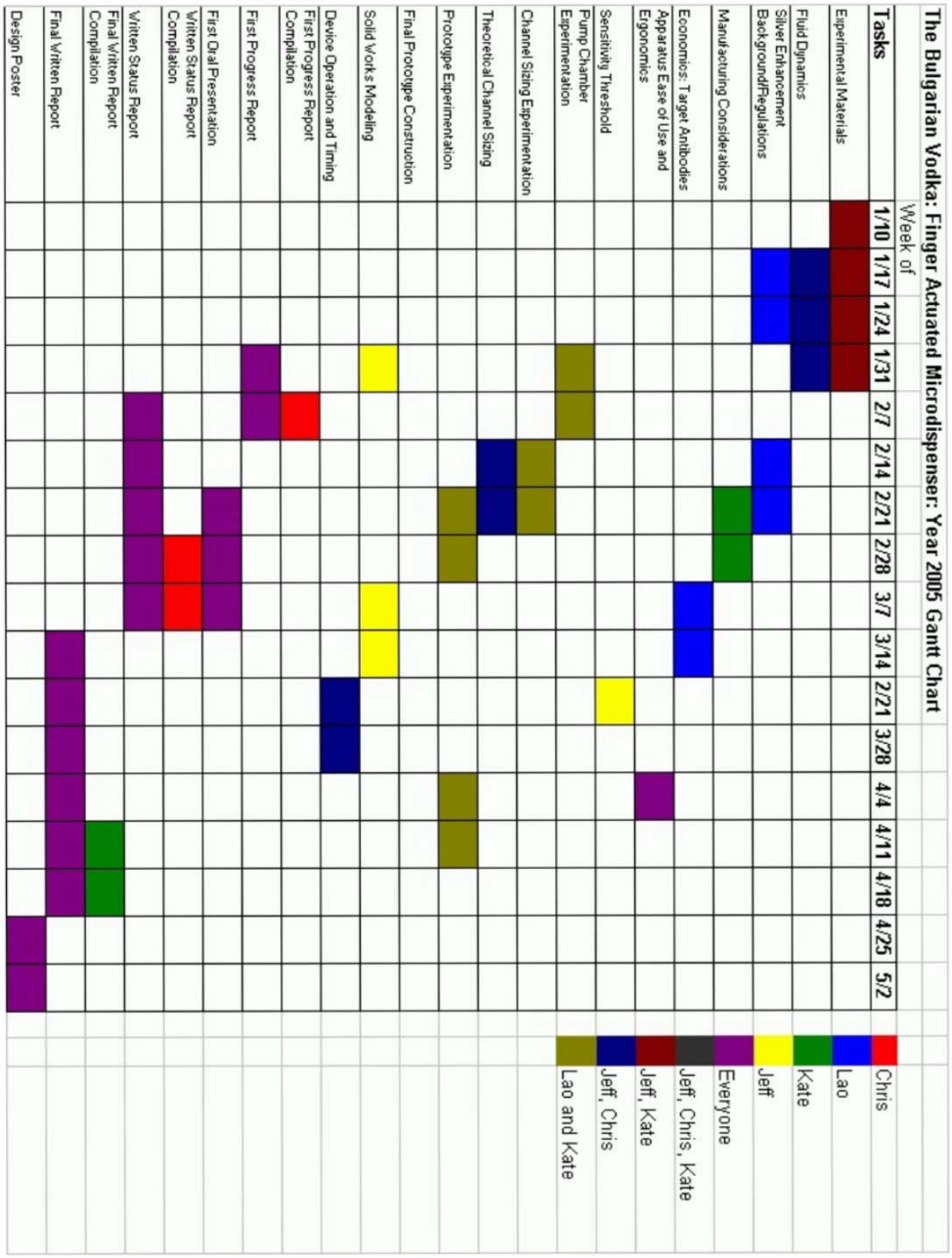
Despite minor unresolved problems, this device has the potential to forge a new market. The demand for point-of-care diagnostic equipment that is inexpensive, quick, and portable from tourists, health officials, and local residents in developing countries is undeniable, and devices of the type proposed in this report could meet their needs.

Recommendations

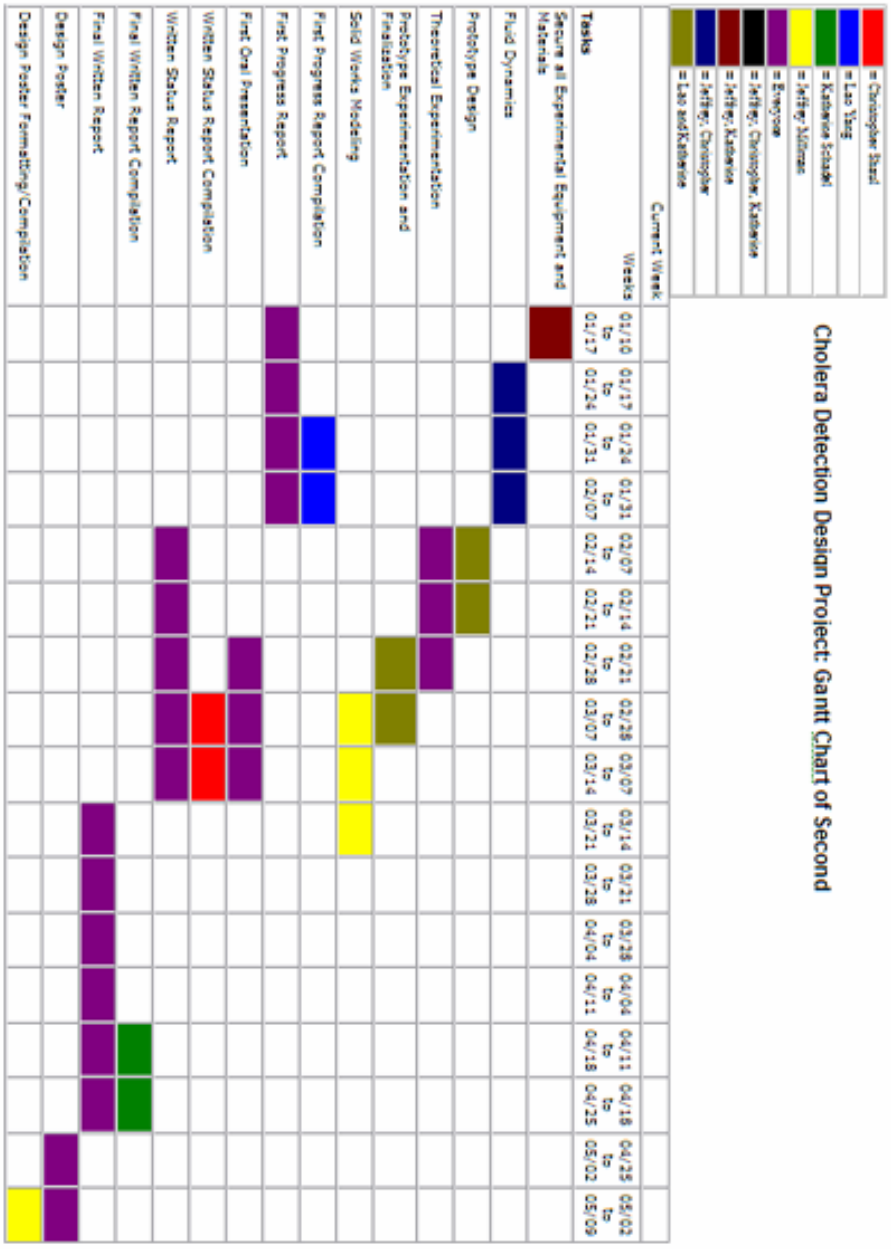
This senior design project completed by Cholera Microbusters, Inc. could serve as the starting point for development of a feasible industrial product. The technical results, derived from reasonable estimates and as much sound experimental work as possible, appear to be favorable for implementation of the design commercially. Furthermore, there are no known applicable governmental regulations to restrict the design, and the cost of materials compared to that of the closest competitor, the SMART, is an indication that the overall price will be reasonable once more thorough pricing information is available, including manufacturing costs.

The following suggestions have been made for development and production of a commercial device. Because the accuracy of many of the values presented in the technical results were limited by a lack of relevant research in the literature, experiments should be conducted to determine how *V. cholerae* would behave in the device, especially during silver enhancement. From these experiments, optimized values for device timing and reagent concentrations could be obtained. To obtain a more accurate estimate of the cost per device, a detailed study on the manufacturing of the device must be done. This will require proprietary information no company wishes to divulge to a senior design team. Finally, a working prototype, complete with immunoassay and silver-enhancement functions, must be constructed and evaluated because the prototypes that have been developed throughout the course of this project are restricted to testing the performance of the pumping mechanisms only.

Appendix 1: *Spring 2005 GANTT Chart*



Appendix 2: *Old Spring 2005 GANTT Chart*



Appendix 3: *Transport Phenomena*

Fluid Dynamics

The purpose of the valves and microstructures discussed in the technical background is to manipulate small volumes of liquid. Fluid dynamics is the study of fluid flow through the transport of momentum. As manipulation of small volumes of liquid is at the center of microfluidics, fluid dynamics plays an important role in the design of microfluidic devices. A scenario helpful for beginning the discussion of fluid dynamics begins with a fluid resting between two parallel plates. As the bottom plate begins to move with a constant velocity, the fluid moves with it as depicted in **Figure 21**.

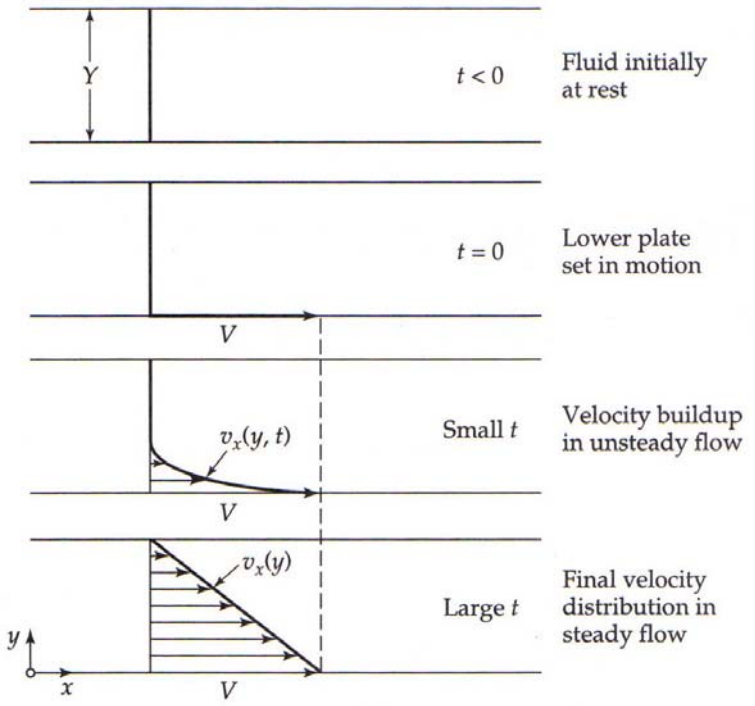


Figure 28: Fluid velocity profile of fluid between parallel plates caused by movement of bottom plate[206].

The fully developed velocity profile can be described by Newton's law of viscosity (**Eq. 14**)[207].

$$\tau_{yx} = -\mu \frac{dv_x}{dy} \qquad \textbf{(Eq. 14)}$$

[206] Bird *et al*. 2002. p. 12.
[207] Bird *et al*. 2002. p. 12.

The symbol τ_{yx} is the shear stress in the x direction on an area normal to the y direction, μ is the viscosity, and dv_x/dy is the change in x direction velocity with respect to the y direction. In general, the motion of any fluid, Newtonian or Non-Newtonian, can be described by the equation of motion (**Eq. 15**), which is expressed as[208]:

$$\frac{\partial}{\partial t}\rho\vec{v} = -\left[\nabla\cdot\rho\vec{v}\vec{v}\right] - \nabla p - \nabla\cdot\vec{\vec{\tau}} + \rho\vec{g} \qquad \textbf{(Eq. 15)}$$

For this equation, ρ is density, $\vec{v}$ is the velocity vector, $\vec{v}\vec{v}$ is the velocity tensor, p is pressure, $\vec{\vec{\tau}}$ is the shear stress tensor, and $\vec{g}$ is the gravitational constant.

The fluid flow velocity profile in microfluidic channels is laminar[209]. This arises from the fact that the flow occurs at low Reynolds numbers (Re). Re is defined for channels as follows in **Eq. 16**[210]:

$$\mathrm{Re} = \frac{D\langle v_x\rangle\rho}{\mu} \qquad \textbf{(Eq. 16)}$$

D is the diameter and $\langle v_x\rangle$ is the average velocity in the x direction. For Re values less than 2100, the flow is considered laminar; above this value, the flow is turbulent. Laminar flow in microfluidic devices results from the small channel diameter and is characterized by a parabolic velocity profile (**Figure 22**), in which the motion of the fluid particles is only in the direction of the bulk flow (**Figure 23**)[211].

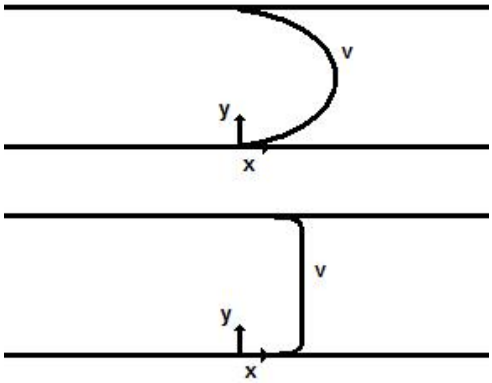


Figure 29: Velocity profile comparison between laminar and turbulent flow. The top picture represents laminar flow, while the bottom picture represents turbulent plug flow in a tube.

[208] Bird *et al*. 2002. p. 80.
[209] Locascio, L. E. 2004. p. 325.
[210] Bird *et al*. 2002. p. 52.
[211] Bird *et al*. 2002. p. 41.

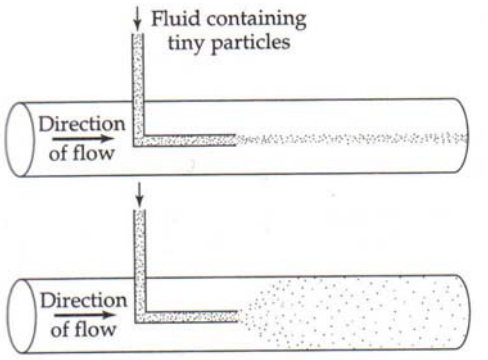


Figure 30: Velocity direction comparison between laminar and turbulent flow. The top pipe has a laminar flowing fluid; therefore the motion of the tiny particles is only parallel with the direction of flow. The bottom pipe has a turbulent flowing fluid; therefore the motion of the tiny particles is not only parallel with the direction of flow[212].

Fluid flow in the proposed microfluidic device will be driven by pressure gradients and can be characterized by the equation of continuity and the Bernoulli equation. The equation of continuity relies upon the principle of conservation of mass and thus is derived from a mass balance on the system (**Eq. 17**)[213].

$$\frac{\partial \rho}{\partial t} = -\left(\nabla \cdot \rho \bar{v}\right) \qquad \textbf{(Eq. 17)}$$

The Bernoulli equation (**Eq. 18**) relates the pressure change to the velocity through an energy balance.

$$\frac{d}{dt}\left(K_{tot} + \Phi_{tot}\right) = -\Delta\left(\frac{1}{2}\frac{\langle v^3 \rangle}{\langle v \rangle} + \hat{\Phi} + \frac{p}{\rho}\right)w + W_m - E_c - E_v \qquad \textbf{(Eq. 18)}$$

In Eq.X, K_{tot} is the kinetic energy, Φ potential energy, $\hat{\Phi}$ potential energy per mass, w mass, W_m the work done by the surroundings, E_c the compression term, and E_v the energy loss due to friction. This equation is only valid for an isothermal system.

Mass Transport

As flow in microfluidic devices is laminar, diffusion is the only source of mixing, unless some form of agitation is added[214]. Diffusion is the random movement of particles from high

[212] Bird *et al*. 2002. p. 41.
[213] Bird *et al*. 2002. p. 77.
[214] Locascio, L. E. 2004. p. 325.

concentrations to low concentrations. Fick's first law of diffusion (**Eq. 19**) is a one-dimensional equation describing diffusion at steady state of a binary mixture[215].

$$j_{Ax} = -\rho D_{AB} \frac{d\omega_A}{dy} \qquad \textbf{(Eq. 19)}$$

The symbol j_{Ax} is the mass flux of species A in the x direction, D_{AB} is the diffusivity constant of species A in species B, and ω_A is the mass fraction of species A. The diffusivity constant is dependent on temperature; the higher the temperature the higher the constant[216].

The equations governing molecular diffusion have to be modified for modeling colloidal suspension diffusion, such as cells. Diffusion of colloidal suspensions result from a random motion called Brownian motion[217]. The equation of motion for these particles is called the Langevin equation and is found in **Eq. 20**[218].

$$m\frac{d\vec{u}_A}{dt} = -\zeta\vec{u}_A + \vec{F}(t) \qquad \textbf{(Eq. 20)}$$

This equation describes particles A of mass m moving at an instantaneous velocity $\vec{u}_A$. The term ζ is the friction coefficient of the Stokes' law drag force and is defined in **Eq. 21**[219].

$$\zeta = 6\pi\mu R_A \qquad \textbf{(Eq. 21)}$$

R_A is the radius of particle A. $\vec{F}(t)$ is a varying force term, and because of this term, the Langevin equation cannot be used directly. However, a probability function on position can be derived assuming the position distribution of the particles reaches a Maxwellian distribution after a long time and is seen in **Eq. 22**[220].

$$W(\vec{r},t;\vec{r}_0,\vec{u}_{A0}) = \left(\frac{\zeta}{4\pi KTt}\right)^{3/2} \exp\left(\frac{-\zeta(r-r_0)^2}{4\pi KTt}\right)d\vec{r} \qquad \textbf{(Eq. 22)}$$

W is the probability function, *K* is Boltzmann's constant, and *r* is the position.

[215] Bird *et al*. 2002. p. 515.
[216] Bird *et al*. 2002. p. 521.
[217] Bird *et al*. 2002. p. 531.
[218] Bird *et al*. 2002. p. 531.
[219] Bird *et al*. 2002. p. 531.
[220] Bird *et al*. 2002. p. 531.

Appendix 4: *Theoretical Pump Analysis*

Single point contact

diameter (in)	contact dia (in)	height (in)	total vol (in^3)	active vol (in^3)	dead vol (in^3)	percent dead vol.
1.25N/A		0.0625	0.076699039	0.025566346	0.051132693	66.66666667
1N/A		0.0625	0.049087385	0.016362462	0.032724923	66.66666667
0.75N/A		0.0625	0.027611654	0.009203885	0.018407769	66.66666667
0.5N/A		0.0625	0.012271846	0.004090615	0.008181231	66.66666667
1.25N/A		0.03125	0.03834952	0.012783173	0.025566346	66.66666667
1N/A		0.03125	0.024543693	0.008181231	0.016362462	66.66666667
0.75N/A		0.03125	0.013805827	0.004601942	0.009203885	66.66666667
0.5N/A		0.03125	0.006135923	0.002045308	0.004090615	66.66666667
1.25N/A		0.02	0.024543693	0.008181231	0.016362462	66.66666667
1N/A		0.02	0.015707963	0.005235988	0.010471976	66.66666667
0.75N/A		0.02	0.008835729	0.002945243	0.005890486	66.66666667
0.5N/A		0.02	0.003926991	0.001308997	0.002617994	66.66666667

Circular contact (both sides squeezed)

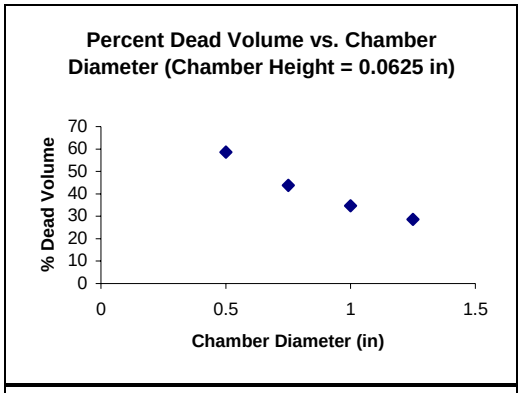
diameter (in)	contact dia (in)	height (in)	total vol (in^3)	active vol (in^3)	dead vol (in^3)	percent dead vol.
1.25	0.85	0.0625	0.076699039	0.054773341	0.021925699	28.58666667
1	0.6	0.0625	0.049087385	0.032070425	0.01701696	34.66666667
0.75	0.35	0.0625	0.027611654	0.015503432	0.012108222	43.85185185
0.5	0.1	0.0625	0.012271846	0.005072363	0.007199483	58.66666667
1.25	0.85	0.03125	0.03834952	0.02738667	0.010962849	28.58666667
1	0.6	0.03125	0.024543693	0.016035213	0.00850848	34.66666667
0.75	0.35	0.03125	0.013805827	0.007751716	0.006054111	43.85185185
0.5	0.1	0.03125	0.006135923	0.002536182	0.003599742	58.66666667
1.25	0.85	0.02	0.024543693	0.017527469	0.007016224	28.58666667
1	0.6	0.02	0.015707963	0.010262536	0.005445427	34.66666667
0.75	0.35	0.02	0.008835729	0.004961098	0.003874631	43.85185185
0.5	0.1	0.02	0.003926991	0.001623156	0.002303835	58.66666667

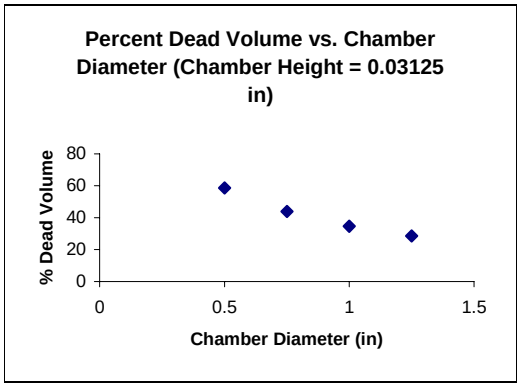
circular contact (one side squeezed, one size rugged).

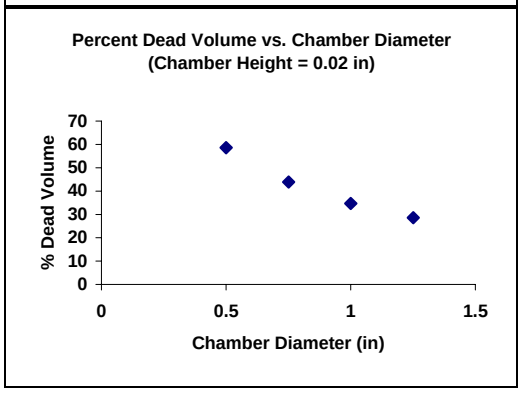
diameter (in)	contact dia (in)	height (in)	total vol (in^3)	active vol (in^3)	dead vol (in^3)	percent dead vol.
1.25	0.85	0.0625	0.076699039	0.054773341	0.021925699	28.58666667
1	0.6	0.0625	0.049087385	0.032070425	0.01701696	34.66666667
0.75	0.35	0.0625	0.027611654	0.015503432	0.012108222	43.85185185
0.5	0.1	0.0625	0.012271846	0.005072363	0.007199483	58.66666667
1.25	0.85	0.03125	0.03834952	0.02738667	0.010962849	28.58666667
1	0.6	0.03125	0.024543693	0.016035213	0.00850848	34.66666667
0.75	0.35	0.03125	0.013805827	0.007751716	0.006054111	43.85185185

0.5	0.1	0.03125	0.006135923	0.002536182	0.003599742	58.66666667
1.25	0.85	0.02	0.024543693	0.017527469	0.007016224	28.58666667
1	0.6	0.02	0.015707963	0.010262536	0.005445427	34.66666667
0.75	0.35	0.02	0.008835729	0.004961098	0.003874631	43.85185185
0.5	0.1	0.02	0.003926991	0.001623156	0.002303835	58.66666667

The following graphs apply to the assumption of a circular contact area between the top and bottom layers.



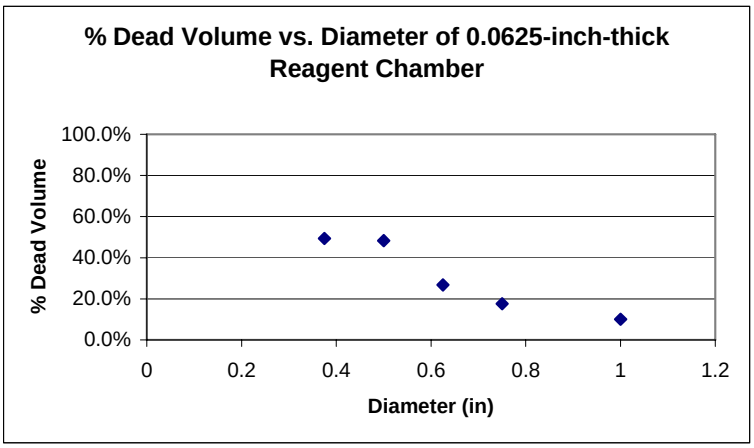


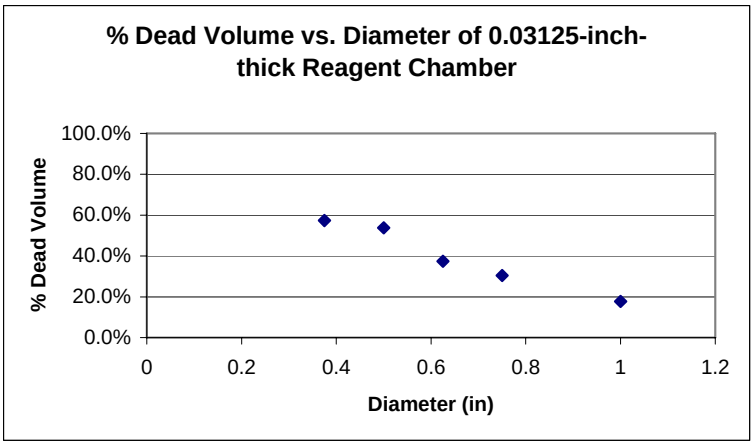


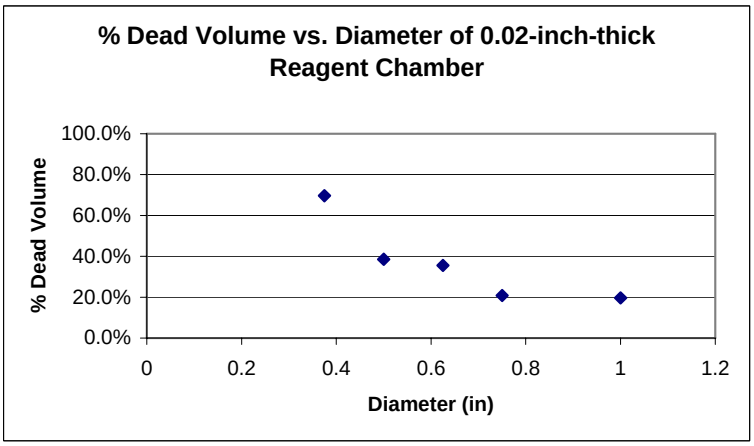
Appendix 5: *Experimental Pump Analysis*

Reagent Chamber Data

Chamber Dimensions		Average Dead Volume (mL)	Total Volume (mL)	% Dead Volume	Average Volume Dispensed (mL)
Height (in)	Diameter (in)				
0.0625	1	9.00E-02	9.00E-01	10.0%	8.03E-01
0.0625	0.75	9.67E-02	5.48E-01	17.6%	4.50E-01
0.0625	0.625	1.10E-01	4.10E-01	26.8%	2.77E-01
0.0625	0.5	1.43E-01	2.97E-01	48.2%	1.70E-01
0.0625	0.375	1.03E-01	2.09E-01	49.4%	7.67E-02
0.03125	1	8.00E-02	4.50E-01	17.8%	4.97E-01
0.03125	0.75	8.33E-02	2.74E-01	30.4%	2.20E-01
0.03125	0.625	7.67E-02	2.05E-01	37.4%	1.60E-01
0.03125	0.5	8.00E-02	1.49E-01	53.9%	8.33E-02
0.03125	0.375	6.00E-02	1.05E-01	57.4%	4.67E-02
0.02	1	5.67E-02	2.88E-01	19.7%	4.67E-01
0.02	0.75	3.67E-02	1.76E-01	20.9%	1.60E-01
0.02	0.625	4.67E-02	1.31E-01	35.5%	1.10E-01
0.02	0.5	3.67E-02	9.51E-02	38.6%	7.33E-02
0.02	0.375	4.67E-02	6.69E-02	69.7%	3.33E-02

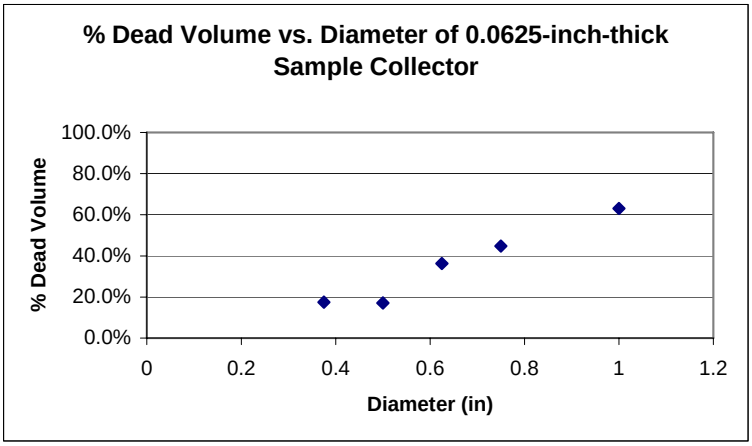


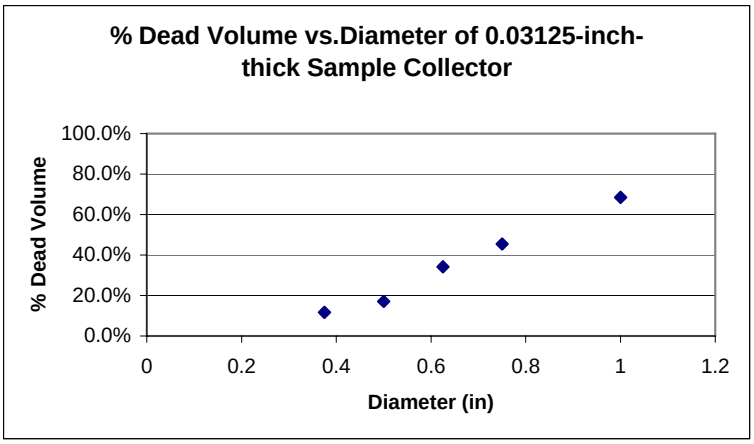

% Dead Volume vs. Diameter of 0.03125-inch-thick Reagent Chamber
% Dead Volume
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
0
0.2
0.4
0.6
0.8
1
1.2
Diameter (in)

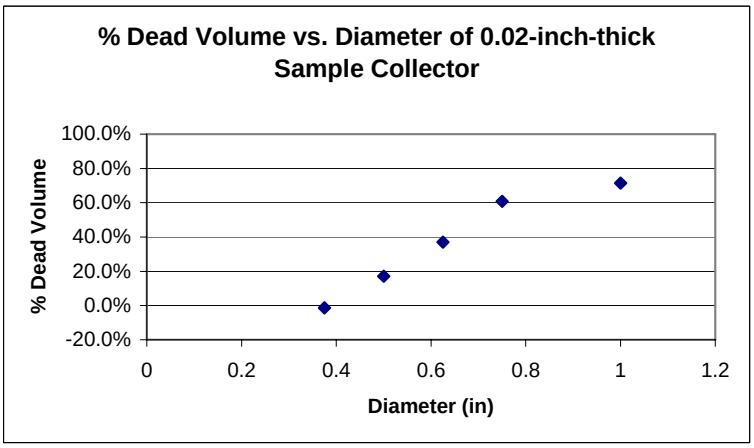

% Dead Volume vs. Diameter of 0.02-inch-thick Reagent Chamber
% Dead Volume
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
0
0.2
0.4
0.6
0.8
1
1.2
Diameter (in)

Sample Collector Chamber Data

Chamber Dimensions		Average Volume Intake (mL)	Total Volume (mL)	Dead Volume (mL)	% Dead Vol.
Height (in)	Diameter (in)				
0.0625	1	2.97E-01	8.04E-01	5.08E-01	63.1%
0.0625	0.75	2.50E-01	4.52E-01	2.02E-01	44.7%
0.0625	0.625	2.00E-01	3.14E-01	1.14E-01	36.3%
0.0625	0.50	1.67E-01	2.01E-01	3.44E-02	17.1%
0.0625	0.375	9.33E-02	1.13E-01	1.98E-02	17.5%
0.03125	1	1.27E-01	4.02E-01	2.76E-01	68.5%
0.03125	0.75	1.23E-01	2.26E-01	1.03E-01	45.5%
0.03125	0.625	1.03E-01	1.57E-01	5.38E-02	34.2%
0.0313	0.50	8.33E-02	1.01E-01	1.72E-02	17.1%
0.03125	0.375	5.00E-02	5.66E-02	6.56E-03	11.6%
0.02	1	7.33E-02	2.57E-01	1.84E-01	71.5%
0.02	0.75	5.67E-02	1.45E-01	8.81E-02	60.9%
0.02	0.625	6.33E-02	1.01E-01	3.72E-02	37.0%
0.0200	0.50	5.33E-02	6.44E-02	1.10E-02	17.1%
0.02	0.375	3.67E-02	3.62E-02	-4.69E-04	-1.3%




% Dead Volume vs.Diameter of 0.03125-inch-thick Sample Collector
% Dead Volume
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
0
0.2
0.4
0.6
0.8
1
1.2
Diameter (in)


% Dead Volume vs. Diameter of 0.02-inch-thick Sample Collector
% Dead Volume
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
-20.0%
0
0.2
0.4
0.6
0.8
1
1.2
Diameter (in)

Data Comparison

Chamber Dimensions		% Dead Volume	
Thickness (in)	Diameter (in)	Sample Collector Scenario	Reagent Chamber Scenario
0.0625	1	63.1%	10.0%
0.0625	0.75	44.7%	17.6%
0.0625	0.625	36.3%	26.8%
0.0625	0.50	17.1%	48.2%
0.0625	0.375	17.5%	49.4%
0.03125	1	68.5%	17.8%
0.03125	0.75	45.5%	30.4%
0.03125	0.625	34.2%	37.4%
0.03125	0.50	17.1%	53.9%
0.03125	0.375	11.6%	57.4%
0.02	1	71.5%	19.7%
0.02	0.75	60.9%	20.9%
0.02	0.625	37.0%	35.5%
0.02	0.375	17.1%	38.6%
0.02	0.50	-1.3%	69.7%

Appendix 6: *Channel Width Calculations*

Experimental Data					
Time	0.25	s	Viscosity	0.000894	Pa-s
Length	0.03810008	m	Density	991	kg/m^3
Width	0.0015875	m			
Height	0.0015875	m	Velocity	0.1524	m/s
Delta P	654.76757	Pa			
Mechanical E bal.					
Velocity	0.26349299	m/s	z	0.02	m
Guess Vel.	0.2639	m/s	alpha	0.5	
Guess Vol Flow Rate	4.1894E-07	m^3/s	sum(F)	0.395285	
Volumetric Flow Rate	4.183E-07	m^3/s	F length	0.045328	
			F expand	0.034822	
			F contract	0.019152	
			F bend	0.295984	
			Length	0.04	m
Pump Dimensions					
Diameter	0.02540005	m			
Height	0.0015875	m			
Volume	8.044E-07	m^3/s			
Dead vol	0.63	fraction			
Volume	2.9763E-07	m^3/s			

Width	Fill Time (s)				
1	0.71042955				
0.9	0.78936617				
0.8	0.88803694				
0.7	1.01489936				
0.6	1.18404925				
0.5	1.4208591				
1.1	0.64584505				
1.2	0.59202463				
1.3	0.54648427				
1.4	0.50744968				
1.5	0.4736197				

Appendix 7: *MSDS*

The following is a list of URLs to find current MSDS information for chemicals of interest in this design. Copies of these each MSDS is also provided in this appendix.

Citric Acid MSDS
http://www.jtbaker.com/msds/englishhtml/c4730.htm

Sodium Citrate MSDS
http://www.jtbaker.com/msds/englishhtml/s3386.htm

Hydroquinone MSDS
http://www.jtbaker.com/msds/englishhtml/h4108.htm

Silver Nitrate MSDS
http://www.jtbaker.com/msds/englishhtml/S2282.htm

MSDS Material Safety Data Sheet

From: Mallinckrodt Baker, Inc.
222 Red School Lane
Phillipsburg, NJ 08865

Mallinckrodt CHEMICALS J.T.Baker

24 Hour Emergency Telephone: 908-859-2151
CHEMTREC: 1-800-424-9300

National Response in Canada
CANUTEC: 613-996-6666

Outside U.S. and Canada
Chemtrec: 703-527-3887

NOTE: CHEMTREC, CANUTEC and National Response Center emergency numbers to be used only in the event of chemical emergencies involving a spill, leak, fire, exposure or accident involving chemicals.

All non-emergency questions should be directed to Customer Service (1-800-582-2537) for assistance.

CITRIC ACID

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1. Product Identification

Synonyms: 2-Hydroxy-1,2,3-propanetricarboxylic acid, monohydrate
CAS No.: 77-92-9 (Anhydrous) 5949-29-1 (Monohydrate)
Molecular Weight: 210.14
Chemical Formula: H3C6H5O7.H2O
Product Codes:
J.T. Baker: 0110, 0115, 0116, 0118, 0119, 0120
Mallinckrodt: 0616, 0627, 7788

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2. Composition/Information on Ingredients

Ingredient	CAS No	Percent	Hazardous
Citric Acid	77-92-9	99 - 100%	Yes

3. Hazards Identification

Emergency Overview

WARNING! CAUSES SEVERE EYE IRRITATION. CAUSES IRRITATION TO SKIN AND RESPIRATORY TRACT.

SAF-T-DATA(tm) Ratings (Provided here for your convenience)

Health Rating: 1 - Slight
Flammability Rating: 1 - Slight
Reactivity Rating: 2 - Moderate
Contact Rating: 3 - Severe
Lab Protective Equip: GOGGLES & SHIELD; LAB COAT & APRON; VENT HOOD; PROPER GLOVES
Storage Color Code: Green (General Storage)

Potential Health Effects

Inhalation:
Causes irritation to the respiratory tract. Symptoms may include coughing, shortness of breath.
Ingestion:
Causes irritation to the gastrointestinal tract. Symptoms may include nausea, vomiting and diarrhea. Extremely large oral dosages may produce gastrointestinal disturbances. Calcium deficiency in blood may result in severe cases of ingestion.
Skin Contact:
Causes irritation to skin. Symptoms include redness, itching, and pain.
Eye Contact:
Highly irritating; may also be abrasive.
Chronic Exposure:
Chronic or heavy acute ingestion may cause tooth enamel erosion.
Aggravation of Pre-existing Conditions:
No adverse health effects expected.

4. First Aid Measures

Inhalation:
Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.
Ingestion:
Induce vomiting immediately as directed by medical personnel. Never give anything by mouth to an unconscious person.
Skin Contact:
Immediately flush skin with plenty of water for at least 15 minutes. Remove contaminated clothing and shoes. Get medical attention. Wash clothing before reuse. Thoroughly clean shoes before reuse.
Eye Contact:
Immediately flush eyes with plenty of water for at least 15 minutes, lifting lower and upper eyelids occasionally. Get medical attention immediately.

5. Fire Fighting Measures

Fire:
Autoignition temperature: 1011C (1852F)
As with most organic solids, fire is possible at elevated temperatures or by contact with an ignition source.
Explosion:
Fine dust dispersed in air in sufficient concentrations, and in the presence of an ignition source is a potential dust explosion hazard.
Fire Extinguishing Media:
Water spray, dry chemical, alcohol foam, or carbon dioxide.
Special Information:
In the event of a fire, wear full protective clothing and NIOSH-approved self-contained breathing apparatus with full facepiece operated in the pressure demand or other positive pressure mode.

6. Accidental Release Measures

Ventilate area of leak or spill. Wear appropriate personal protective equipment as specified in Section 8. Spills: Sweep up and containerize for reclamation or disposal. Vacuuming or wet sweeping may be used to avoid dust dispersal.

7. Handling and Storage

Keep in a tightly closed container, stored in a cool, dry, ventilated area. Protect against physical damage. Containers of this material may be hazardous when empty since they retain product residues (dust, solids); observe all warnings and precautions listed for the product.

8. Exposure Controls/Personal Protection

Airborne Exposure Limits:
None established.
Ventilation System:
A system of local and/or general exhaust is recommended to keep employee exposures as low as possible. Local exhaust ventilation is generally preferred because it can control the emissions of the contaminant at its source, preventing dispersion of it into the general work area. Please refer to the ACGIH document, *Industrial Ventilation, A Manual of Recommended Practices*, most recent edition, for details.
Personal Respirators (NIOSH Approved):
For conditions of use where exposure to dust or mist is apparent and engineering controls are not feasible, a particulate respirator (NIOSH type N95 or better filters) may be worn. If oil particles (e.g. lubricants, cutting fluids, glycerine, etc.) are present, use a NIOSH type R or P filter. For emergencies or instances where the exposure levels are not known, use a full-face positive-pressure, air-supplied respirator. WARNING: Air-purifying respirators do not protect workers in oxygen-deficient atmospheres.
Skin Protection:
Wear impervious protective clothing, including boots, gloves, lab coat, apron or coveralls, as appropriate, to prevent skin contact.
Eye Protection:
Use chemical safety goggles and/or full face shield where dusting or splashing of solutions is possible. Maintain eye wash fountain and quick-drench facilities in work area.

9. Physical and Chemical Properties

Appearance:
White granules.
Odor:
Odorless.
Solubility:
ca. 60 g/100 ml @ 20C (Anhydrous)
Density:

1.542
pH:
2.2 (0.1 N sol)
% Volatiles by volume @ 21C (70F):
0
Boiling Point:
No information found.
Melting Point:
ca. 100C (ca. 212F)
Vapor Density (Air=1):
No information found.
Vapor Pressure (mm Hg):
No information found.
Evaporation Rate (BuAc=1):
No information found.

10. Stability and Reactivity

Stability:
Stable under ordinary conditions of use and storage.
Hazardous Decomposition Products:
Carbon dioxide and carbon monoxide may form when heated to decomposition.
Hazardous Polymerization:
Will not occur.
Incompatibilities:
Metal nitrates (potentially explosive reaction), alkali carbonates and bicarbonates, potassium tartrate. Will corrode copper, zinc, aluminum and their alloys.
Conditions to Avoid:
Heat, flames, ignition sources and incompatibles.

11. Toxicological Information

Oral rat LD50: 3 g/kg; irritation skin rabbit: 500 mg/24H mild; eye rabbit: 750 ug/24H severe.

--------\Cancer Lists\----------

Ingredient	---NTP Carcinogen--- Known	Anticipated	IARC Category
Citric Acid (77-92-9)	No	No	None

12. Ecological Information

Environmental Fate:
No information found.
Environmental Toxicity:
No information found.

13. Disposal Considerations

Whatever cannot be saved for recovery or recycling should be managed in an appropriate and approved waste disposal facility. Processing, use or contamination of this product may change the waste management options. State and local disposal regulations may differ from federal disposal regulations. Dispose of container and unused contents in accordance with federal, state and local requirements.

14. Transport Information

Not regulated.

15. Regulatory Information

--------\Chemical Inventory Status - Part 1\--------

Ingredient	TSCA	EC	Japan	Australia
Citric Acid (77-92-9)	Yes	Yes	Yes	Yes

--------\Chemical Inventory Status - Part 2\--------

Ingredient	Korea	--Canada-- DSL	NDSL	Phil.
Citric Acid (77-92-9)	Yes	Yes	No	Yes

--------\Federal, State & International Regulations - Part 1\--------

Ingredient	-SARA 302- RQ	TPQ	------SARA 313---- List	Chemical Catg.
Citric Acid (77-92-9)	No	No	No	No

```
  --------\Federal, State & International Regulations - Part 2\--------------
--
                                                          -RCRA-    -TSCA-
  Ingredient                                  CERCLA      261.33     8(d)
  ------------------------------------------  ------     ------    ------
  Citric Acid (77-92-9)                       No         No         No


Chemical Weapons Convention:  No     TSCA 12(b):  No     CDTA:  No
SARA 311/312:  Acute: Yes      Chronic: No   Fire: No  Pressure: No
Reactivity: No          (Pure / Solid)
```

Australian Hazchem Code: None allocated.
Poison Schedule: None allocated.
WHMIS:
This MSDS has been prepared according to the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all of the information required by the CPR.

16. Other Information

NFPA Ratings: Health: **2** Flammability: **1** Reactivity: **0**
Label Hazard Warning:
WARNING! CAUSES SEVERE EYE IRRITATION. CAUSES IRRITATION TO SKIN AND RESPIRATORY TRACT.
Label Precautions:
Avoid contact with eyes, skin and clothing.
Avoid breathing dust.
Keep container closed.
Use only with adequate ventilation.
Wash thoroughly after handling.
Label First Aid:
In case of contact, immediately flush eyes or skin with plenty of water for at least 15 minutes. Remove contaminated clothing and shoes. Wash clothing before reuse. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. In all cases, get medical attention.
Product Use:
Laboratory Reagent.
Revision Information:
MSDS Section(s) changed since last revision of document include: 3.
Disclaimer:
****
****
Mallinckrodt Baker, Inc. provides the information contained herein in good

faith but makes no representation as to its comprehensiveness or accuracy. This document is intended only as a guide to the appropriate precautionary handling of the material by a properly trained person using this product. Individuals receiving the information must exercise their independent judgment in determining its appropriateness for a particular purpose. MALLINCKRODT BAKER, INC. MAKES NO REPRESENTATIONS OR WARRANTIES, EITHER EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION ANY WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE WITH RESPECT TO THE INFORMATION SET FORTH HEREIN OR THE PRODUCT TO WHICH THE INFORMATION REFERS. ACCORDINGLY, MALLINCKRODT BAKER, INC. WILL NOT BE RESPONSIBLE FOR DAMAGES RESULTING FROM USE OF OR RELIANCE UPON THIS INFORMATION.

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Prepared by: Environmental Health & Safety
Phone Number: (314) 654-1600 (U.S.A.)

SODIUM CITRATE

1. Product Identification

Synonyms: Citrosodine; trisodium citrate; citric acid, trisodium salt; 2-hydroxy-1,2,3-propanetricarboxylic acid, trisodium salt, dihydrate;sodium citrate dihydrate
CAS No.: 68-04-2 (Anhydrous); 6132-04-3 (Dihydrate)
Molecular Weight: 294.10
Chemical Formula: HOC (COONa) (CH2COONa)2.2H2O
Product Codes:
J.T. Baker: 3646, 3647, 3648, 3649, 3650, 4093
Mallinckrodt: 0634, 0734, 0754, 7773

2. Composition/Information on Ingredients

Ingredient	CAS No	Percent	Hazardous
Sodium Citrate	68-04-2	90 - 100%	Yes

3. Hazards Identification

Emergency Overview

CAUTION! MAY CAUSE IRRITATION TO SKIN, EYES, AND RESPIRATORY TRACT.

SAF-T-DATA(tm) Ratings (Provided here for your convenience)

Health Rating: 1 - Slight
Flammability Rating: 1 - Slight
Reactivity Rating: 0 - None
Contact Rating: 1 - Slight
Lab Protective Equip: GOGGLES; LAB COAT; PROPER GLOVES
Storage Color Code: Green (General Storage)

Potential Health Effects

Inhalation:
Inhalation of large amounts of dust may cause irritation to the respiratory tract.
Ingestion:
Extremely large oral dosages may produce gastrointestinal disturbances.
Skin Contact:
Possible irritation on prolonged contact with moist or sensitive areas of the skin.
Eye Contact:
No adverse effects expected but dust may cause mechanical irritation.
Chronic Exposure:
No information found.
Aggravation of Pre-existing Conditions:
No information found.

4. First Aid Measures

Inhalation:
Remove to fresh air. Get medical attention for any breathing difficulty.
Ingestion:
Give several glasses of water to drink to dilute. If large amounts were swallowed, get medical advice.
Skin Contact:
Wash exposed area with soap and water. Get medical advice if irritation develops.
Eye Contact:
Wash thoroughly with running water. Get medical advice if irritation develops.

5. Fire Fighting Measures

Fire:
As with most organic solids, fire is possible at elevated temperatures or by contact with an ignition source.
Explosion:
Fine dust dispersed in air in sufficient concentrations, and in the presence of an ignition source is a potential dust explosion hazard.
Fire Extinguishing Media:
Water spray, dry chemical, alcohol foam, or carbon dioxide.
Special Information:
In the event of a fire, wear full protective clothing and NIOSH-approved self-contained breathing apparatus with full facepiece operated in the pressure demand or other positive pressure mode.

6. Accidental Release Measures

Remove all sources of ignition. Ventilate area of leak or spill. Wear appropriate personal protective equipment as specified in Section 8. Spills: Clean up spills in a manner that does not disperse dust into the air. Use non-sparking tools and equipment. Reduce airborne dust and prevent scattering by moistening with water. Pick up spill for recovery or disposal and place in a closed container.

7. Handling and Storage

Keep in a tightly closed container, stored in a cool, dry, ventilated area. Protect against physical damage. Isolate from incompatible substances. Containers of this material may be hazardous when empty since they retain product residues (dust, solids); observe all warnings and precautions listed for the product.

8. Exposure Controls/Personal Protection

Airborne Exposure Limits:
None established.
Ventilation System:
In general, dilution ventilation is a satisfactory health hazard control for this substance. However, if conditions of use create discomfort to the worker, a local exhaust system should be considered.
Personal Respirators (NIOSH Approved):
For conditions of use where exposure to dust or mist is apparent and engineering controls are not feasible, a particulate respirator (NIOSH type N95 or better filters) may be worn. If oil particles (e.g. lubricants, cutting fluids, glycerine, etc.) are present, use a NIOSH type R or P filter. For emergencies or instances where the exposure levels are not known, use a full-face positive-pressure, air-supplied respirator. WARNING: Air-purifying respirators do not protect workers in oxygen-deficient atmospheres.
Skin Protection:
Wear protective gloves and clean body-covering clothing.
Eye Protection:
Use chemical safety goggles. Maintain eye wash fountain and quick-drench facilities in work area.

9. Physical and Chemical Properties

Appearance:
White crystals.
Odor:

Odorless.
Solubility:
72 g/100 g of water.
Density:
ca. 1.7
pH:
ca. 8.0
% Volatiles by volume @ 21C (70F):
0
Boiling Point:
Decomposes at red heat.
Melting Point:
150C (302F)
Vapor Density (Air=1):
No information found.
Vapor Pressure (mm Hg):
No information found.
Evaporation Rate (BuAc=1):
Not applicable.

10. Stability and Reactivity

Stability:
Stable under ordinary conditions of use and storage.
Hazardous Decomposition Products:
Carbon dioxide and carbon monoxide may form when heated to decomposition.
Hazardous Polymerization:
Will not occur.
Incompatibilities:
Strong oxidizers.
Conditions to Avoid:
Heat, flame, ignition sources, dusting and incompatibles.

11. Toxicological Information

No LD50/LC50 information found relating to normal routes of occupational exposure.

```
  --------\Cancer Lists\----------------------------------------------------
-
                                        ---NTP Carcinogen---
  Ingredient                            Known    Anticipated    IARC
Category
```

Sodium Citrate (68-04-2)	No	No	None

12. Ecological Information

Environmental Fate:
No information found.
Environmental Toxicity:
No information found.

13. Disposal Considerations

Whatever cannot be saved for recovery or recycling should be managed in an appropriate and approved waste disposal facility. Processing, use or contamination of this product may change the waste management options. State and local disposal regulations may differ from federal disposal regulations. Dispose of container and unused contents in accordance with federal, state and local requirements.

14. Transport Information

Not regulated.

15. Regulatory Information

\-------\Chemical Inventory Status - Part 1\--------

Ingredient	TSCA	EC	Japan	Australia
Sodium Citrate (68-04-2)	Yes	Yes	Yes	Yes

\-------\Chemical Inventory Status - Part 2\--------

Ingredient	Korea	--Canada-- DSL	NDSL	Phil.
Sodium Citrate (68-04-2)	Yes	Yes	No	Yes

\-------\Federal, State & International Regulations - Part 1\--------

Ingredient	-SARA 302- RQ	TPQ	------SARA 313---- List	Chemical Catg.

```
  ---------------------------------------  ---   -----   ----  ------------
--
  Sodium Citrate (68-04-2)                 No    No      No         No

  --------\Federal, State & International Regulations - Part 2\--------------
--
                                                          -RCRA-    -TSCA-
  Ingredient                               CERCLA     261.33     8(d)
  ---------------------------------------  ------     ------    ------
  Sodium Citrate (68-04-2)                 No         No         No


Chemical Weapons Convention:  No     TSCA 12(b):  No     CDTA:  No
SARA 311/312:  Acute: No       Chronic: No   Fire: No  Pressure: No
Reactivity: No          (Pure / Solid)
```

Australian Hazchem Code: None allocated.
Poison Schedule: None allocated.
WHMIS:
This MSDS has been prepared according to the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all of the information required by the CPR.

16. Other Information

NFPA Ratings: Health: **1** Flammability: **0** Reactivity: **0**
Label Hazard Warning:
CAUTION! MAY CAUSE IRRITATION TO SKIN, EYES, AND RESPIRATORY TRACT.
Label Precautions:
Avoid contact with eyes, skin and clothing.
Avoid breathing dust.
Use with adequate ventilation.
Keep container closed.
Wash thoroughly after handling.
Label First Aid:
In case of contact, immediately flush eyes or skin with plenty of water for at least 15 minutes. Get medical attention if irritation develops or persists. If inhaled, remove to fresh air. Get medical attention for any breathing difficulty.
Product Use:
Laboratory Reagent.
Revision Information:
MSDS Section(s) changed since last revision of document include: 8.
Disclaimer:
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Prepared by: Environmental Health & Safety
Phone Number: (314) 654-1600 (U.S.A.)

HYDROQUINONE

1. Product Identification

Synonyms: 1,4-Dihydroxybenzene; p-Dihydroxybenzene; 1,4-Benzenediol; Dihydroxybenzene; Quinol
CAS No.: 123-31-9
Molecular Weight: 110.11
Chemical Formula: C6H4(OH)2
Product Codes:
J.T. Baker: N449
Mallinckrodt: 5256

2. Composition/Information on Ingredients

Ingredient	CAS No	Percent	Hazardous
Hydroquinone	123-31-9	90 - 100%	Yes

3. Hazards Identification

Emergency Overview

DANGER! MAY BE FATAL IF SWALLOWED. AFFECTS CENTRAL NERVOUS SYSTEM. CAUSES SEVERE SKIN AND EYE IRRITATION. HARMFUL IF INHALED. MAY CAUSE ALLERGIC SKIN REACTION. CAUSES IRRITATION TO RESPIRATORY TRACT.

J.T. Baker SAF-T-DATA(tm) Ratings (Provided here for your convenience)

Health Rating: 2 - Moderate
Flammability Rating: 1 - Slight
Reactivity Rating: 1 - Slight
Contact Rating: 2 - Moderate
Lab Protective Equip: GOGGLES; LAB COAT; VENT HOOD; PROPER GLOVES
Storage Color Code: Orange (General Storage)

Potential Health Effects

Inhalation:
Causes irritation to the respiratory tract. Symptoms may include coughing, shortness of breath. Systemic effects have not been proven by this route.
Ingestion:
Highly Toxic. May cause hyperactivity, stupor, fall in blood pressure, hyperpnea, abdominal pain, diarrhea, intense thirst, sweating, tinnitis, nausea, dizziness, a sensation of suffocation, an increased rate of respiration, vomiting, pallor, muscular twitching, headache, cyanosis, delirium, and collapse (from respiratory failure). Estimated lethal dose lies between 5 to 12 grams (usually because of respiratory failure from methemoglobin formation which leaves the blood unable to carry oxygen). May cause green to brownish-green urine.
Skin Contact:
Causes severe irritation, redness and pain. Alkaline solutions can cause skin sensitization.
Eye Contact:
Causes severe irritation and possible corneal ulceration.
Chronic Exposure:
Repeated exposure to vapor or dust (typically 10 to 30 mg/m3) for > 5 years has caused brownish staining of the conjunctiva which may be followed by changes to the cornea leading to loss of visual acuity. Repeated exposure may also cause skin effects.
Aggravation of Pre-existing Conditions:
Persons with pre-existing skin or eye disorders or impaired respiratory function may be more susceptible to the effects of this substance.

4. First Aid Measures

Inhalation:
Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.
Ingestion:
If swallowed, DO NOT INDUCE VOMITING. Give large quantities of water. Never give anything by mouth to an unconscious person. Get medical attention immediately.
Skin Contact:
Wipe off excess material from skin then immediately flush skin with plenty of soap and water for at least 15 minutes. Remove contaminated clothing and shoes. Get medical attention. Wash clothing before reuse. Thoroughly clean shoes before reuse.
Eye Contact:

Immediately flush eyes with plenty of water for at least 15 minutes, lifting upper and lower eyelids occasionally. Get medical attention.

5. Fire Fighting Measures

Fire:
Flash point: 165C (329F) CC
Autoignition temperature: 516C (961F)
May pose a fire hazard when exposed to heat, flame, or oxidizing agents.
Explosion:
Fine dust dispersed in air in sufficient concentrations, and in the presence of an ignition source is a potential dust explosion hazard.
Fire Extinguishing Media:
Dry chemical, alcohol foam or carbon dioxide. Water or foam may cause frothing.
Special Information:
In the event of a fire, wear full protective clothing and NIOSH-approved self-contained breathing apparatus with full facepiece operated in the pressure demand or other positive pressure mode.

6. Accidental Release Measures

Remove all sources of ignition. Ventilate area of leak or spill. Wear appropriate personal protective equipment as specified in Section 8. Spills: Clean up spills in a manner that does not disperse dust into the air. Use non-sparking tools and equipment. Reduce airborne dust and prevent scattering by moistening with water. Pick up spill for recovery or disposal and place in a closed container. US Regulations (CERCLA) require reporting spills and releases to soil, water and air in excess of reportable quantities. The toll free number for the US Coast Guard National Response Center is (800) 424-8802.

7. Handling and Storage

Keep in a tightly closed container, stored in a cool, dry, ventilated area. Protect against physical damage. Isolate from any source of heat or ignition. Isolate from oxidizing materials. Protect from direct sunlight. Containers of this material may be hazardous when empty since they retain product residues (dust, solids); observe all warnings and precautions listed for the product.

8. Exposure Controls/Personal Protection

Airborne Exposure Limits:
-OSHA Permissible Exposure Limit (PEL):
2 mg/m3 (TWA)

-ACGIH Threshold Limit Value (TLV):
2 mg/m3 (TWA)
Ventilation System:
A system of local and/or general exhaust is recommended to keep employee exposures below the Airborne Exposure Limits. Local exhaust ventilation is generally preferred because it can control the emissions of the contaminant at its source, preventing dispersion of it into the general work area. Please refer to the ACGIH document, *Industrial Ventilation, A Manual of Recommended Practices*, most recent edition, for details.
Personal Respirators (NIOSH Approved):
If the exposure limit is exceeded, and engineering controls are not feasible, a full-face piece respirator with an organic vapor cartridge and particulate filter (NIOSH type N100 filter) may be worn up to 50 times the exposure limit, or the maximum use concentration specified by the appropriate regulatory agency or respirator supplier, whichever is lowest. If oil particles (e.g. lubricants, cutting fluids, glycerine, etc.) are present, use a NIOSH type R or P particulate filter. For emergencies or instances where the exposure levels are not known, use a full-face piece positive-pressure, air-supplied respirator. WARNING: Air-purifying respirators do not protect workers in oxygen-deficient atmospheres. This compound possibly exists in both particulate and vapor phase. A gas/vapor cartridge should be used in addition to the particulate filter (NIOSH type N95 or better filter). If the vapor concentration alone exceeds the exposure limits, use a supplied air respirator, because warning properties are unknown for these compounds. Breathing air quality must meet the requirements of the OSHA respiratory protection standard (29CFR1910.134).
Skin Protection:
Wear impervious protective clothing, including boots, gloves, lab coat, apron or coveralls, as appropriate, to prevent skin contact.
Eye Protection:
Use chemical safety goggles and/or full face shield where dusting or splashing of solutions is possible. Maintain eye wash fountain and quick-drench facilities in work area.

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9. Physical and Chemical Properties

Appearance:
White crystals.
Odor:

Odorless.
Solubility:
7g/100g water @ 25C (77F).
Specific Gravity:
1.33 @ 15C
pH:
No information found.
% Volatiles by volume @ 21C (70F):
0
Boiling Point:
285C (545F)
Melting Point:
170C (338F)
Vapor Density (Air=1):
3.81
Vapor Pressure (mm Hg):
4 @ 150C (302F)
Evaporation Rate (BuAc=1):
No information found.

10. Stability and Reactivity

Stability:
Stable under ordinary conditions of use and storage. Solution becomes brown in air due to oxidation.
Hazardous Decomposition Products:
Quinone and oxides of carbon may be formed when this material is heated to decomposition.
Hazardous Polymerization:
Will not occur.
Incompatibilities:
Sodium hydroxide, strong alkalis, and oxidizers.
Conditions to Avoid:
Heat, flame, ignition sources, incompatibles, light, and air.

11. Toxicological Information

Oral rat LD50: 320 mg/kg; investigated as a tumorigen, mutagen, reproductive effector.

--------\Cancer Lists\----------
-
---NTP Carcinogen---

Ingredient	Known	Anticipated	IARC Category
Hydroquinone (123-31-9)	No	No	3

12. Ecological Information

Environmental Fate:
When released into the soil, this material may biodegrade to a moderate extent. When released into the soil, this material is expected to leach into groundwater. When released into the soil, this material is not expected to evaporate significantly. When released into water, this material is not expected to evaporate significantly. When released into water, this material may biodegrade to a moderate extent. This material has an experimentally-determined bioconcentration factor (BCF) of less than 100. This material is not expected to significantly bioaccumulate. When released into the air, this material may be moderately degraded by reaction with photochemically produced hydroxyl radicals. When released into the air, this material may be removed from the atmosphere to a moderate extent by wet deposition.
Environmental Toxicity:
No information found.

13. Disposal Considerations

Whatever cannot be saved for recovery or recycling should be managed in an appropriate and approved waste disposal facility. Processing, use or contamination of this product may change the waste management options. State and local disposal regulations may differ from federal disposal regulations. Dispose of container and unused contents in accordance with federal, state and local requirements.

14. Transport Information

Domestic (Land, D.O.T.)

Proper Shipping Name: HYDROQUINONE
Hazard Class: 6.1
UN/NA: UN2662
Packing Group: III
Information reported for product/size: 12KG

International (Water, I.M.O.)

Proper Shipping Name: HYDROQUINONE, SOLID
Hazard Class: 6.1
UN/NA: UN2662
Packing Group: III
Information reported for product/size: 12KG

International (Air, I.C.A.O.)

Proper Shipping Name: HYDROQUINONE
Hazard Class: 6.1
UN/NA: UN2662
Packing Group: III
Information reported for product/size: 12KG

15. Regulatory Information

--------\Chemical Inventory Status - Part 1\--------

Ingredient	TSCA	EC	Japan	Australia
Hydroquinone (123-31-9)	Yes	Yes	Yes	Yes

--------\Chemical Inventory Status - Part 2\--------

Ingredient	Korea	--Canada-- DSL	NDSL	Phil.
Hydroquinone (123-31-9)	Yes	Yes	No	Yes

--------\Federal, State & International Regulations - Part 1\--------

Ingredient	-SARA 302- RQ	TPQ	------SARA 313---- List	Chemical Catg.
Hydroquinone (123-31-9)	100	500*	Yes	No

--------\Federal, State & International Regulations - Part 2\--------

Ingredient	CERCLA	-RCRA- 261.33	-TSCA- 8(d)
Hydroquinone (123-31-9)	100	No	No

Chemical Weapons Convention: No TSCA 12(b): No CDTA: Yes
SARA 311/312: Acute: Yes Chronic: Yes Fire: No Pressure: No
Reactivity: No (Pure / Solid)

Australian Hazchem Code: 2Z
Poison Schedule: None allocated.
WHMIS:
This MSDS has been prepared according to the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all of the information required by the CPR.

16. Other Information

NFPA Ratings: Health: **3** Flammability: **1** Reactivity: **0**
Label Hazard Warning:
DANGER! MAY BE FATAL IF SWALLOWED. AFFECTS CENTRAL NERVOUS SYSTEM. CAUSES SEVERE SKIN AND EYE IRRITATION. HARMFUL IF INHALED. MAY CAUSE ALLERGIC SKIN REACTION. CAUSES IRRITATION TO RESPIRATORY TRACT.
Label Precautions:
Avoid contact with eyes, skin and clothing.
Avoid breathing dust.
Keep container closed.
Use only with adequate ventilation.
Wash thoroughly after handling.
Label First Aid:
If swallowed, DO NOT INDUCE VOMITING. Give large quantities of water. Never give anything by mouth to an unconscious person. Get medical attention immediately. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention. In case of contact, wipe off excess material from skin then immediately flush eyes or skin with plenty of water for at least 15 minutes. Remove contaminated clothing and shoes. Wash clothing before reuse. Get medical attention.
Product Use:
Laboratory Reagent.
Revision Information:
MSDS Section(s) changed since last revision of document include: 8.
Disclaimer:
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Prepared by: Environmental Health & Safety
Phone Number: (314) 654-1600 (U.S.A.)

SILVER NITRATE

1. Product Identification

Synonyms: Lunar caustic; silver nitrate toughened; Nitric Acid, Silver (I) Salt
CAS No.: 7761-88-8
Molecular Weight: 169.87
Chemical Formula: AgNO3
Product Codes:
J.T. Baker: 3426, 3429
Mallinckrodt: 2160, 2169, 7992

2. Composition/Information on Ingredients

Ingredient	CAS No	Percent	Hazardous
Silver Nitrate	7761-88-8	99 - 100%	Yes

3. Hazards Identification

Emergency Overview

POISON! DANGER! CORROSIVE. CAUSES BURNS TO ANY AREA OF CONTACT. MAY BE FATAL IF SWALLOWED. HARMFUL IF INHALED. STRONG OXIDIZER. CONTACT WITH OTHER MATERIAL MAY CAUSE FIRE.

J.T. Baker SAF-T-DATA(tm) Ratings (Provided here for your convenience)

Health Rating: 3 - Severe (Poison)
Flammability Rating: 0 - None
Reactivity Rating: 3 - Severe (Oxidizer)
Contact Rating: 3 - Severe (Corrosive)
Lab Protective Equip: GOGGLES; LAB COAT; PROPER GLOVES
Storage Color Code: Yellow (Reactive)

Potential Health Effects

Inhalation:
Extremely destructive to tissues of the mucous membranes and upper respiratory tract. Symptoms may include burning sensation, coughing, wheezing, laryngitis, shortness of breath, headache, nausea and vomiting. May be absorbed into the body following inhalation with symptoms paralleling those from ingestion exposure. Dust deposits in the lungs may resemble a form of pneumoconiosis.
Ingestion:
Corrosive. Swallowing can cause severe burns of the mouth, throat, and stomach. Can cause sore throat, vomiting, diarrhea. Poison. Symptoms include pain and burning in the mouth, blackening of the skin and mucous membranes, throat, and abdomen, salivation, vomiting of black material, diarrhea, collapse, shock, coma and death.
Skin Contact:
Corrosive. Symptoms of redness, pain, and severe burn can occur.
Eye Contact:
Corrosive. Can cause blurred vision, redness, pain, severe tissue burns and eye damage.
Chronic Exposure:
Repeated application or ingestion causes a permanent bluish discoloration of the skin, conjunctiva, and mucous membranes. Repeated inhalation may cause lung disease.
Aggravation of Pre-existing Conditions:
Persons with pre-existing skin disorders or eye problems or impaired respiratory function may be more susceptible to the effects of the substance.

4. First Aid Measures

Inhalation:
Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention immediately.
Ingestion:
If swallowed, DO NOT INDUCE VOMITING. Give large quantities of water. Never give anything by mouth to an unconscious person. Get medical attention immediately.
Skin Contact:
Immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Get medical attention immediately. Wash clothing before reuse. Thoroughly clean shoes before reuse.
Eye Contact:
Immediately flush eyes with plenty of water for at least 15 minutes, lifting lower and upper eyelids occasionally. Get medical attention immediately.

5. Fire Fighting Measures

Fire:
This oxidizing material can increase the flammability of adjacent combustible materials.
Explosion:
Many reactions may cause explosion. Reacts with ammonia to form compounds that are sensitive to mechanical shock.
Fire Extinguishing Media:
Use flooding amounts of water. Do not use dry chemical, carbon dioxide or Halon. Do not allow water runoff to enter sewers or waterways.
Special Information:
In the event of a fire, wear full protective clothing and NIOSH-approved self-contained breathing apparatus with full facepiece operated in the pressure demand or other positive pressure mode.

6. Accidental Release Measures

Remove all sources of ignition. Ventilate area of leak or spill. Wear appropriate personal protective equipment as specified in Section 8. Spills: Clean up spills in a manner that does not disperse dust into the air. Use non-sparking tools and equipment. Reduce airborne dust and prevent scattering by moistening with water. Pick up spill for recovery or disposal and place in a closed container. US Regulations (CERCLA) require reporting spills and releases to soil, water and air in excess of reportable quantities. The toll free number for the US Coast Guard National Response Center is (800) 424-8802.

7. Handling and Storage

Keep in a tightly closed container, stored in a cool, dry, ventilated area. Protect against physical damage and moisture. Isolate from any source of heat or ignition. Avoid storage on wood floors. Separate from incompatibles, combustibles, organic or other readily oxidizable materials. Protect from light. Containers of this material may be hazardous when empty since they retain product residues (dust, solids); observe all warnings and precautions listed for the product.

8. Exposure Controls/Personal Protection

Airborne Exposure Limits:
-OSHA Permissible Exposure Limit (PEL):
0.01 mg/m3 (TWA) for silver metal dust and fume as Ag

-ACGIH Threshold Limit Value (TLV):
0.01 mg /m3 (TWA) for soluble silver compounds as Ag

Ventilation System:

A system of local and/or general exhaust is recommended to keep employee exposures below the Airborne Exposure Limits. Local exhaust ventilation is generally preferred because it can control the emissions of the contaminant at its source, preventing dispersion of it into the general work area. Please refer to the ACGIH document, *Industrial Ventilation, A Manual of Recommended Practices*, most recent edition, for details.

Personal Respirators (NIOSH Approved):

If the exposure limit is exceeded and engineering controls are not feasible, a full facepiece particulate respirator (NIOSH type N100 filters) may be worn for up to 50 times the exposure limit or the maximum use concentration specified by the appropriate regulatory agency or respirator supplier, whichever is lowest. If oil particles (e.g. lubricants, cutting fluids. glycerine, etc.) are present, use a NIOSH type R or P filter. For emergencies or instances where the exposure levels are not known, use a full-facepiece positive-pressure, air-supplied respirator.
WARNING: Air-purifying respirators do not protect workers in oxygen-deficient atmospheres.

Skin Protection:

Wear impervious protective clothing, including boots, gloves, lab coat, apron or coveralls, as appropriate, to prevent skin contact.

Eye Protection:

Use chemical safety goggles and/or full face shield where dusting or splashing of solutions is possible. Maintain eye wash fountain and quick-drench facilities in work area.

9. Physical and Chemical Properties

Appearance:
Transparent, colorless crystals.

Odor:
Odorless.

Solubility:
219g/100g water @ 20C (68F).

Specific Gravity:
4.352

pH:
ca. 6 (neutral to litmus)

% Volatiles by volume @ 21C (70F):
0

Boiling Point:
444C (831F) Decomposes.

Melting Point:
212C (414F)
Vapor Density (Air=1):
4.4
Vapor Pressure (mm Hg):
Very low.
Evaporation Rate (BuAc=1):
No information found.

10. Stability and Reactivity

Stability:
Stable at room temperature in sealed containers. Discolors on exposure to light.
Hazardous Decomposition Products:
Oxides of nitrogen.
Hazardous Polymerization:
Will not occur.
Incompatibilities:
Ammonia, alkalis, antimony salts, arsenites, bromides, carbonates, chlorides, iodides, thiocyanates, ferrous salts, phosphates, tannic acid and tartrates.
Conditions to Avoid:
Heat, flame, sources of ignition, light and incompatibles.

11. Toxicological Information

Oral rat LD50: 1173 mg/kg. Irritation data, rabbit, std Draize: eye= 1 mg, severe. Investigated as a tumorigen, mutagen, reproductive effector.

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  --------\Cancer Lists\-----------------------------------------------------
-
                                       ---NTP Carcinogen---
  Ingredient                           Known    Anticipated    IARC
Category
  ----------------------------------   -----    -----------    ------------
-
  Silver Nitrate (7761-88-8)            No          No            None
```

12. Ecological Information

Environmental Fate:
No information found.
Environmental Toxicity:
No information found.

13. Disposal Considerations

Whatever cannot be saved for recovery or recycling should be handled as hazardous waste and sent to a RCRA approved waste facility. Processing, use or contamination of this product may change the waste management options. State and local disposal regulations may differ from federal disposal regulations. Dispose of container and unused contents in accordance with federal, state and local requirements.

14. Transport Information

Domestic (Land, D.O.T.)

Proper Shipping Name: RQ, SILVER NITRATE
Hazard Class: 5.1
UN/NA: UN1493
Packing Group: II
Information reported for product/size: 80OZ

International (Water, I.M.O.)

Proper Shipping Name: SILVER NITRATE
Hazard Class: 5.1
UN/NA: UN1493
Packing Group: II
Information reported for product/size: 80OZ

15. Regulatory Information

--------\Chemical Inventory Status - Part 1\--------

Ingredient	TSCA	EC	Japan	Australia
Silver Nitrate (7761-88-8)	Yes	Yes	Yes	Yes

--------\Chemical Inventory Status - Part 2\--------

Ingredient	Korea	--Canada-- DSL	NDSL	Phil.
Silver Nitrate (7761-88-8)	Yes	Yes	No	Yes

--------\Federal, State & International Regulations - Part 1\--------

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                                          -SARA 302-    ------SARA 313----
--
  Ingredient                              RQ    TPQ     List  Chemical
Catg.
  ----------------------------------------  ---   -----   ----  ------------
--
  Silver Nitrate (7761-88-8)              No    No      No    Sliver cmpd/

  --------\Federal, State & International Regulations - Part 2\--------------
--
                                                          -RCRA-    -TSCA-
  Ingredient                              CERCLA     261.33     8(d)
  ----------------------------------------  ------     ------    ------
  Silver Nitrate (7761-88-8)              1          No         No


Chemical Weapons Convention:  No     TSCA 12(b):  No     CDTA:  No
SARA 311/312:  Acute: Yes      Chronic: Yes  Fire: No  Pressure: No
Reactivity: Yes         (Pure / Solid)
```

Australian Hazchem Code: 2X
Poison Schedule: S6
WHMIS:
This MSDS has been prepared according to the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all of the information required by the CPR.

16. Other Information

NFPA Ratings: Health: **4** Flammability: **0** Reactivity: **0** Other: **Oxidizer**
Label Hazard Warning:
POISON! DANGER! CORROSIVE. CAUSES BURNS TO ANY AREA OF CONTACT. MAY BE FATAL IF SWALLOWED. HARMFUL IF INHALED. STRONG OXIDIZER. CONTACT WITH OTHER MATERIAL MAY CAUSE FIRE.
Label Precautions:
Keep from contact with clothing and other combustible materials.
Do not get in eyes, on skin, or on clothing.
Do not breathe dust.
Keep container closed.
Use only with adequate ventilation.
Wash thoroughly after handling.
Store in a tightly closed container.
Do not store near combustible materials.
Label First Aid:
If swallowed, DO NOT INDUCE VOMITING. Give large quantities of water.

Never give anything by mouth to an unconscious person. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. In case of contact, immediately flush eyes or skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. In all cases get medical attention immediately.

Product Use:

Laboratory Reagent.

Revision Information:

No Changes.

Disclaimer:

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Symbol Nomenclature

t	Time
v	Velocity
τ_{yx}	Shear stress
μ	Viscosity
ρ	Density
$\vec{v}$	Velocity vector
$\vec{v}\vec{v}$	Velocity tensor
p	Pressure
$\vec{\vec{\tau}}$	Shear stress tensor
$\vec{g}$	Gravitational constant
Re	Reynolds number
D	Diameter
$\langle v_x \rangle$	Average velocity
K_{tot}	Kinetic energy
Φ	Potential energy
$\hat{\Phi}$	Potential energy per mass
w	Mass
W_m	Work done by the surroundings
E_c	Compression term
E_v	Energy loss due to friction
j_{Ax}	Mass flux
D_{AB}	Diffusivity constant
ω_A	Mass fraction
$\vec{u}_A$	Particle instantaneous velocity
m	Particle mass
ζ	Friction coefficient of the Stokes' law drag force
R_A	Radius
$\vec{F}(t)$	Varying force term
T	Temperature
W	Probability function
K	Boltzmann's constant
r	Position

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