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Novel Low-Cost Smartphone Enabled Contaminant Detection Device

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# Table of Contents

Authorship .......................................................................................................................... 1  
Project Report .................................................................................................................. 1  
Project Management ........................................................................................................ 1  
Acknowledgements ........................................................................................................... 2  
Abstract ............................................................................................................................ 3  
Chapter 1: Introduction – Significance of Cleaning Assessment ........................................ 4  
Chapter 2: Literature Review ............................................................................................ 7  
  Chapter 2.1 – Importance of Ambulatory Transport ....................................................... 7  
    2.1.1 – Overall Prevalence .............................................................................................. 7  
    2.1.2 – Critical Conditions Requiring Transport ............................................................ 9  
    2.1.3 – Variance by Demographics ................................................................................ 9  
  Chapter 2.2 – Contaminants .......................................................................................... 12  
    2.2.1 – Drug Resistant Pathogens ............................................................................... 12  
    2.2.2 – Equipment classification corresponding and bacterial contamination risk ......... 13  
    2.2.3 – Areas of concern within the ambulance ............................................................. 18  
    2.2.4 – MRSA prevalence in ambulances ..................................................................... 18  
  Chapter 2.3 – Ambulance Cleaning Methods .................................................................. 19  
    2.3.1 – Ambulance Protocol ....................................................................................... 20  
    2.3.2 – State of the art protocol ................................................................................... 21  
  Chapter 2.4 – Comparison of Risk: Ambulances vs. Hospitals ....................................... 22  
    2.4.1 – Risk Evaluation ............................................................................................... 23  
    2.4.2 – Variances in Protocol ...................................................................................... 27  
    2.4.3 – Other Industries Worth Considering ................................................................. 28  
  Chapter 2.5 – Biological Contaminant Monitoring ....................................................... 28  
    2.5.1 – Rapid Antigen Detection Test ......................................................................... 28  
    2.5.2 – Airborne Bacteria Monitoring Systems ............................................................. 31  
    2.5.3 – ATP Monitoring systems ................................................................................. 32
Chapter 3: Project Strategy ........................................................................................................... 37
  3.1 – Client Statement & General Framework .............................................................................. 37
     3.1.2 – Objectives .................................................................................................................. 38
     3.1.3 – Constraints ............................................................................................................... 41
  3.2 – Project Approach ............................................................................................................. 43
     3.2.1 – Technical Approach ............................................................................................... 44
     3.2.2 – Management Approach ......................................................................................... 49
     3.2.3 – Financial Approach ............................................................................................... 51
Chapter 4: Alternative Designs .................................................................................................. 54
  4.1 Needs Analysis .................................................................................................................... 54
  4.2 Design Process ................................................................................................................... 54
     4.2.1 Device Functions ....................................................................................................... 54
     4.2.2 Device Specifications ................................................................................................. 61
Chapter 5: Final Design ............................................................................................................ 64
  5.1 Proposed Housing Design .................................................................................................. 64
  5.2 Photodiode Circuit ............................................................................................................. 66
  5.3 Data Collection/Processing ............................................................................................... 67
  5.4 Application/Interface Design ............................................................................................ 68
     5.4.1 Android™ Application ............................................................................................. 68
     5.4.2 Cloud Application .................................................................................................... 72
Chapter 6: Design Verification .................................................................................................. 74
  6.1 Materials and Methods ...................................................................................................... 74
  6.2 Results ................................................................................................................................ 75
     6.2.1 Variable Output LED ................................................................................................. 75
     6.2.2 Biological Validation ................................................................................................. 80
     6.2.3 Smartphone Application ........................................................................................... 81
     6.2.4 Cloud Application .................................................................................................... 87
List of Figures

Figure 1: Rapid Antigen Detection Test lateral flow diagram ..........................................................29

Figure 2: Objectives Tree ..................................................................................................................38

Figure 3: Work Breakdown Structure ................................................................................................49

Figure 4: Gantt Chart .........................................................................................................................50

Figure 5: Initial Cost Analysis ...........................................................................................................51

Figure 6: Original concept sketch for the Smartphone application ..................................................60

Figure 7: Isometric view and cutaway view (left to right) of the sample housing ..................................64

Figure 8: Photodiode circuit used in conjunction with an Arduino™ microcontroller ..........................66

Figure 9: Final Smartphone application design with specifications ..................................................69

Figure 10: Final prototype screens of Smartphone application ..........................................................71

Figure 11: Cloud applications with graphing features for trend analysis ..........................................73

Figure 12: Circuit diagram for the variable LED circuit used in validation testing .............................75

Figure 13: Output voltage for the sensor as a function of light intensity .......................................... 79

Figure 14: RLU output as a function of light intensity ....................................................................... 79

Figure 15: Comparison between the LODs of our design and the Charm NovaLum™ system ........... 80

Figure 16: The Ambulops application was successfully installed on the Genymotion emulator device ..81

Figure 17: Cloud Application: Organization Selection ......................................................................... 82

Figure 18: Smartphone application selection options ......................................................................... 83

Figure 19: Troubleshooting photodiode circuit .................................................................................. 83

Figure 20: Smartphone application: simulated readings .................................................................... 84

Figure 21: A parse.com database for data collection ......................................................................... 85

Figure 22: Smartphone Application: Results dialog .......................................................................... 85

Figure 23: After collection, data is successfully pushed to the Cloud for later retrieval ....................... 86

Figure 24: Cloud application home screen ......................................................................................... 87

Figure 25: Cloud application login screen ......................................................................................... 88
Figure 26: Cloud application: Organization selection.................................................................88
Figure 27: Cloud application: Organization details .................................................................89
Figure 28: Cloud application: Vehicle information can also be viewed or edited.....................90
Figure 29: Cloud application: Editing .......................................................................................90
Figure 30: Cloud application: Vehicle additions ........................................................................91
Figure 31: Budget analysis: SB Photodiode vs. Avalanche Photodiode ........................................97
Figure 32: Enhanced luciferin/luciferase reaction (Haenish et al., 2011) ....................................98
Figure 33: ADK-PPK-AMP reaction. (Satoh et al., 2004) ............................................................99
Figure 34: Highcharts is recommended for future expansion. (Highcharts, 2013) .................101
List of Tables

Table 1: HBV – Commercial Rapid Tests ................................................................. 30
Table 2: HBV Commercial Rapid Tests: Required amounts & Processing Times ...................... 30
Table 3: Alternative Designs: Numerical Evaluation Matrix ....................................................... 47
Table 4: Function-Means analysis of the initial design. ............................................................. 56
Table 5: Function-means analysis of the photodiode circuit component of the team’s design .......... 58
Table 6: Function-means analysis of the microprocessing component of our design .................... 59
Table 7: Function-means analysis of the Smartphone application ................................................... 60
Table 8: Function-means analysis of the Cloud application ........................................................... 61
Table 9: Numerical and calculated values for the variable LED circuit. ........................................... 76
Table 10: Variable LED validation testing results .............................................................................77
Table 11: Dialog Prompts for Cleaning Results ...............................................................................86
List of Equations

Equation 1: Luciferase assay volume optimization ................................................................. 36
Equation 2: Photodiode circuit: Capacitance value calculation ................................................. 66
Equation 3: Resistance across the LED in a variable LED circuit ........................................... 76
Equation 4: Current across the LED in a variable LED circuit ................................................ 76
Equation 5: Power across LED in a variable LED circuit ....................................................... 77
Equation 6: Light intensity emitted by LED in a variable LED circuit ..................................... 77
Equation 7: RLU to ATP conversion ......................................................................................... 80
Authorship

Project Report

Cassandra Clift – This author was responsible for the following chapters: Abstract, Introduction, Literature Review, Project Strategy, Alternative Designs, Final Designs, Design Verification, Discussion, Conclusions and Recommendations.

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Project Management

Cassandra Clift – This author was responsible for the design in relation to the SolidWorks® and 3D printing design of the housing chamber along with the photodiode circuit design, circuit simulations, and prototype assembly.

Alexandria Powell – This author was responsible for the design in relation to the Smartphone application and the Cloud application along with prototype assembly.
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Abstract

Now more than ever, ambulance acquired infections are of increasing concern as the scope of procedures that emergency medical services (EMS) personnel can perform has increased. Cleaning assessment, following cleaning and sterilization protocols, has been proven to increase cleaning efficacy and reduce acquired infections – however due to high initial cost, ambulances cannot afford the devices required to perform this assessment step. To address this concern, the team has created a low cost alternative to Adenosine Tri-Phosphate (ATP) detection systems currently on the market. ATP found in contaminants, when combined with the enzyme luciferase, emits a light with intensity proportional to the ATP concentration, indicating contamination. The team’s novel design uses a photovoltaic photodiode along with a Smartphone application for processing in order to quantify that light emission and provide user friendly and speedy assessment results. The light intensity this instrument is capable of detecting is between 0.2 and 50 mW/cm² at 520nm, a viable range for ATP detection.
Chapter 1: Introduction – Significance of Cleaning Assessment

As the scope of medical procedures continues to increase, ambulance and hospital cleanliness is a top priority. Drug resistant pathogens, such as Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* (E. Coli), and Vancomycin-resistant *Enterococcus faecium* (VRE), can live in an ambulance despite routine daily cleaning with disinfectant agents. In one study examining 25 ambulances near a Level 1 Trauma Center, 28% were found to be positive for MRSA (Semmons et al., 2010). Reducing contamination should be a priority for not only the safety of patients but for safety of personnel as well. Hepatitis B can live in dried blood up to 7 days – even up to four weeks in optimal conditions – putting EMS personnel and patients at risk for infection (Lee et al., 2006). While personnel acknowledge occupational hazards, the last thing a patient would want is to contract an illness while transported in an ambulance.

When used appropriately, the current cleaning solutions and methodologies are highly effective in cleaning all contaminants within the ambulance. Unfortunately, even when correct protocol is followed, significant amounts of contaminants, such as dried blood and bacteria, may remain. The ambulance presents a challenging environment with difficult to clean materials and crevices, which do not facilitate easy cleaning. Furthermore, due to the nature of EMS treatment, personnel are largely confronted with trauma patients with a variety of conditions and injury. Particularly if dealing with a volatile patient, contaminants can become widely spread across the cabin. Even if cleaning and sterilization are conducted properly, particularly in these cases, contaminants may remain if cleaning agents are not working properly on the given materials. A second cleaning may be necessary, given the nature of the surfaces with the ambulance, but there is no means to verify the presence of remnant contaminants, as microscopic blood and pathogen
cannot be seen with the human eye alone. Thus, the use of a biological indicator plays an important role in assessing cleaning efficacy in hospitals to verify effective cleaning and identify breakdowns and failure and protocol. Ambulances do not currently implement such a step due to cost reasons, making it more difficult for EMS personnel to validate successful cleaning. Contaminant monitoring systems are used regularly in biohazardous clean-up as well as the food industry for the purpose of cleaning assessment. Cleaning assessment has been proven as an important tool for verifying protocol adherence and monitoring and evaluating effectiveness of utilized methods and cleaning/disinfecting agents. However, even these devices, at current market value, stretch beyond a price range that most ambulance depots can afford. Furthermore, EMS personnel do not have significant amounts of extra time to allocate to these steps. The personnel, and their ambulances, need to be back in the field and available for dispatch response as soon as possible as trauma cases are severely time sensitive. The goal of this qualifying project, therefore, is to design an inexpensive and easy to use contaminant detection system in order to be used as a quick and easy validation step at the end of a cleaning protocol.

To best address this current need, the team researched current cleaning protocol, contaminants, and evaluation methods of sterilization in hospitals and emergency rooms, and patents for current commercially available rapid detection and ATP hygiene tests. Based on the conducted research and analysis, a final design incorporating ATP detection with luciferin/luciferase was chosen based on its ability to detect a wide range of contaminants. Next, the team brainstormed preliminary designs that meet as many of the client’s objectives as possible to create an ATP detection system that would be inexpensive and time efficient, as well as environmentally friendly and easy to use, thus, creating a device that would be feasible for EMS implementation. Aspects of the design that were taken into consideration were the
mechanics of the method of detection, sensor circuitry, and materials used for the detection device housing. Smartphone and web applications were also designed for data processing to really capitalize on use of this device as a trend assessment tool to capture breakdowns in protocol and identify possible pathogenic outbreaks. After final design and testing, the device was verified using a variable output LED and vials which contained biological samples reacted with a commercially available luciferin/luciferase assay. A cost analysis was also completed in order to ensure viability. Although the limit of detection was not as low as the team had hoped, this novel design provides proof of concept that creation of a device that is accessible to EMS personnel, by remaining low in cost and easy to use, is absolutely feasible.
Chapter 2: Literature Review

Chapter 2.1 – Importance of Ambulatory Transport

In the following section, the importance and prevalence of ambulatory transport in America will be discussed. Also discussed are the types of critical conditions being treated in ambulances today, as well as how the scope of these treatments has increased over time. Finally variance in treatment by demographic will be reviewed.

2.1.1 – Overall Prevalence

As A. K. Leppaniemi discusses, trauma is an ongoing problem worldwide. In the United States, the cost of trauma is about 3.8 times greater than cancer treatment and 6.6 times that of cardiovascular disease (Leppaniemi, 2004). Ambulance use constitutes a significant portion of the cost of health care, and ambulance expenditures were estimated at $7 billion in 1994 (Rucker et al., 1997). Nearly a decade later, this estimation is likely even higher.

While trauma treatment includes resources in house (or in hospital, rather), a major part of trauma treatment occurs in ambulances. Ambulances play a crucial role in time-sensitive traumas and in pre-emergency department preparation. This is a significant number of people that not only expect to receive adequate care but expect to return home safe and healthier than when the ambulance arrived.

Rucker et al. conducted a study evaluating ambulance use across five urban emergency departments in the northeast. The study was carried out between February and May of 1993, allocating one month to each emergency department. Chief complaints, or primary reason for medical attention, of abdominal pain, chest pain, head trauma, shortness of breath, hand lacerations, and vaginal bleeding during the first trimester were examined due to their overall prevalence and higher potential for malpractice claims. If able to respond, patients were given a
questionnaire to complete. These questionnaires collected data on race, ethnicity, language, household income, work status, and insurance status. Medical records were reviewed for all patients, extracting information on age, sex, clinical severity, insurance status, and means of transport (Rucker et al., 1997).

In total, type of transport was identified for over 4900 patients. Of these, about 2300 patients were surveyed for type of transport. In the surveyed group, 480 patients (21%) were transported by ambulance, while about 1300 patients (26%) were transported by ambulance in the entire group of patients included. Form of transportation was unknown for about 4% of patients in each group (Rucker et al., 1997).

Main predictors of ambulance use included type of insurance, level of income, age, level of severity, and type of complaint. Patients were more likely to undergo ambulance transport if they were over 65, had a higher level of severity, and/or if chest pain, abdominal pain, head trauma, and/or shortness of breath constituted chief complaint. Rucker et al. recognized bias present, as the sickest patients were unable to complete the survey. Thus, the higher severity group showed greater correlation to ambulance use with socioeconomic factors; meanwhile, in the lower severity group, ambulance use was associated more with clinical predictors and poor physical function (e.g. limited mobility) (Rucker et al., 1997).

While a number of factors impact the choice for ambulance transport, one sentiment is consistently expressed, “ambulance services play an important part in the continuum of health care” (Clark & FitzGerald, 1999). Thus, one would only expect ambulances to be held to the same level of standards as other healthcare facilities whenever possible.
2.1.2 – Critical Conditions Requiring Transport

Traumas, and other conditions, are often very time-sensitive, thus necessitating transport by ambulance. Cardiac arrest patients, for example, often experience “re-arrest” or some critical event, including hypertension or hypoxemia, after the initial arrest (Hartke et al., 2010). In cases of severe chest or abdominal trauma, time is also of the essence (Gopinath, 2004; Brooks & Simpson, 2009). Trauma deaths, more generally, are divided into immediate, early, and late trauma (Datena, 1998). While there is little hope in preventing immediate trauma deaths (death within seconds to minutes after injury), ambulances play a crucial role in minimizing early trauma deaths (deaths that occur within two to three hours after injury) (Datena, 1998). In these cases especially, ambulatory transport is crucial as self-transport or non-ambulance transport may not be optimal, if available at all.

During transport, a patient may or may not be conscious and may or may not have any form of identification. Therefore, it is incredibly difficult to be aware of preexisting conditions. Both trauma and preexisting conditions lead to an increased vulnerability, as patients’ immune systems are either fully compromised or otherwise occupied by addressing the immediate injury. Particularly in cases of severe trauma, contaminants have a direct means of entering the body if in contact with these tissues, as the skin (the body’s first line of defense) has been breached. Furthermore, abdominal patients are at increased risk for infection and sepsis, requiring close monitoring before and after surgery (Brooks & Simpson, 2009). Given these vulnerabilities, it is necessary to minimize known risks in the ambulatory environment, despite the dynamic basis and limited control on such an environment (Ro et al., 2012).
### 2.1.3 – Variance by Demographics

Between July 1995 and June 1996, Clark and FitzGerald examined 351,000 ambulance transport cases in Queensland. Of the 351,000 cases, 5565 cases were excluded. In Australia, coding is comparable to the priority index in the United States. Code 1 and 2 indicate life-threatening and urgent cases, while Code 3 indicates a non-urgent transport. Clark and Fitzgerald found that, generally, the elderly (age 65+) constituted 12% of the population but accounted for about 1/3 (35.6%) of emergency transport cases and about 2/3 (64.5%) of non-urgent transport (Clark & FitzGerald, 1999).

Overall, Clark and FitzGerald found that use of ambulance increases with age after the first 4 years of life. A 30% increase in urgent care ambulance transport and a 55% increase in non-urgent transport were observed between ages 60-64 to 65-69. Males were also more likely to undergo ambulance transport as they were more likely to postpone seeking medical assistance, thus resulting in increased urgency when medical help was finally requested. Age-related statistics were attributed largely to limited mobility, existence of chronic condition and deteriorating health, and lack of available transport (either by self or family/neighbors) (Clark & FitzGerald, 1999).

Broxterman et al. found single transports often correspond with traumatic events while repeat transports are often product of chronic illnesses that require emergent care more frequently (e.g. asthma, seizures) (Broxterman et al., 2000). As with elderly patients, care may require more regular emergency transport for patients with chronic conditions. In any case, immune systems may be compromised, and, in repeat transports, this immunological
vulnerability becomes subjected to more frequent ambulance exposure and a subsequent higher risk of infection, thus the need to ensure a clean ambulance environment.

Meanwhile, Beillon et al. examine the correspondence of ambulance use in relation to population density. In Sweden, one of three priority levels are assigned to any incoming call based on level of severity. Top priority goes to life-threatening, time-sensitive conditions such as a heart attack. Incoming calls are placed into one of thirty categories for chief complaint (Beillon et al, 2009).

In their study, Beillon et al. examined frequency of ambulance use and correlation of chief complaint in four geographical areas (classified by population density): urban, suburban, rural, and remote rural areas. Two dispatch centers were responsible for all areas in the study. Each of the first three regions housed three ambulance depots, while the remote rural area contained seven. Twenty-two chief complaints were tallied, and likelihood of ambulance use was compared to geographical location and to chief complaint, in order to derive correlations between ambulance use and population density. Beillon et al. found that non-urban patients with chief complaint of chest pain or similar symptoms were more likely to receive transport (90%) than urban patients with similar complaints (~67%). Overuse of ambulance transport was more evident in urban areas (39% of calls) compared to rural areas (16%) (Beillon et al., 2009).

Overall, Beillon et al. concluded that remote areas correspond with more critical transport. Thus, while sheer numbers may be higher in urban areas due to population size, ambulance transport in rural areas may be transporting more critical patient who are thus more vulnerable to infection. Hence, the need for a consistent protocol and assurance of proper decontamination exists for all areas in which ambulance transportation is available.
Chapter 2.2 – Contaminants

In the following section, the types of contaminants will be explored, followed by a discussed on why these contaminants affect overall health and safety of EMS personnel and patients. High risk contaminants, such as drug resistant pathogens, and their prevalence will be reviewed along with equipment classification and areas of concern within the ambulance.

2.2.1 – Drug Resistant Pathogens

In his article on drug-resistant pathogens, Flickinger discusses the increasing prevalence of drug resistant pathogens in the hospital setting, what he specifically refers to as hospital acquired infections (HACI) and expresses his concern for addressing the prevalence of these pathogens in both the healthcare and biotech/pharmaceutical domains. He notes that MRSA increased 19-fold between 1990 and 2001 in the UK. In the US, he also notes the Infectious Disease Society of America (IDSA) statistics that 4 out of every 1000 patients discharged from hospitals between 2001 and 2004 returned with infections (Flickinger, 2006).

Flickinger highlights the IDSA’s “most wanted” list which includes the following: MRSA, *E. coli*, the *Klebsiella* species, *Acinetobacter baumannii; Aspergillus* fungi, VRE, and *Pseudomonas arruginosa*. He then goes on to discuss the findings at the UK’s Ambulance Association’s conference (AMBEX) that traditional cleaning is not effective. The conference had found dry vaporized hydrogen peroxide (VHP) to be more effective. As later discussed, however, fumigation can be timely, difficult, and expensive to reach proper levels (Flickinger, 2006).

Ro et al. highlight three top pathogens for examination, MRSA, VRE, and Tuberculosis (TB). MRSA was responsible for 126,000 hospital admissions from 1999 to 2000 and an additional 94,000+ over 2005 in the United States. In Canada, MRSA and VRE were responsible
for 8.62 and 1.32 of every 1000 hospital admissions, respectively in 2007. According to the World Health Organization, tuberculosis accounted for 9.2 million infections and 1.7 million deaths worldwide (Ro et al., 2012).

In the health care sector, pathogens often spread from patient to patient through contact with peripheral surfaces and contact with health care workers. As Ro et al. point out, knowledge of a patient’s infectious status is often unknown, and “ambulances [often] transport patients without having a prior knowledge of a patient’s risk factors or of the colonization or infection state of the ambulance” (Ro et al., 2012). Though direct links cannot be drawn from ambulance transmission and hospital acquired infections, the compilation of these unknowns result in a very high risk scenario.

2.2.2 – Equipment classification corresponding to bacterial contamination risk

Noh et al. conducted a study examining bacterial contamination of metropolitan ambulances. A total of 429 sample sites were taken across 13 ambulances, resulting in 33 sample sites per ambulance. Each sample was collected using a soft rayon swab, and samples were cultured in blood agar and MacConkey agar plates. The plates were then screened for MRSA and VRE (No et al., 2011).

During the course of their study, Noh et al. also classify all the equipment present into critical, semi-critical, and noncritical categories. Critical equipment includes any device that makes contact with the inner, sterile parts of the body, such as an intubation tube. Semi-critical equipment is classified as any device that mostly makes contact with non-sterile mucous membranes. Devices listed in this category include the facial mask and laryngoscope blade. Meanwhile, noncritical equipment includes any device that makes contact with intact skin and
any non-medical device housed within the ambulance. Thus, noncritical equipment includes the ECG line, air conditioner, steering wheel, cabinets, etc. (No et al., 2011).

In addition to swabbing various sites within the ambulance, Noh et al. also tested fluid from the oxygen filter tank and suction bottle, but for the purposes of this study, we are more interested in the results from the noncritical swabs. Contamination was determined at percentage levels with a 95% confidence interval. Considering all classes of devices present, contamination was determined to be highest amongst circulation devices at a level of 69.2% (C.I.: 56.6 to 80.1%). Noncritical devices were contaminated at a rate of 59.1% (C.I. 44 to 57.9%). Noh et al. observed that noncritical contamination was not a cause for alarm; however, this number, combined with the other findings, suggests a potential risk factor for infections. Among sites swabbed, *Acinebacter baumannii* was present at 3 sites, *Staphylococcus aureus* was present at 4 sites, and *Klebsiella pneumoniae* was present at 2 sites, all species on the ISDA’s most wanted. More specifically, the study found 73.1% contamination at the driver’s side, including one positive MRSA swab. Four of the ambulances had been cleaned with alcohol and tap water before samples were collected (No et al., 2011).

As McDonell notes, the amount of procedures, or the scope of what paramedics can do, has increased immensely over the past 50 years. Thus, he suggests more of an interest should be taken in infection control during pre-hospital (ambulatory/paramedic) care. Specifically, he examines the contamination levels of monitoring cables used for vitals screening and respiratory equipment within the ambulance. Successful infection control requires the practice of proper procedures and work practices, an understanding of various pathogens and their modes of
transmission (e.g. air, contact, etc.), and an understanding of hospital dynamics and their effect on levels of transmission, in order to minimize patient and personnel risk (McDonell, 2008).

Four modes or potential origins of patient exposure and subsequent risk of infection are identified within the ambulance as follows:

1. blood and fluid deposited from other patients
2. blood and fluid from health care workers, e.g. paramedics
3. cross contamination of equipment
4. contact with contaminated equipment during medical procedures

McDonell then references the same categorical breakdown (as discussed by No et al.) of critical, semi-critical, and noncritical equipment within the ambulance. Subsequently, he highlights a potential risk scenario stemming from the routine practice of monitoring vital signs en route. Monitoring equipment is utilized in almost any transport, and its use is particularly necessary in cases of trauma and cardiopulmonary distress. Thus, this equipment faces potential exposure to blood which may or may not be visible (e.g. if a patient is emitting blood from the respiratory tract). McDonell acknowledges and warns, for example, that cable cleaning may be omitted unless visible contamination by blood or other fluids presents. (McDonell, 2008)

Specifically, McDonell highlights two studies on noncritical equipment that can be easily contaminated in cases of trauma, pulse oximeter probes and tourniquets. One study that McDonell highlights discovered 29 of 44 “clean” probes tested positive for bacteria despite the fact that 20 of these had been cleaned with alcohol or an antibacterial/antiviral agent prior to testing. Similarly, another study highlighted by McDonell found 25 of 50 tourniquets had visible bloodstains and 17 (34%) tested positive for pathogenic bacteria. As mentioned, McDonell
stresses that, in cases of trauma and/or cardiac arrest, blood may be present on equipment but not immediately noticeable (McDonell, 2008).

Another scenario McDonell discusses is use of the laryngoscope blade. As this makes contact with mucous membranes, this piece of equipment could be considered semi-critical and should, thus, be mostly sterile. The equipment is, however, often subjected to saliva, vomit, blood, mucus, and other bodily fluids. As McDonell points out, reusable equipment that comes in contact with sterile viscera or mucous membranes should be cleaned as soon as possible before disinfection. Sterilization techniques and standards are fairly well established for procedures and corresponding reusable equipment. However, this protocol becomes essentially ineffective if procedures are not conducted within a relatively sterile environment. Again, as the scope of what paramedics are licensed to do has increased, so have the amount of critical and semi-critical procedures and, thus, the possibilities of potential exposure to harmful pathogens (McDonell, 2008).

Rutala and Weber draw attention to Earle H. Sprague’s theory that the disinfecting process could be better understood by dividing equipment into three categories. This organization has since been adapted and employed by the Center for Disease Control and Prevention (CDC). This classification is slightly different from those discussed in McDonell’s and Noh et al.’s works. The organizational taxonomy Rutala and Weber recall is based on those previously extracted but more specifically details the level of cleanliness that must be achieved for each class of equipment (Rutala & Weber, 2007).

Critical equipment, as classified by Sprague and the CDC, includes any equipment that presents a high risk of infection if any contaminants (even spores) are present, and this class
should be fully sterilized. Semi-critical equipment includes anything that may be decontaminated with disinfectants as this class “should be free of all microorganisms… [though] small numbers of… spores may be present” (Rutala & Weber 2007). Even equipment in this class, however, “minimally requires high-level disinfection using chemical disinfectants” (Rutala & Weber, 2007). Glutaraldehyde, hydrogen peroxide, ortho-phthalaldehyde, peracetic acid with hydrogen peroxide, and chlorine can all be considered high-level disinfectants in the right concentrations (Rutala & Weber, 2007).

Finally, noncritical equipment includes any remaining medical devices, ventilation, etc. which makes contact with skin only. Rutala and Weber note that noncritical equipment generally may be decontaminated where used and that this equipment may not always be subject to the same high-level disinfectants. They further warn, as do both McDonell and Noh et al., that even when protocol exists for noncritical equipment, personnel may not always adhere to guidelines.

These findings help to demonstrate that current protocols cannot ensure a MRSA-free environment and that more attention should be allocated to the cleaning and sanitation of noncritical devices. Despite dismissal of this equipment, these devices pose, theoretically, as much, if not greater, risk for contamination. This risk factor also presents an even greater need to identify contaminants. If contaminants are consistently identified on noncritical items by EMS personnel, firsthand, more regular cleaning of these equipment may become commonplace. It is also possible that this class of equipment is decontaminated regularly but that the materials of certain equipment (e.g. a seatbelt) prove incredibly difficult to adequately decontaminate. With this in mind, use of a quality assurance device could be targeted to particularly high risk and high traffic areas and equipment.
2.2.3 – Areas of concern within the ambulance

Nigam and Cutter conducted a yearlong study of Welsh ambulances. Each month, 14 sample sites were swabbed, 7 before and 7 after cleaning. Their study found that solutions within crews varies from generic purpose detergent, phenolic pine disinfectant, and alcohol and chlorhexidine wipes to Tiket, 10% bleach solution, Domestos, 2% stericol, .02% chlorhexide gluconate, and hypochlorite solution. Cleaning practices and materials also varied greatly (Nigam & Cutter, 2003).

Some areas were even found to show higher levels of contamination after cleaning. For example, contamination levels at the steering wheel were estimated at 33.4% before and 41.7% after. The authors also emphasized specific bacteria and pathogens observed, including *Staphylococcus aureus* (*S. aureus*) found on the track floor and *Streptococcus viridans* (*S. viridans*) found in multiple sample areas. Nigam and Cutter identify *S. viridans* as a potential pathogen. While they list *S. aureus* as a normal species, this is once again on the IDSA’s most wanted. Once again, as in Noh et al.’s study, noncritical areas showed levels of contamination that were cause for concern as potential risk factors for exposure (Nigam & Cutter, 2003).

2.2.4 – MRSA prevalence in ambulances

Semmons et al. conducted a study of ambulances near a Level 1 Trauma Center, examining specifically contamination by MRSA. The trauma center receives about 60 EMS transports each day, and samples were taken from 25 ambulances upon arrival to the emergency department. Samples were taken with a sterile saline swab from the steering wheel, stretcher hand rails, the oxygen container knob, and the inside door handle to the cabin (Semmons et al., 2010).
The samples were then plated on BBL CHROMagar plates, and growth was observed at 24 hour intervals, at 24, 48, and 72 hours from plating. Of the 25 ambulances examined, a total of 8 out of 100 samples (12.5%) tested positive for MRSA, and 7 of the 25 ambulances tested positive, indicating that one of the ambulances had 2 positive growth sites (Semmons et al., 2010).

For cleaning, the ambulances had each been sprayed and wiped with an antibacterial. Of the 25 ambulances, 19 had been cleaned at least 8 hours prior to sample collection, and 6 had been cleaned less than 8 hours prior to sample collection. None of the ambulances tested had been cleaned within 2 hours prior. This time table of last reported cleanings suggests that either recent transports had been carrying MRSA or, more likely, that cleaning measures were not sufficient to completely sterilize the ambulance. No correlation was observed between time from cleaning and a positive culture, once again supporting the hypothesis that current cleaning and sterilization in ambulances are either not enough or not done properly (Semmons et al., 2010).

While not explicitly discussed, full high-level sterilization cleanings presumably had not been conducted prior to testing; the vehicles were simply wiped down. This study raises concern for possible exposure risk for both personnel and patients. Semmons et al.’s findings also suggest that either more rigorous cleaning should happen more regularly or that there is a need for an improved method for regular cleaning between transports. (Semmons et al., 2010)

Chapter 2.3 – Ambulance Cleaning Methods
In this section, ambulance cleaning methods will be reviewed. Current ambulance protocol, including cleaning and sterilization, will be reviewed along with the efficacy of that
cleaning. The team needed to evaluate if ambulances are at increased risk due to faulty protocols, and if so how those protocols could be improved?

2.3.1 – Ambulance Protocol

North Dakota Ambulance Services have created a detailed outline on how to limit exposure control through their methodology and cleaning supplies. This depot recognizes that contaminants can be transmitted through a variety of bodily fluids such as semen, synovial fluid, and saliva. In this case, it is mandatory for all EMTs to treat every situation with high risk and wear the maximum level of personal protective equipment (PPE) – but unfortunately this isn’t always the case (AIPC, 2013).

Non sterile gloves are worn at all times when handling bodily fluids, but in some cases there are high level contaminants even when bodily fluids cannot be seen. The protocol also suggests washing hands immediately, but as there is no hand washing station in an ambulance this suggestion is not always followed either (AIPC, 2013).

While EMS personnel are required to receive HBV immunizations, exposure can still occur when the virus comes into contact with non-intact skin, eyes, mouth, nose, or mucous membranes. There is strict OSHA-approved protocol to follow whenever bodily secretions are known to be within the cabin. The first step, after filling out an incident report, is to decontaminate the ambulance. For low level cleaning, hydrogen peroxide is used. When HBV or MRSA is known to be present, however, a stronger disinfectant with a shorter cure time known as Hepacide or BH38 is used (AIPC, 2013).

The procedure instructs the personnel to spray all surfaces and then wipe immediately. Linen is to be removed and labeled as biohazardous. Once fluids, such as vomit, blood, or urine,
are absorbed, disinfectants are then used. The high level decontamination is only recommended to be used once a month, or when bodily fluids are present. This is another case where the protocol is not necessarily optimal, as most ambulances encounter high level contaminants much more frequently than once a month (AIPC, 2013).

Reusable equipment such as laryngoscope blades is cleaned with soap and water and then placed in an approved cleaner for 10 minutes. Following this, the equipment is washed with soap and water again. While this technique is appropriate for small reusable items, it is impossible to clean large items such as the stretcher or countertops in this manner. We hypothesize that the use of a quality assurance device after patients as an indicator of contamination will encourage more frequently cleaning. This device will also service as a validation, in order to indicate when disinfection has been performed inadequately (AIPC, 2013).

2.3.2 – State of the art protocol

In some cases, third party companies are hired to clean and sanitize ambulances in special circumstances, such as a known outbreak of a harmful pathogen. One such third party company is Aftermath Incorporated. The team spoke with Bill Ciaccio of Aftermath Inc. in order to obtain a better understanding of 3rd party cleaning methodology. Third party biohazardous cleaning companies generally attend to suicide, deaths, crime scenes, industrial accidents, ambulances, and jail cells amongst other sites.

The third party company’s cleaning procedure is more regulated than what is required of an ambulance. “First, universal precaution is always used. The highest level of personal protection equipment, or PPE, is used and thrown away after each used. All cloth and porous materials are removed from the contaminated site and destroyed. A degreasing agent is then used with rags; then the surface is left to dry. All rags used for degreasing are disposed of.
Disinfectant is then sprayed with a pump sprayer. The cure time for the disinfectant is 20 minutes. Residue is then removed with a standard household cleaning agent, and a validation step is performed” describes Bill Ciaccio of Aftermath Inc.

For insurance reasons, several validation steps with an ATP Hygiene Monitoring Device are performed during the cleaning timeline. Hard surfaces are sampled for bacterial contamination and measured with the ATP-HMD to give an output of RLU – or relative light units. An acceptable reading of RLU is ~4000 RLU’s, and a contaminated site containing blood can have an RLU reading of over 2,000,000, according to Ciaccio.

The third party cleaning company has more time to attribute to disinfection; the whole process takes two men 1.5 hours. Unfortunately, ambulance depots are reluctant to spare the time and labor if it is not absolutely necessary. This is one contributing factor to the ongoing prevalence of harmful pathogens within the ambulance environment (Ciaccio, 2013).

One important thing to note is the consistent use of the validation step in this Gold Standard cleaning methodology. In this case, if a disinfection method does not attribute to an ATP-HMD reading of less than 4000 RLU, the entire process is repeated, wasting time and resources. This validation encourages technicians to work efficiently and follow all protocol precisely.

Chapter 2.4 – Comparison of Risk: Ambulances vs. Hospitals
In this section, a risk evaluation of ambulances compared to hospitals will be done in order to answer questions like “Why do ambulances have a higher rate of infection than emergency rooms?” and “What costs restrictions are there, in terms of cleaning budget, in ambulances compared to hospitals. Here, variances in cleaning protocol between hospitals and
ambulances will be evaluated. Then, other industries’ cleaning practices will be reviewed as a model for change within the health care industry.

2.4.1 – Risk Evaluation

McDonell depicts a side-by-side comparison of the viability and subsequent transmission efficiency of pathogens, noting that Hepatitis B is more efficiently transmitted than Hepatitis C, which is in turn more efficiently transmitted than Human Immunodeficiency Virus. According to McDonell, HBV can remain viable in dried blood for up to one week at room temperature, sometimes longer, hence, resulting in numerous opportunities for exposure and infection, both for patients and personnel. Again, as blood can remain undetected, these pathogens are of considerable concern (McDonell, 2008).

Many factors can affect the efficacy of disinfecting/sterilizing agents, including organic load (what is physically present/visible), the type of contaminant (which pathogen is present), the physical characteristics of the equipment (grooves, material, etc.), and the characteristics of the disinfectant/sterilant (concentration, temperature, pH level, physical form, etc.). McDonell breaks down disinfectants into four classes: phenolic solutions, hypochlorites, alcohols, and aldehydes. Each class, however, has limitations and needs to be used effectively. Hypochlorites, for example, can be corrosive, have a limited shelf life, and can be deactivated by organic material, such as blood. Alcohols in 70% concentrations can be effective at destroying HIV and HBV but must be in contact for a minimal amount of time (McDonell, 2008).

These limitations can severely hinder the disinfectant process if not used properly. Thus, the need for a quality assurance (some sort of biological indicator) is a crucial gap currently in ambulance protocol. As later discussed, if biological indicators are used as a means of quality
assurance and risk assessment in hospitals, why not apply similar measures to ambulatory/pre-hospital care?

Kei and Richards’ study examines the prevalence and corresponding exposure risk in a large, urban emergency department between May and August of 2006. Twenty objects in the ED were sampled, including telephones, desktops, security keypads, ultrasound probes, and blood pressure cuffs. Each object was sampled twice, once in May and again in August to avoid bias based on time of sampling, resulting in a total of 40 sample swabs. MRSA has been well-established as a communal pathogen among daycare students, professional athletes, prisoners, and exotic dancers, best spread through skin to skin contact and entering via some exposed wound or membrane (Kei & Richards, 2011).

Fewer studies have perhaps been closely studied about the spread of MRSA in a hospital setting, as the primary concern is stopping the outbreak. Some of the objects tested, however, have been linked to MRSA spread in intensive care units (ICUs). Samples were collected by spreading and collecting 0.9% normal saline on a section of each object using a BBL™ CultureSwab™. The swabs were rolled over a 5% sheep blood agar plate 4-mm deep, coating all sides of the swab. This same method was repeated using a mannitol salt agar plate of equal depth. Samples were incubated overnight at 35 C and observed for pathogens over two days (Kei & Richards, 2011).

The study yielded only one positive sample, originating from the swab used at the security pad in the ambulance bay. Kei and Richards discuss the rational as to why the positive sample actually originated from outside the ED. The ED sampled was located in a high-volume, Level 1 urban trauma center, thus exposing the bay key pad to a high level of traffic, including
hospital personnel, EMS staff, and even law enforcement. They theorize that the open ED may be too harsh for MRSA to survive for lengthy periods. On the other hand, the bay is a less controlled environment, and the pad may be cleaned less than other objects. EMS personnel also have limited hand washing resources. While the authors acknowledge limitations in the small size of the study (stressing that other positive sites may exist but were not sampled), the location of the one positive sample suggests either a potential gap between ambulatory and hospital protocol and/or an overall higher risk of exposure during ambulatory transport due to the uncontrolled environment (Kei & Richards, 2011).

Resulting from their observations of high risk scenarios, Ro et al. conducted an observational study of a tertiary academic hospital to estimate the prevalence of positive transports of TB, MRSA, and VRE. The study spanned from January 2007 to December 2008 and all ambulances in the study consisted of fire department crews with 2 to 3 crew members (either EMT or paramedics). Patients in the study were divided into three groups based on mode of transport: pre-hospital transport by ambulance, inter-facility (hospital to hospital) transport by ambulance, and non-ambulance transport. Doctors ordered cultures based on sign of infection, and cultures were derived from blood and other bodily fluids to determine infection. (Ro et al., 2012)

From these data, odds ratios were calculated for risk of infection due to transport, assigning non-ambulance transport with a value 1.00 for reference and comparison. The odds ratios for TB, MRSA, and VRE in the pre-hospital transport group were, respectively, calculated to be 1.23, 2.41, and 2.73, with 2.13 as an overall odds ratio. More drastically, the odds ratios for these same pathogenic infections in the inter-facility transport group were calculated to be 2.58, 6.50, and 10.29, with 5.94 as an overall ratio (Ro et al., 2012).
These ratios suggest at least a two-fold greater risk of exposure of infection from pre-hospital transport than from non-ambulance transport. Inter-facility transport had the highest odds ratio, with a 6.5 and more than 10 times greater risk of contracting MRSA and VRE, respectively (Ro et al., 2012). This is interesting to note, as the greater prevalence and odds ratios derived from inter-facility transport suggest that ambulances may not be a direct source of MRSA and other pathogens, but are, rather, a propagating factor within the community. It is also possible that ambulances transporting patients from one hospital to another may have the same amount of pathogens, but patients being transported from one hospital to another are more likely to be immune-compromised due to worsening of an injury or longstanding illness, thus making them more susceptible to infection. It is acknowledged that possible confounding factors may exist, including infection prior to transport.

Lee et al., as described in greater depth in Section 2.6.2, tested ambulance equipment for blood contamination. In their study, they found 15% of equipment was visibly contaminated and another 42% tested positive for less obvious contamination. Both HIV and Hepatitis B, as previously mentioned, can live in blood for prolonged periods, and the ambulance provides the optimal setting for these. Cold water may harden fats of lipid viruses (such as Hepatitis B and HIV). Hot water may erode surfaces and cause adherence of proteins to materials. Alcohols are only effective at killing viruses if in contact with the surface for at least five minutes. Lee et al. conclude that cleaning and sterilization are only effective if done properly. Without any means to verify, however, there is no obvious assurance of what may be “proper” and “necessary” after each call (Lee et al., 2006).
2.4.2 – Variances in Protocol

According to Rutala and Weber, health care professionals perform approximately 46.5 million surgical procedures annually and even more other “critical” or invasive medical procedures. During both operation and recovery, there is a risk of infection and death. A major portion of this risk stems from pathogenic bacteria and other microbes. Effective protocols are in place to minimize infections. However, deficiencies in these protocols arise from both failure of adherence and failures in the process. For example, a batch of inactive glutaraldehyde was used in 60 hospitals in Belgium, placing over 34,000 patients at increased risk due to the defective disinfectant. Failures in the process include failures due to human error in amounts, equipment failure, and systemic or operational issues. If a disinfectant process is not communicated properly and used properly with fully functioning equipment, it is basically ineffective (Rutala & Weber, 2007).

Sterilizing/disinfecting agents can be broken down into two main categories: sterilants and disinfectants. Disinfectants, contrary to popular opinion, kill most microbial life except for bacterial spores. Sterilants, on the other hand, destroy all microbial life, including these spores. Rutala and Weber emphasize that before sterilization, cleaning (the removal of all visible soil) must occur. They emphasize that “thorough cleaning is essential, because inorganic and organic materials that remain… interfere with the effectiveness” of disinfection and sterilization (Rutala & Weber, 2007).

Rutala and Weber establish a 14-step process for confirming failures in the decontamination process and evaluating the established failure. Biological indicators are often used to confirm suspected and/or unknown failures; for example, they may indicate that not all spores have been killed during a sterilization cycle. A large portion of Rutala and Weber’s
analysis then discusses means to determine whether disclosure of a failure is needed, based on types of failure, epidemiological statistics, and calculated risk of direction contamination and possible infection. Rutala and Weber reaffirm that regardless of calculated risk, and even if disclosure is unnecessary, “it is important to rapidly identify potential exposure and instigate corrective action for the… [failed] procedure” (Rutala & Weber, 2007).

2.4.3 – Other Industries Worth Considering
Flickinger suggests looking to sister industries in biotech/pharmaceuticals for top-line standards for maintaining cleanliness and sterilization in a health care setting. No matter what the setting and the process selected, hospital and ambulatory crews “need a mechanical action of cleaning first, followed by an effective disinfectant” and that “the cleaning process… should be built in from the beginning,” and not a mere afterthought of treatment/production. This is another industry that also relies on quality control methods for ensuring proper cleaning and sterilization (Flickinger, 2006).

Chapter 2.5 – Biological Contaminant Monitoring
Finally, current biological contaminant monitoring systems on the market today will be evaluated in order to determine which would best meet the needs of the client. Feasibility and costs were considered, along with accuracy of each system. Here, three types of systems were reviewed – rapid antigen detection tests, airborne bacteria monitoring systems, and ATP monitoring systems.

2.5.1 – Rapid Antigen Detection Test
Rapid Antigen Detection Tests, or Guardian Lateral Flow Tests, were introduced in 1980’s and ever since have been an inexpensive means for semi-quantitatively measuring analytes including antigens, antibodies, and nucleic acid amplification tests. Urine, saliva, serum, plasma, and blood can be used as specimens.
Test sensitivity can indicate antigens with as low of a concentration as 1.0 ng or less. Tests use a colloidal gold, dye, or latex bead conjugates to generate a positive test signal. Test strips have a shelf life of several months when protected from moisture and heat. Depending on the antigens used for detection, a kit can cost anywhere from $20-$50.

Figure 1: The lateral flow diagram shows each component of a rapid antigen detection test.

A sample, either alone or combined with a running buffer, is placed on the sample pad, such as in Figure 1 above. The signal is solubilized and binds to the antigen or antibody in the sample and moved through the membrane by capillary flow. If a specific analyte is present, the signal reagent binds to it and a second antibody or antigen-immobilized as a line in the nitrocellulose, then the complex is captured. The total read time of the instrument is approximately 5 to 10 minutes.
This method would be a cost effective way for EMS personnel to easily test ambulances after, or before, each cleaning procedure. A disadvantage of the rapid antigen detection test is that the concentration required may not be able to adequately detect contaminants. This method is also limited in the type of antigen or antibody it can detect (Point-of-Care Technologies, 2007).

Finny et al., in their study, examine three different commercial kits currently on the market for detection of Hepatitis B. Brand name and assay type of the three kits examined can be summarized in Table 1 below. All three rapid detection kits were compared to IMx HBsAg assay, which has a lower detection limit between 0.2 and 0.5 ng/mL. According to Finny et al., Hepatitis B is considered to be relatively contained in western countries. However, it is still a contaminant of concern, particularly to the health of EMS personnel (Finny et al., 1996).

Table 1: HBV – Commercial Rapid Tests

<table>
<thead>
<tr>
<th>HBV Commercial Rapid Tests</th>
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</thead>
<tbody>
<tr>
<td>Immunocomb-II</td>
<td>enzyme immunodot assay</td>
</tr>
<tr>
<td>Austragen</td>
<td>latex agglutination assay</td>
</tr>
<tr>
<td>Daina Screen</td>
<td>immunochromatographic assay</td>
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Finny et al. tested for HBV in 500 blood donors in Southern India. From these donors, 100 sera were selected for comparison, including 31 positive sera and 69 negative sera. The kits required between 25 µL and 75 µL and readout times varied between 7 minutes and 1.5 hours as summarized in Table 2. None of the kits were determined to be great at detecting low virus load in positive sera.
If our team were to create our own rapid antigen detection test, the target time and sera ranges would line up with the Daina Screen and Austragen tests, respectively. After thoroughly cleaning the ambulance once through, EMS personnel would want to know within a matter of minutes, not hours, whether or not more high level disinfection needed to occur. Also, following initial cleaning and sterilization, there should not be significant amounts of bodily fluids, thus making it difficult to collect significant amounts of sera or liquid for testing (Finny et al., 1996).

2.5.2 – Airborne Bacteria Monitoring Systems

Bioaerosols monitors on the market today are capable of evaluating complex microorganisms, such as culturable, nonculturable, and dead microorganisms, fragments of microorganisms, and toxins. These devices are reliable and capable of collecting and enumerating airborne organisms using culture based analysis. Some require vacuum pumps making them noisy, large, heavy, and in need of constant electrical source. Portable devices available are smaller in size and weight, but are limited in their filtration and culturing capabilities (Mehta, et al., 2000).

Airborne contaminant monitors use agar in order to culture bacteria within the air. Due to their limited flow rate, 100 Liters of air, which is the suggested sample size for a reading, can take several days to obtain. After the agar has been subjected to 100 L of air, the plates are then incubated at 35°C for 48 to 72 hours. After incubation, the colonies are transferred to blood agar plates, and an experienced biologist uses a Biolog Microstation® system in order to identify types
of bacteria with supplemental tests, such as gram stain, catalase, coagulase, and oxidase. These varying tests are performed as needed (Mehta, et al., 2000).

Due to the long process of each airborne contamination test, this would not be an efficient means for daily monitoring of cleaning activity within an ambulance. Another limiting factor of this technique in ambulance use is difficult of use and analysis of the current airborne monitors. These tests require wet lab trained biologists in order to interpret these results, and adequately avoid contamination in lab. As ambulance depots do not have a lab on hand, tests would constantly have to be sent away to be examined – increasing the total investment of the quality assurance step (Mehta, et al., 2000).

2.5.3 – ATP Monitoring systems

ATP test is a way to rapidly monitor the presence of microorganisms through detection of adenosine triphosphate, or ATP. ATP monitoring systems usually involve some sort of chemical treatment of a particulate sample in order to isolate microorganisms.

ATP is the primary energy carrier for all life forms – including contaminants such as bacteria. As such, the measurement of ATP has a direct correlation to the concentration of biological contaminants and their health.

ATP tests quantify ATP detection through the measurement of light produced through ATP’s reaction with the naturally occurring firefly enzyme Luciferase. The measurement is taken using a luminometer, or photometer. A luminometer, or photometer, uses a photodiode, which is a type of photodetector that is capable of converting light into either current or voltage (IUPAC, 1997). This measurement is converted into RLU’s, or relative light units. In
biotechnology, a reading of 4000 or less indicates a properly cleaned surface, while a reading above 4000 would indicate the need for further sterilization.

There are two types of ATP to be measured: 1) Intra-cellular ATP that is contained within living biological cells, and Extra-cellular ATP that is located outside of living biological cells. The extra-cellular ATP is released from dead or compromised organisms. For a comparative table of 7 different brands of ATP Hygiene Monitoring Tests, see Appendix A (LuminUltra, 2012).

ATP Hygiene monitors are capable of detecting multiple types of contaminants, making them ideal for identification within an ambulance environment. The total test time, from reading to result, is less than five minutes. This quick test time would allow EMTs to be able to perform quality assurance several times a day if needed. Most tests on the market are able to detect a small amount of ATP – between 0.97 and 10,000 femtomoles. These devices were originally intended for the food industry, so they are easy to use and require very little training in order to interpret results. Devices on the market today require an initial investment between $1800 - $5000 depending on the model. Each swab required for testing costs between $1.85 - $3.00, depending on the brand. If the team were able to design an ATP monitor for a low cost, this system would be ideal for contamination monitoring within the ambulance.

2.5.3.1 – The Luciferase Assay

Most ATP hygiene tests on the market use luciferase in combination with luciferin, an enzyme and substrate derived from fireflies, in order to detect ATP. The Luciferase assay is very sensitive, because its light production has the highest quantum efficiency known for any chemiluminescent reaction. It has the added benefit of no background luminescence found in the host cells or the assay chemistry, significantly reducing noise and simplifying analysis. The
assay is also rapid, requiring only a few seconds per sample as the protein does not require post-translational processing (Promega, 2002).

Light is produced by converting the chemical energy of luciferin oxidation through an electron transition, forming the product molecule oxyluciferin. Luciferase, the enzyme derived from fireflies, catalyzes luciferin oxidation using ATP and magnesium as a co-substrate. Once this reaction occurs, light is generated. However, this late decays rapidly, with most of the light intensity gone by 15 seconds. Some assays incorporate a coenzyme for improved kinetics between the enzyme and the substrate (Promega, 2002).

These assays also contain a cell lysis buffer. This buffer allows for the breaking down of the cell membrane and therefore the release of ATP, creating a stronger reaction. Different lysis buffers are used for different applications (i.e., bacterial cells, mammalian cells, etc.). A design alternative to consider is the ratio of the components of this assay – the luciferin, luciferase, and the lysis buffer (Promega, 2002).

In order to modify this assay to be done quickly, a room-temperature stable solution would have to be found. In some assay kits, luciferin and luciferase and stored in a freezer before use. However, both liquid stable and freeze-dried room temperature stable enzymes are available. In order to be used quickly by EMT’s, the protocol would have to be altered in order to be completed in less than 5 minutes. This would also involve finding a coenzyme that would improve the kinetics of the luciferin-luciferase reaction, and an appropriate lysis buffer. One such enzyme is dehydroluciferyl (Promega, 2002).

2.5.3.2 – Bioluminescence Quantification
Visible light consists of billions of photons, which are emitted from bioluminescent reactions such as that emitted from the Luciferase assay. This reaction is typically measured with
a luminometer, which is designed to measure light output. Luminometers consist of a sample chamber, detector, signal processing method, and signal output display.

Luminometer chambers are completely sealed in order to prevent ambient light from entering the chamber and influencing the reading. The chamber and sample should be as close to the detector as possible in order to further reduce interference.

The detectors in luminometers are either photodiodes or photomultiplier tubes (PMTs). Recent improvements in photodiodes have made them an inexpensive and accurate alternative to expensive PMTs. However, for more sensitive readings PMTs are the detector of choice, as they can measure extremely low levels of light. PMTs can be oriented in two different orientations (side on and end on), relating to two different measurement methods. However, both measurements will display an output in RLUs, or relative light units. While PMTs can sense low levels of light, they are limited in dynamic range – that is, they can only measure a limited amount of photons in a certain amount of time. Because of this, they cannot allow fast enough to respond to high light levels and short durations of fast reactions such as the luciferase assay.

In the case of luminometers, light output is directly proportional to the sample volume when the concentrations and ratio of the reactants are held constant. So, in the case of the luciferase assay, if the ATP and luciferin-luciferase ratio is constant and the volume is doubled, the light output will also double. Concentrations of reactants are also important to consider because light output is directly proportional to the concentration-limiting reaction in the system. The light output of the reaction is also proportional to the ratio of reagent volume to total volume in the same way. This optimization can be quantified by the following equation:
where “L” = the total light reaching the detector, “Cr” = the concentration of the reagent, in excess, “Vr” = the volume of the reagent, “Vt” = the volume of the reagent plus the volume of the unknown (total volume), and “N” = the effective order of the reaction. Therefore, it is possible to maximize the absolute sensitivity of the assay by using a highly concentrated reagent and by using a large volume of reagent compared to the volume of the unknown (Comm Tech, 2012).
Chapter 3: Project Strategy

3.1 – Client Statement & General Framework

For this design project, the client stated a need for an evaluation of the current contaminants within the ambulance environment, as well as a design which could help prevent and minimize cross contamination between the ambulance, EMT personnel, and patients. From this need, the following initial problem statement was made:

“The goal of this project is to develop a method for evaluating the current efficiency of decontamination within the ambulance environment. With this information, the team will design a new cleaning solution that effectively removes contaminants, is less costly, saves time, and is environmentally safe and minimally toxic to the cleaning staff, EMS personnel, and patients.”

While this problem statement clarifies certain objectives, such as understanding decontamination methods and the need to improve upon current cleaning protocol, many issues are not addressed. The team realized through client interviews, team discussions and background research that with proper use, current solution and methodology is effective against removing harmful contaminants within the ambulance environment. This is why the team decided to focus on creating a quality assurance device in order to validate cleaning and eventually modify protocol as a preventative measure for cross contamination.

Due to this realization about the industry and the team’s establishment of revised objectives and constraints, the team created a revised client statement:

“The goal of this project is to evaluate the current ambulance cleaning methodology, as well as the types of contaminants found within an ambulance environment in order to create a quality assurance device. This device will be able to validate cleaning methodology and be used as an indicator for cleaning need. The design should be able to detect and alert EMS personnel of contaminant indication quickly, process a small concentration and sample size
while remaining non-toxic, inexpensive, and meeting all FDA and EPA regulations.”

This revised client statement provides a clear outline of what the manufactured device is expected to do and the requirements it is expected to meet.

3.1.2 – Objectives

With a one academic year time constraint and a budget of 312 dollars, the quality assurance device needed to meet certain objectives. The device should be able to leave no harmful residue, meet all EPA and FDA regulations, easy to dispose of, and inexpensive. Furthermore, the device must be easy to use (while emitting easy-to-read results) and, most importantly, be able to detect contaminants. We also wanted the device to be reliable, meaning the device should be consistent and accurate in its readings. Once these criteria were established, an objectives tree was then created, summarizing the goals of the device as seen in Figure 2 below.

![Objectives Tree Diagram]

**Figure 2:** The above diagram highlights top objectives for the team’s design.
As with any device in the medical industry, the device used should be nontoxic to users and to the environment. Ideally, minimal personal protection equipment (PPE) would be required, as it is difficult to regulate PPE use. Therefore, our first main objective is solely for the device to be “nontoxic” – or to be as biologically inert as possible during use. Particularly, the device should not cause any remaining harmful residue after use. Harmful residue could pose a threat to the long-term health of both EMS personnel and patients. In this respect, the device should comply with any FDA regulations on toxicity, allowable materials, and allowable concentrations of chemicals. Our device intends to help protect patients, thus the ability to be nontoxic is an important objective.

The device should not only be personally safe to use but should also be environmentally safe. It should be easy and safe to dispose of, producing minimal biohazard waste. Materials used and all disposed waste should fall in line with EPA standards as well.

Even more importantly, the device should be inexpensive. Current quality assurance devices that are available for ambulances are adequately sensitive, but fall out of range of the typical depot budget. A major goal of this project is to create a comparable or improved device that is within a lower price range. As discussed with the clients, depots operate on a strict budget, so any additions to the followed standards and/or equipment should be relatively inexpensive.

Furthermore, the device should be easy to use and the device results should be easy to read and analyze. Currently, paramedics are often responsible for cleaning and decontaminating the ambulances they work in. Therefore, the device and results should be simple enough for a paramedic, EMS director, or other EMS personnel to use and interpret with minimal training. Our device is intended for rapid use as a quality assurance of current cleaning protocol. As the
EMS personnel would like to know as soon as possible whether or not more cleaning is needed, sufficient time is not available to send out a culture sample to a trained biologist. For this reason a device is needed that can indicate contamination quickly and easily.

Overall, the device should be reliable. In order to be reliable, the device should be relatively consistent and accurate. If the device is not consistent and does not provide a relatively accurate depiction of contaminants, this defeats the purpose of use. Without reliability, there can be no confidence in the significance of a positive or negative readout.

The main goal of our device should be to detect contaminants. Contaminants may include, but are not limited to, pathogenic bacteria cellular biohazards. As our background literature supports, many contaminants may remain after initial cleaning and are often no visible to the naked eye. Therefore, a quality assurance device would indicate if and where contaminants were still present and if further cleaning and decontamination of some areas are necessary. By identifying when and where extra decontamination needs to occur, the cleaning and decontamination process may become more efficient and be able to reduce overall risk of infection.

Once fully established, these objectives were then compared using a Pairwise comparison chart. The main objective or goal as a detection device and each of the sub-objectives were included, with the exception of minimal PPE. Minimal PPE was replaced with the objective “to be nontoxic.” Each chosen objective or sub-objective was compared to one another in a chart form. An objective or sub-objective in the first column was selected and compared to each objective or sub-objective in each subsequent column on a 0 to 1 scale. If the objective in the first column was determined to be more important, it received a score of 1 in that row under the
named column of the objective it was being compared to. If the objective in the first column was less important, it received a 0 in the same placement. If the objectives were considered to have equal weight, a 0.5 was marked. Any box comparing an objective or sub-objective to itself was blacked out for simplicity purposes. The full chart can be seen in Appendix B.

After each objective was rated in the chart, each row was totaled in order to assign a numeric score to each objective. Once scores were assigned, the objectives were ranked, based on score. Top objectives were established as follows:

1. To be inexpensive.

2. To effectively determine presence of contaminants and pathogens and identify the areas that must be decontaminated again in order to reduce the risk of cross contamination and infection.

3. To be able to meet all regulations (FDA, EPA, etc.) while remaining non-toxic during use

Establishing top objectives is an important step to refining design ideas. Functions detailing what the device will do cannot be established until it has been determined what the general objectives are.

3.1.3 – Constraints

Constraints of the design were then established. It was determined the device must be table-top, able to detect a small amount of contaminant, and be available at a cost that is reasonable for the budget allocated to an ambulance depot. Simultaneously, the materials, chemicals, and biological components used must fall within the framework of allowed amounts and uses established by the FDA and EPA, and necessary use should fall within labor guidelines established by the union. Use of the device must also be completed within a few minutes, in
order to swiftly determine any additional cleaning/sterilization necessary and to minimize the amount of time the ambulance is out of active commission. If the device is too costly and/or too timely, ambulance depots will be less likely to implement the device and comply with proper use.

The device should be table-top. If the device is too large and/or lacks portability, it may be difficult to easily test sites within each of the ambulances. By creating a device that is table-top, this limits both the weight and the size, making testing possible at the ambulance hanger after regularly scheduled cleaning. Opting for a table-top over handheld device, however, leaves the paramedic’s hands free to access the information through a laptop or Smartphone and/or begin preparing the next sample site.

As this device is intended to pick up remaining contaminants, the device should be able to test a small sample size. If the device is unable to detect a small amount of contaminant, the sensitivity would be inefficient in determining harmful levels of contaminants. Currently available ATP hygiene tests can test a sample between 1.98 and 100,000 femtomoles. Our device would need to be at least within this range of sensitivity. Further detailed specifications will be later discussed.

Cost of the device should be limited to a price that falls reasonably within an ambulance depot’s budget. Current ambulance depots often work with limited resources, including supplies and labor. The same EMS staff that treat patients are often the same staff that clean the ambulances. Extra budget allocated to outsource cleaning (i.e. to a 3rd party company such as Aftermath Inc.) is not regular practice, mainly due to cost. Generally, this outsourcing falls within the price range of a few hundred dollars while some current quality assurance devices, as
previously discussed, fall in the range of a few thousand. The team would ideally like to cut down the cost of our device by 10-fold compared to current QA devices and keep the range of our device within a one-time investment of a few hundred dollars. If contaminants can be identified and cleaning methods thus improved, this outsourced cleaning could possibly be cut out or severely reduced.

With the constraint of cost in mind, labor can also be very costly. As mentioned, EMS personnel are often responsible for their own upkeep of equipment and thus would be likely candidates for conducting QA steps. With this extra responsibility, extra labor must be allocated, which leads up to the constraint of time. Time is an essential of constraint for a number of reasons. Minimizing the time of use minimizes extra cost of labor, if any. Shorter time of use also allows contaminants to be removed more swiftly. Furthermore, whenever an ambulance must be thoroughly cleaned, it is pulled out of active commission. The sooner an ambulance can be returned to active commission the better, as patients lives may be relying on that ambulance for critical transport.

Finally, while the FDA and EPA does not necessarily outline specification for the design of QA devices, all materials used must be easy to recycle, or incinerate upon coming into contact with hazardous materials. All residue left by the testing must also meet these regulations by being non-toxic and easy to clean.

3.2 – Project Approach

In order to create this design, the team conducted research about contaminants within the ambulance, current cleaning methodology, as well as bacteria and antigen detection devices that are currently on the market. Once we understand the limitations of current detection devices, the team will create design alternatives and choose a final design that best both meets the objectives
and falls within the constraints outlined. Upon fabrication of the design, the team will perform validation testing by comparing the results and functionality of our design to detection via assays, microscopy, and other evaluation methods within a controlled environment with a relatively benign strain of bacteria. From this information, the team will draw conclusions, and discuss further improvements that could be made. A sister methodology will then be constructed for a more harmful pathogen, and a protocol will be written to accompany the device during actual depot implementation.

3.2.1 – Technical Approach

Three types of QA devices were researched and analyzed to determine which type of device might be the best fit for an ambulatory setting. Various ATP hygiene tests, airborne detectors, and rapid antigen tests were researched and are discussed in Chapter 2. From this information, the team discussed advantages and disadvantages to each class of detection method. The ATP hygiene test was determined to be good at identifying overall contaminant level and is not contaminant specific, allowing it to test for any different types of contaminants. Airborne detection methods were found to be accurate and a good indicator of contaminants in the air within the cabin, but required extensive culturing skills and were not considered simple enough or timely enough for efficient use by EMS personnel. Rapid antigen tests are good for detecting specific antigens, but could only be used for one type or one specific class of contaminants. Neither the airborne nor the rapid antigen means would be able to identify non-bacterial/viral contaminants such as blood and other bodily fluids. A summary of the advantages and disadvantages of each class of device can be summarized in Appendix D.

After determining the advantages and disadvantages of each type, an evaluation matrix was created to determine the type of device would be best suited for implementation in an
ambulance. Parameters for this matrix were created based on the objectives and sub-objectives discussed in Section 3.1.2 and constraints identified in Section 3.1.3 of this chapter. Specifically, to establish which type of device would best meet our objectives, scores were given based on ease of disposal, ease of use, and ability to detect contaminants at low concentrations. For constraints, each class of device was scored based on portability (i.e. easily transportable, either handheld or table-top), cost, how well regulations were met, and length of time needed to conduct the test.

3.2.1.1 – Numeric Evaluation Matrix

The rating system included in Appendix C was used as a guideline for creating the numerical evaluation matrix seen in Table 3. Ability to meet each objective was scored between zero and 100 (from worst to best). First, the device should be easily disposable. This means that trash produced from each test should be a small amount, minimizing biohazardous waste. For this objective, both the ATP test and the rapid detection test types received a score of 80; some waste would be produced, but this waste would be minimal, equivalent to a swab and cartridge or a swab and test stip. Airborne test type received a score of 50, as this would produce significantly more waste, including an agar plate, pipette tips, and microscope slides.

For ease of use, scores were assigned based on how intuitive use of the class of device is and how much training would be required beyond EMS training. Minimal training and intuitive use warranted a score of 100, while excessive training outside the scope of EMS and nursing professions would earn a score of zero, for example. Based on this rating system, ATP hygiene test received a score of 70, as this class of device does require some training to learn how to read the device, but this training is not excessive. Rapid antigen tests were decided to be slightly easier to use, with a readout similar to a pregnancy test, so this received a score of 80.
Meanwhile, airborne tests received a score of 20 as this type of testing requires more extensive microbiology training.

Initially, the team wanted to create a rapid antigen detection type of test. After completing this evaluation and compiling a matrix comparing the three types, we discovered that this class of device is incredibly limited at detection. This test method requires sera, can only detect one type of contaminant and often doesn’t do so reliably, as discovered in our background research. Thus, this type of test received a score of 30. ATP and airborne test types, on the other hand, detect contaminants very well. The ATP test type was given a score of 90, and not 100, as it cannot differentiate between normal and pathogenic flora. Similarly, the airborne test type also received a score of 90, this time, because this type of test relies exclusively on human interpretation of the sample.

To score portability, the higher the score, the greater the portability, ranging from 0 (a fixed device) to 100 (completely table top or handheld). Based on this score system, the airborne test type received a score of 0, because the housing is completely fixed and would have to be installed within the interior of the cabin. Both the ATP and rapid antigen test types received a score of 100, as both are considered completely handheld devices and could be easily adapted to a tabletop device.

Cost was scored with the consideration of both initial investment and regular cost in mind from 0 (completely outside of a depot’s budget) to 100 (completely low cost and within a depot’s budget). Rapid antigen test type was assigned a score of 80, as these test types are relatively cheap; however, some of the antibodies required can be pricier, depending on the antigen that is being detected. The ATP test received a score of 50, due to the heavy upfront initial investment
on the electronics component—however, our incorporation of the Smartphone application and new sensor does decrease initial investment. Airborne test type was given a score of 30, due to cost of installation and cost of extra training and/or outside labor.

All device types received a score of 100 for the regulations objective. Each device type is in accordance with current regulations and used in some aspect of the health care industry and/or related and supporting networks (i.e. pharmaceutics, biological clean-up crews).

Finally, time required for testing was scored. Scores were gauged on a scale of 0 (test time greater than a few hours) to 100 (test time less than 5 minutes). The ATP test type received a score of 100 as ATP testing can be easily conducted and interpreted in 5 minutes or less. On the other hand, the airborne type was given a score of 0, because culture accumulation and analysis could take hours if not days. The rapid test type was assigned a score of 70, as sample collection would take no longer than sample collection for the ATP test type; however, many tests on the market have a wide range of time required for interpretation.

Table 3: A numerical evaluation matrix was created to compare types of detection systems currently available on the market today.

<table>
<thead>
<tr>
<th>Design Constraints (C) &amp; Objectives (O)</th>
<th>ATP Hygiene</th>
<th>Airborne</th>
<th>RADT</th>
</tr>
</thead>
<tbody>
<tr>
<td>O: Easy to dispose of</td>
<td>80</td>
<td>30</td>
<td>80</td>
</tr>
<tr>
<td>O: Easy to use</td>
<td>70</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>O: Detect Contaminants</td>
<td>90</td>
<td>90</td>
<td>30</td>
</tr>
<tr>
<td>C: Table-top/portable</td>
<td>100</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>C: Inexpensive</td>
<td>50</td>
<td>30</td>
<td>80</td>
</tr>
<tr>
<td>C: Meet regulations</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>C: Test time &lt; 5 minutes</td>
<td>100</td>
<td>0</td>
<td>70</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>590</strong></td>
<td><strong>290</strong></td>
<td><strong>540</strong></td>
</tr>
</tbody>
</table>

After each category was scored, the values for each test type were totaled. Airborne types received a total score of 290. The total score for the rapid antigen test type was 540. The ATP
test type was found to score the highest overall, with a total score of 590. With this information, the ATP test type was determined to be the best class of device to pursue during the design process.

3.2.1.2 – The initial design process

In designing an ATP detection method, the team needed to be careful not to infringe on current patents and other protections of similar technologies. At this point in the design process, brainstorming began, discussing possibilities such as optimizing the ratio of luciferin to Luciferase and lysis buffer, and the use of a lens to focus the light at a receptor.

At this point, the team began to discuss with the clients other ways to differentiate our design from current products on the market. The team, as the design’s objectives reflect, also wanted to create a device that was more user-friendly and accessible to EMS personnel. One means discussed for accomplishing both overall goals was via the creation of a Smartphone application, to make our test results easier to interpret. Specifics as to our final and alternative designs will be discussed in further detail in the body of Chapter 5.

However, even before our designs were fully established, the team did have some idea of supplies that would be needed and what types of testing would need to be conducted. Each aspect of the device and the process would be needed to be tested separately. Aspects that needed to be tested included swab technique, luciferase/luciferin ratio, and means of detecting the illumination produced, means of signal amplification, signal output, and method of interpretation. The final device would be compared to the validation study conducted on Charm Sciences’ product (Turner, et. al., 2010).
3.2.2 – Management Approach

As the team was to establish and test a complete design within one academic year, time management was of particular concern, and time was considered a major project constraint. Work was to be split and assigned to team members as needed throughout the course of the project and design process. However, basic tasks and timeframes were established early on.

3.2.2.1 – Work Breakdown Structure

To establish a framework for the design process that should be engaged, a work breakdown structure was created as seen below in Figure 3. Once all the steps of the design process were established, this gave the team goals to work towards and a framework for keeping the project on target. During the design process, it is easy to expand the scope beyond what is feasibly accomplished with limited time and/or other resources. Thus, a plan and a true understanding of the problem is a crucial step to the design process.

Figure 3: A work breakdown structure was created to establish the needed steps during the design process.
### 3.2.2.2 – Gantt Chart

After a basic framework and research areas were established, a Gantt chart was created to map a timeline of these tasks that needed to be accomplished. A completed Gantt chart can be seen in Figure 4 below and outlines when during the course of the project the task was to be completed. The Gantt chart was divided into four academic terms to compare whether the workload was well balanced across the allotted times.

**Figure 4:** A Gantt chart was created to establish a timeline for assigned tasks and report writing.
This Gantt chart is an example of the team’s progress during the last few weeks of C term. A different color was assigned to each task based on whether it was completed, currently being worked on, or to be completed. As can be seen, the team remained on schedule up until that point.

3.2.3 – Financial Approach

3.2.3.1 – Cost Breakdown

With fewer members than is typical, the project team also had an incredibly limited budget. The Biomedical Engineering Department allots $156 per person, not taking into account a cumulative lab fee of $100, leaving $212 available for supplies needed that are not available within the assigned lab. Based on the technical approach previously outlined, a rough list of supplies was drawn up and approximate costs were assigned based on costs discovered during the research process and prices detailed on supplier sites. The lab fee was included for the cost breakdown to account for all supplies we would likely be using. A full cost breakdown can be seen in Figure 5 below.

<table>
<thead>
<tr>
<th>Item</th>
<th>Order through</th>
<th>Our Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Electronics</strong></td>
<td></td>
<td>$5.00</td>
</tr>
<tr>
<td>Photovoltaic Photodiode PIN-S0P/3B</td>
<td>OSI Electronics</td>
<td>FREE</td>
</tr>
<tr>
<td>Non-Inverting Op-Amp</td>
<td>Professor O’Rourke</td>
<td>FREE</td>
</tr>
<tr>
<td>Circuit board, microprocessor, capacitors</td>
<td>BME Dept.</td>
<td>FREE</td>
</tr>
<tr>
<td>10 Mega-ohm Resistors</td>
<td>WPI</td>
<td>5.00</td>
</tr>
<tr>
<td><strong>Biologics</strong></td>
<td></td>
<td>$11.10</td>
</tr>
<tr>
<td>Sample Swabs</td>
<td>Charm Sciences</td>
<td>FREE</td>
</tr>
<tr>
<td>Rat’s blood, feces, urine, mammalian cells</td>
<td>WPI</td>
<td>FREE</td>
</tr>
<tr>
<td>Pure ATP</td>
<td>Sigma-Aldrich</td>
<td>10.10</td>
</tr>
<tr>
<td><strong>Lab fee</strong></td>
<td></td>
<td>$100.00</td>
</tr>
<tr>
<td>6-Well, 24-Well, 96-Well Plates</td>
<td>Included</td>
<td>--</td>
</tr>
<tr>
<td>D.I. Water, Saline, Media</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Pipettes, Other Equipment</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td>$176.08</td>
</tr>
<tr>
<td>Lens</td>
<td>Edmund Optics</td>
<td>$90.00</td>
</tr>
<tr>
<td>3D Printing/Prototype</td>
<td>WPI RF Machine</td>
<td>$64.08</td>
</tr>
<tr>
<td>Shipping</td>
<td>--</td>
<td>$15.00</td>
</tr>
<tr>
<td>Reflective Spray Paint</td>
<td>Krylon</td>
<td>$7.00</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>$292.18</td>
</tr>
</tbody>
</table>

Figure 5: A cost analysis was completed in order to ensure the teams design was kept within the two person budget.
Cost analysis was broken down into electronics, biologics, lab fee, and any other projected expenses. For electronics, sample parts were priced and identified where they could be obtained. Biologics included the luciferase assay, pure ATP, and body fluids (rat’s blood, urine, etc.). Luciferase was determined to be obtainable from Promega, while any bacteria could be obtained from a supplier such as American Type Culture Collection. Rat fluids could be obtained from WPI’s physiology class, which uses female Sprague Dawley retired breeder rats from Charles River Laboratories for surgeries and monitoring of vital signals.

Total cost was estimated at $292, approximately twenty dollars under budget. At the time the budget was established, we hoped to reduce this cost by ordering through WPI and the help of Lisa Wall, thus permitting the allocation of more funds to electronic components. The $100 lab fee would include media, pipettes, and any other basic supplies needed. Finally, $64 was appropriated to expected rapid prototyping of the housing for the final device. The cost of prototyping was covered by Mustapha Fofana of Mechanical Engineering. However, the generous donation received from Bill Ciaccio of Aftermath Inc. did not provide enough to complete biological testing. Therefore the team had to order a luciferin-luciferase assay that was $167, putting the team about $80 over budget. The entire cost of design and testing was thus estimated at around $457 while prototype assembly costs were estimated at about $200.

3.2.3.2 – Cost Considerations in Industry

During the actual production of the device (i.e. if the device were to ever hit the market), the cost of lab supplies and biologics would be minimal per device, as the device would need to be tested during quality control, but not as extensively as our initial product. The largest cost for production would be the initial cost of any machining equipment needed for the electronics housing. Following this investment, materials and labor per device are projected to remaining
relatively low, due to bulk ordering and minimal complexity of the housing. Thus, the marketed price and cost to ambulance depots would be able to remain within the target of a few hundred dollars, initially, with low cost of maintenance and cost per test.
Chapter 4: Alternative Designs

4.1 Needs Analysis

The device needs to have a sensitivity level reasonably within the same range as similar products on the market. Our device should be able to detect ATP at one less 10-fold dilution than equivalent detection devices. By detecting gram-positive bacteria, the device is intended to detect significant amounts of contaminants still present after cleaning, including gram-negative *S. aureus*.

Test time needs to be comparable to that of other ATP testing devices on the market for a fraction of the cost. Ideally, the testing time should be less than 5 minutes. Overall, testing needs to be easy to complete and should not detract a significant amount of time and resources.

We would like the device to match the sensitivity level of current counterparts on the market. However, given the other constraints and needs, a sensitivity within 10-fold of competitive products is acceptable. Where a certain level of detection can be accounted for by normal flora, the final cutoff value for a positive readout can be adjusted based on final sensitivity level.

4.2 Design Process

4.2.1 Device Functions

Once needs were established, functions were discussed to determine what the device will specifically do. The device was determined to have four main functions that must be met. These functions and potential means were entered into a morphological chart to analyze and confirm some of the team’s initial design ideas. This chart can be seen in Appendix D.
First, the device collects a sample reflecting flora and possible pathogens still present in the environment. As discussed in Chapter 3 under detection test types, there are 3 main ways of accomplishing this. Sample collection may either be conducted via agar plate, a cotton swab, or microfluidics (a small sera sample). For our purposes, as previously discussed, an agar plate would produce excessive waste and would only collect contaminants that are present in the air. Microfluidics, while a promising emerging field in diagnostics, did not seem appropriate given the fact that most contaminants present in the ambulance will have dried and hardened on to surfaces. Pools of blood are easily visible and cleaned. Dried blood (and other fluids) and corresponding pathogens are the contaminants that are overlooked and are of concern for our project. Thus, a cotton swab remained the most viable and logical option. Cotton swabs allow for collection of both wet and dry contaminants, take up minimal space, and produce minimal waste.

Second, the device causes a biochemical reaction with some substrate-enzyme reaction indicating presence of living organisms. Two main means of accomplishing this would be through the use of a flow assay or through a luciferase assay. Florescent tagging could be used to assist in microscopy indication of contaminants. The simplest and cheapest of these means, as determined through the team’s research, appears to be the luciferase assay. There is no cure time or complicated conjugation methods. The components directly react with ATP molecules present in the contaminants.

The device then detects and quantifies the level of the reaction. Corresponding means for the three types of detection tests (airborne, rapid antigen, and ATP hygiene) for this step involve a microbiologist, colored antibodies, and a photometer, respectively. As discussed in Chapter 3, device use should avoid the need for outsourcing samples and labor. Colored antibodies have
limited accuracy and sensitivity. A photometer, on the other hand, can provide varying levels of sensitivity desired depending on the grade of the component.

Finally, the device converts raw data to a positive or negative readout. This readout can be a positive test line, similar to a pregnancy test, can be converted into an electronic message via voltage conversion, or can be sent from a microbiologist. Again, the use of a microbiologist would require labor outsourcing, and the team would rather have all functions of the device self-contained, thus leaving the only human interpretation to read that the ambulance “positive” or “negative” for contaminants based on the device’s outputs. A positive line test is relatively simple and straightforward. However, the use of an electronic means to convert and display the final result does allow for long-term data collection and has the potential to minimize tampering with testing and compliance.

This preliminary analysis of functions and means thus confirmed the team’s initial idea to pursue an ATP hygiene type of detection device. With the creation of this type of device, all of the functions could be self-contained and completed on site within the ambulance hanger. There would be no need for human analysis of contaminants present, only interpretation of an electronic reading indicating a “positive” or “negative” test, with positive indicating the need for further cleaning.

Table 4: Function-Means analysis of the initial design.

<table>
<thead>
<tr>
<th>Function</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collects a sample reflecting flora and pathogens present</td>
<td>Agar Plate</td>
</tr>
<tr>
<td>Causes a biochemical reaction</td>
<td>Flow Assay</td>
</tr>
<tr>
<td>Detects and quantifies level of reaction</td>
<td>Microbiologist</td>
</tr>
<tr>
<td>Converts raw data to a positive/negative readout</td>
<td>USB with software</td>
</tr>
</tbody>
</table>
Now that the type of device had been confirmed, a second morphological chart was created to analyze functions and potential means specifically applicable to the detection of ATP present in energy-containing contaminants. This chart can be seen in Table 5.

In this analysis, the first function remains as previously established; the device collects a sample reflecting flora and possible pathogens still present in the environment. Possible means for this include a cotton swab or a small amount of sera via microfluidics. Of these, a cotton swab was preferred. As previously discussed, most remaining contaminants will have dried and/or be located in crevices of devices within the ambulance. There are not liquid samples readily available. Samples could be collected and mixed with a buffer. However, using this method, there was extensive concern that this methodology would dilute the sample too much and would not produce an accurate depiction of the amount of pathogens still present.

The device must then detect the amount of ATP present. Numerous means for this were considered and researched, including the use of a Luciferase assay, florescent binding nanoparticles, and aptamer chain detection. Using a Luciferase assay was determined to be the simplest of these means, as the amount of bioluminescence emitted is proportional to the amount of contamination present. This method was preferred to microfluidics as each test can be performed relatively inexpensive – approximately $2.50 per test.

This reaction, however, must be instigated by some means. Thus, the device needs to combine detection ingredients with the collected sample. Identifying means for this function, unfortunately, proved substantially more difficult. One means, for example, would be to puncture a cartridge with the cotton swab, thus releasing the assay ingredients with the collected sample.
This means/method, however, has been patented by Charm Sciences®, one of the current market competitors.

The device then concentrates the luminescence created by the reaction onto the sensor. Some of these means, such as the used of mirrors, have been patented. The use of a lens focused at the photomultiplier tube or photodiode was also discussed. The signal produced by the luminescence and measured by the sensor can also be electronically amplified by an operational amplifier, which magnifies the voltage of a signal depending on the resistance used in conjunction with the diode and op-amp. This will later be discussed in the electronic design portion of this chapter.

After the reaction occurs, the device detects the luminescence produced. Two main means of accomplishing this are with a photodiode or a photomultiplier tubs. While a photomultiplier tube is used in most handheld luminometers on the market today, they are expensive – ranging between $1000 - $2000 dollars. For this reason, a photodiode was chosen as the means of detection. A corresponding function-means analysis for the circuit component of the design can be seen in Table 5.

Table 5: Function-means analysis of the photodiode circuit component of the team’s design.

<table>
<thead>
<tr>
<th>Function</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detects luminescence &amp; outputs signal</td>
<td>Avalanche diode</td>
</tr>
<tr>
<td>Amplifies signal</td>
<td>Op-amp</td>
</tr>
<tr>
<td>Reduces noise</td>
<td>Op-amp orientation</td>
</tr>
<tr>
<td>Records analog data</td>
<td>A/D Converter</td>
</tr>
</tbody>
</table>
Finally, the device converts the raw data into a meaningful format to alert the tester of a positive or negative reading. Currently, competing products convert the detected voltage into “RLUs” and display this number on an electronic screen. The team hypothesized this feature and storage abilities account for the high price of current products. Therefore, we discussed alternative means to this feature, which are given in the function-means chart. Due to the accessibility and availability of Smartphones currently on the market, the team decided to use an Android™ application in conjunction with an Arduino™ board as an analog-digital (A/D) converter. This function, however, must be broken down into sub-functions detailed in the function-means table below.

Table 6: Function-means analysis of the microprocessing component of our design.

<table>
<thead>
<tr>
<th>Function</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Records analog data</strong></td>
<td>Arduino™</td>
</tr>
<tr>
<td><strong>Converts to digital data</strong></td>
<td>Arduino™</td>
</tr>
<tr>
<td><strong>Filters data (digital)</strong></td>
<td>Limited intervals</td>
</tr>
<tr>
<td></td>
<td>Processing (before transfer)</td>
</tr>
<tr>
<td></td>
<td>Android™/Java</td>
</tr>
<tr>
<td><strong>Communicates data from Arduino™ to Android™</strong></td>
<td>USB</td>
</tr>
</tbody>
</table>

The Smartphone application itself must perform several functions. It was determined that the application would require a login screen to identify and associate a personnel member with each use of the device. The application would also need to be capable of saving and retrieving data from the Arduino™ and from Cloud storage. Finally, the application would need to process the data and alert the user of the results. The functions required for this application are listed and mapped out in Table 7 below, along with the corresponding means of accomplishing these functions.
Table 7: Function-means analysis of the Smartphone application.

<table>
<thead>
<tr>
<th>Function</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prompts user for identification</strong></td>
<td>Login Screen</td>
</tr>
<tr>
<td></td>
<td>Spinner Menu</td>
</tr>
<tr>
<td></td>
<td>Text Field Inputs</td>
</tr>
<tr>
<td><strong>Retrieves data from Arduino™/Cloud</strong></td>
<td>Bluetooth™ communication</td>
</tr>
<tr>
<td></td>
<td>Wi-Fi</td>
</tr>
<tr>
<td><strong>Interprets data</strong></td>
<td>If… then…</td>
</tr>
<tr>
<td><strong>Displays message with results</strong></td>
<td>Toast</td>
</tr>
<tr>
<td><strong>Backlogs data to Cloud</strong></td>
<td>Alert Dialog</td>
</tr>
<tr>
<td></td>
<td>Wi-Fi</td>
</tr>
</tbody>
</table>

Once functions and means for the app had been established, a concept sketch was also drawn out using a combination of Microsoft PowerPoint and Paint. Figure 6, below, shows this concept sketch of the original application layout design. The main app functions to be initialized by the user, syncing, data collection, and access to backlogged material need to be clearly and visibly accessible by clearly labeled buttons. The initial concept also included a settings menu for calibration and backlogging data.

![Concept Sketch](image)

**Figure 6: Original concept sketch for the Smartphone application.**
The team also chose to incorporate a Cloud data storage application in order to take processing one step further – in that the cleaning personnel isn’t just given an initial cleaning assessment, but also has the capability to follow trends by reviewing stored data. This was done through a Cloud system. A function-means analysis of the Cloud application can be seen in Table 8.

Table 8: Function-means analysis of the Cloud application.

<table>
<thead>
<tr>
<th>Function</th>
<th>Stores user/organization id/data</th>
<th>Backlogs sample data</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stores user/organization id/data</td>
<td>UserID associated w/cleaning</td>
<td></td>
<td>UserID associated w/user</td>
</tr>
<tr>
<td>Backlogs sample data</td>
<td>Save/Retrieve function</td>
<td></td>
<td>Pull from Smartphone app</td>
</tr>
<tr>
<td>Allows graphing by day, ambulance, depot</td>
<td>Single vehicle graphs over time</td>
<td></td>
<td>Series graphs w/multiple vehicles</td>
</tr>
</tbody>
</table>

4.2.2 Device Specifications

In order to be competitive with ATP Hygiene tests on the market today, our design needs to have similar specifications and levels of sensitivity. A main goal of the design is to produce an ATP monitor that can be sold for only a few hundred dollars. The price per test should be less than $2.50. Total test time should be less than 2 minutes, with a instrument read time of less than 30 seconds. Storage temperature of the swabs should be between freezing and 25C. Shelf life of the swabs should be at least 6 months. The instrument should be self-calibrating, and should be able to detect ATP in the range of 1.98-100,000 femtomoles. The instrument should be easy to transport, weighing less than 1 pound and be relatively small (20x10x5 cm).

Testing should require no more than 5 minutes for swab collection and readout. This includes a reaction and read out time within the instrument of less than 60 seconds. With a brief
test time, EMS personnel are more likely to comply with regular use. The testing should be completed promptly after cleaning to determine if more cleaning is necessary. With a short test time, it would also be easy to complete testing before all cleaning materials are returned to storage.

Storage of swabs should be feasible between 0 and 25º C due to the fluctuating temperature of the ambulances and depot. The readout device should be stored within a reasonable range at about room temperature, similar to other electronics. Likely, the housing and electronics for analysis would be stored in an office within the depot or within the depot hanger space. Individual swabs, however, would be likely stored within each ambulance for easy access.

Shelf life of the swabs should be at least 6 months. To reduce cost, ambulance depots would most likely purchase swabs in bulk. In order to minimize shipping waste, each purchase should last a considerable amount of time. The depot would only want as many on hand as could be easily stored, but the less the swabs need to be ordered regularly (weekly or monthly) the better.

4.3 Simulations

Once the team decided to use a photovoltaic photodiode as the detector and an op-amp as the amplifier, simulations were run in order to verify circuit design. The manufacturer of a potential photodiode suggested operating the circuit with a rail-to-rail op amp under 15 volts supply. However, since the team was trying to keep cost low and assembly easy, a 9V battery was the ideal power source for the device. The team ran simulations using Circuit Lab simulation software in order to determine appropriate values for input voltage and other circuit parameters such as resistances and capacitors. Below is a screenshot of the simulation software.
The team determined through simulation that a voltage of 5V input to the op amp could be used, with a high value resistor (in orders of Mega ohms) with minimal effects on the sensitivity of the device. This simulation shows that low voltage can be used in conjunction with high gain. As can be seen in the figure. The minimal input voltage to the LED circuit on the right, 1.9V, results in a detectible output from the photovoltaic diode circuit (25mV). While the sensitivity is decreased from the 15V supply, the team determined that this was an acceptable range to begin testing.

Figure 7: Circuit simulation using Circuit Lab software.
Chapter 5: Final Design

5.1 Proposed Housing Design

For the scope of this project, the team decided to focus on the actual detection portion of the device and to design this around the current Charm PocketSwab®, as opposed to redesigning both the ATP-assay system and the detection. Below is a SolidWorks® diagram of the proposed swab containment chamber or housing, based upon the functions and specifications outlined in Chapter 4.

![Figure 8: Isometric view and cutaway view (left to right) of the sample housing](image)

The main function of the housing is to hold the swab purchase by Charm Sciences Inc., containing the bioluminescent reaction and keep the photodiode in a consistent location. In order to hold the swab, the chamber had to be about 1 inch wide and 4 inches tall. A rubber washer would be used to keep the swab in place. To keep the diode in place, two small holes were
drilled through the electronics housing (the base of the design). The column of the swab chamber would be attached to the electronics housing via screws for ease of assembly.

The sample chamber, or housing, must also concentrate the light emitted from that reaction onto the photovoltaic photodiode. One means of doing this is through the use of a polycarbonate film coated with a highly reflective paint on the interior of the chamber. Another means is through the use of a concentrator lens, with an input diameter equal to that of the output diameter of the vial (1cm) and an output diameter equal to that of the input diameter of the photodiode (2.5mm). The housing must also hold the circuit and microcontroller used to measure the light intensity, along with a power supply. The housing must be easy to take apart and reassemble for quality assurance and repair purposes, if needed.

The placement of the swab mechanism in relation to the concentrator lens is important, as the bottom of the vial should be as close to the top of the lens as possible. This is achieved through various lips within the interior of the chamber as well as securing the vial with a rubber gasket.

The housing must also keep out all ambient light and dust in order to prevent contamination of the lens and over-powering the circuit. For the prototype, this is achieved through the use of a simple cap. However, for the next design a mechanical or electric switch should be used that make it impossible for the circuit to be powered if the swab is not present within the chamber. To prevent any room light from getting in to the chamber during the reaction and reading collection, a rubber washer was used. Thus, the washer serves not only to keep the swab in place, but to keep out ambient light as well.
5.2 Photodiode Circuit

The main function of the circuit is to detect the bioluminescent react and convert that light intensity into a proportional output voltage. Since the light intensity is so small in this reaction, the signal must also be amplified by means of a non-inverting op amp. This op amp, along with the use of a microcontroller and high pass filters such as capacitors, serve to reduce noise found within that signal. The circuit also incorporates a microcontroller in order to record the analog data measured from the output voltage terminal and convert this into a digital signal for further processing by said microcontroller. The final circuit design can be seen in Figure 9 below.

![Photodiode Circuit Diagram](image)

Figure 9: Photodiode circuit used in conjunction with an Arduino™ microcontroller.

Here, the values for $C_F$ and $C_A$ were calculated using the following equation:

$$\sqrt{\frac{GBP}{2\pi R_F(C_F+C_A)}} > \frac{1}{2\pi R_F C_F}$$

Equation 2

where GBP is the Gain-Bandwidth Product, $R_F$ is the feedback resistor, $C_F$ is the feedback capacitor, and $C_A$ is the amplifier capacitor. The means of detection selected for this circuit is a photovoltaic photodiode. This photodiode must be able to detect a light intensity between .01 and 1 mW/cm$^2$. 
This is achieved both through the use of appropriate resistance values, and in the housing design previously mentioned. Specifically, a blue-enhanced diode was chosen due to the more linear behavior the photodiode displays over the 400 to 600 nanometer range, compared to a normal photodiode. Though the luciferase reaction often consistently emits at 520 nm, there is a range over which the reaction occurs. Due to the good range and higher sensitivity over this range, the blue enhanced series photodiode was chosen as the best alternative to an avalanche diode, which could not be afforded by the team due to strict budget constraints.

5.3 Data Collection/Processing

The Arduino™’s main function is to convert the analog signal retrieved from the circuit and convert this into digital data that could eventually be accessed by the Smartphone application. Specifically, the Arduino™ achieves this function by initiating a serial connection and looping input collection. The code for the Arduino processing can be seen in Appendix E.

Before the Smartphone application can retrieve this data, however, filtering and preprocessing must occur. Filtering should be done in order to further eliminate noise that was not already filtered by the capacitors, as well as perform readings on limited, pre-set intervals. Here, input collection relies pre-set interval of one tenth (1/10) seconds or collection every 100 milliseconds. Baud rate on the microprocessor is set to 9600, to optimize performance. Preprocessing must be done in order to limit the processing required by the application. Here, the raw data value is converted to a float value representing a voltage between 1 and 5 volts before being transferred to a Smartphone application. Min, max, and average values will later be calculated.
5.4 Application/Interface Design

5.4.1 Android™ Application

To replace and expand upon the Charm® system’s current on-board computing, a Smartphone application was designed to communicate with the Arduino™ via Bluetooth™ serial communication. Coding was conducted using a combination of Java, to dictate sequencing of activities, and XML, to format the layout of the graphical user interface.

The Android™ application serves to facilitate user integration although proof of concept can be established without the use of a complex graphic user interface. For proof of concept and validation purposes, the Arduino™ serial monitor and/or a java applet were used. To verify that the Android™ application was working, on the other hand, a basic circuit was used in conjunction with an application emulator and a Motorola Droid phone. However, the Smartphone application is still a crucial component of the final design. Once data has been collected by and recorded on the Arduino™, this information must be retrieved and translated into data that is meaningful to and readable by the user (i.e. the paramedic).

There are a number of steps or functions that must occur in order for this to happen. First, once opened, the application must initiate communication via a Bluetooth™ connection. Once the channels are open between the Arduino™ and the Android™ application, data can be transferred in either direction. Hardware ports are utilized on the Arduino™ to facilitate Bluetooth™ communication, and a baud rate of 9600 is again used. The same connection necessity applies for parsing the data after collection; the connection must be initialized in order for data to past freely between the phone and the Cloud. Figure 10 below show the final prototyped main screen for the application, along with a summary of specifications.
Figure 10: Final application design with specifications

Once the connection has been established, the Android™ sends data to the Arduino™ to initiate the needed Arduino™ sketch, in this case, ReadAnalogVoltage, which performs the data collection and pre-processing functions earlier described. As the sketch runs, the Android™ retrieves data from this sketch in the form of a float. Data is collected for approximately 30 seconds, at 10 readings per second. The data is then processed, selecting the minimum and maximum values and applying a series of “if… then…” statements to determine if the level of voltage is high enough to require a second cleaning. These voltages could also be converted to a series of relative light units (RLUs), which relate the reading to a base level. Once data is collected and processed, the application then displays these values (reading number, maximum reading, and minimum reading) on-screen and prompts an alert mechanism notifying the user what to do. An alert dialog box is dispatched to prompt the user, informing the user of one of three options, that a) cleaning is considered sufficient, b) cleaning should be repeated, or c) the results were inconclusive and a second sample/reading is required. These dialogs are also color-coordinated similarly to a street-light for easy interpretation (i.e. green indicates that cleaning is sufficient, while red indicates potentially hazardous levels of remaining contamination). After
the reading is complete, the application gives the user the opportunity to dismiss the alert, and a “zero” button is available to reset the min/max values.

Beyond the main function of the Android™ application, data collection and user notification, there are a number of other tasks/functions the application must complete and that must be accessible to the user. Upon opening the application, the device prompts the user with a login screen, which allows the user to pick one of two options. The user can either register as a user within an organization or log in to the application. This register/login function allows any readings to be associated with a UserID tied to that specific personnel and an OrganizationID which ties the user to their respective depot or organization. This screen and set of activities is to provide an organizational structure under which the collected data is organized as well as provides a level of security to the application.

One logged into the main screen, the application grants the user the opportunity to select which ambulance is being sampled and where the sample was collected from via a series of drop-down menus known as spinner widgets. The list of vehicles is pulled from the Cloud based on the organization with which the user has been associated. The location menu, on the other hand, is a set list of locations, primarily the areas of the ambulance that are most prone to residual contamination (i.e. the bench, seatbelt, etc.). However, if necessary, this list could also be modified upon user request. User inputs also allow the user to note any particular circumstances that preceded (i.e. during dispatch) or occurred during the cleaning (e.g. excessive blood present due to a gunshot wound (GSW), volatile patient, different cleaner used, longer cleaning time than usual). This input is perhaps the most important piece of information for later administrative monitoring and cleaning analysis, as this helps identify trends in cleaning and faulty equipment and protocol as will be later explained. This information associated with the TestID assigned to
each reading. All this information is also linked to the selected vehicle via a VehicleID, allowing further organization of all collected data. Figure 11 shows the login and main screens side by side. The “Sync” button initializes communications, while the “Read” button initializes actual data collection. User input at the top allow for custom notes to be associated with each reading.

![Figure 11: Final prototype screens of Smartphone application](image)

On the device, application records allow the user to view the past 20 readings that have been collected, in case they are curious about overall cleaning effectiveness or are wondering why some reading was so far off from another. This feature is accessible via the “Prev. Tests” button. Graphing capabilities and overall trends, however, will be reserved for administrative access and organization via the Cloud, through the online application platform, Parse. Data, however, is initially saved on the phone before the results are pushed to the Cloud application.
5.4.2 Cloud Application

As mentioned, an online platform and Cloud application will be responsible for administrative oversight of the operation and backlogging of collected data. The Cloud application stores all registered data associated with each UserID as well as their corresponding organization. Through a series of save/retrieve commands, this complementary application also records and stored backlogged data sent from the Android™ application. Once uploaded, each cleaning results package of data is associated with a date, time, user, vehicle, device, and organization. These associations will later make it possible to organize data and to identify problems within the current protocols within an organization. To design the structure and organization of this Cloud application, a combination of JavaScript, HTML, and CSS languages with Bootstrap and Boostrap-editable libraries were used for data storage and retrieval, parse communication, and aesthetics.

The Cloud application also allows the depot head (i.e. head/senior paramedic) to input and modify information about the depot and each of the depot’s vehicles. This way the ground user (i.e. paramedic conducting testing) need only select which organization he/she belongs to and which vehicle is being cleaned. Similarly, the Cloud app allows the depot head to make any special note about vehicles, such as any modifications made and/or any damages to equipment that may affect effectiveness of cleaning solutions and protocols. Again, these functionalities will allow the administration to better identify trends and breakdowns in cleaning protocols so they may be remedied as swiftly as possible.

Since all data is saved within the Cloud, this app grants access to all previous data since the purchase of the main device. With guiding user input, the app also graphs trends amongst readings, examining either one vehicle or the entire depot. This function allows the admin to
observe trends amongst users, within vehicles, and across the depot overall. Thus, the app also tracks and raises any red flags (i.e. repeated readings indicating excessive bacteria, coincidence between multiple vehicles). Figure 12 demonstrates the graphing feature of this application.

![Graph of trends](image)

**Figure 12: Cloud applications with graphing features for trend analysis**

With this feature, any problematic trends will be brought to the depot head’s attention, and the application gives the admin the tools to determine whether the red flags are indicative of a possible outbreak and/or if these trends are symbolic of a faulty cleaner, inconsistent protocol, and/or vehicles that are more susceptible to bacterial accumulation. The sooner problems with protocol, materials, and cleaning solutions can be established, the sooner they may be remedied. The sooner these problems are remedied, the lower the risk of pathogenic infection both to personnel and patients. Thus, the Cloud app was designed to really serve as a public health assessment tool and to maximize the impact of this device.
Chapter 6: Design Verification

In order to validate our designs, two types of validation experiments were performed. The first was a variable output LED calibration, where the device measured a known light emission from the LED, shown in mW/cm². The second was a series of biological luminescent reactions with a luciferase-luciferin reaction testing five different supplies of ATP – pure ATP, red blood cells, rat urine, and rat feces.

6.1 Materials and Methods

Animal use. All tissues were collected from animals maintained according to approved protocols. All samples were collected after euthanasia.

RLU determination. A 10-µL aliquot of each test substance, with 5 replicates per substance, was micropipetted into the tube of the PocketSwab Plus® collection chamber.

ATP. Purified ATP (Sigma-Aldrich, St. Louis, MO) was used to determine the limit of detection, in terms of device sensitivity, of our design. The manufacturer supplied kit contained lyophilized standard disodium salt trihydrate ATP and dilutions were prepared according to the manufacturer’s recommendation.

Laboratory contaminants. Blood was collected from a freshly euthanized rat, placed into a 15 mL centrifuge tube, and combined with an anti-clotting agent and stored at -20C until testing was performed. Upon thawing, blood was serially diluted with PBS in order to form concentrations of red blood cells of 7.0x10⁷, 7000, and 700.

Feces were diluted 1:1 (w:v) with PBS and homogenized. Serial dilutions were for concentrations of 10⁻⁴g, 10⁻⁵g, and 10⁻⁶g of feces.
Approximately 3 mL of urine was collected from a freshly euthanized rate and placed into a 15 mL centrifuge tube. The sample was serially diluted to concentrations of 1:100 and 1:10.

6.2 Results

In this section, the results of the validation testing will be presented. As mentioned, three types of validation were performed – a variable output LED calibration, biological validation testing for limit of detection, and a Smartphone application verification.

6.2.1 Variable Output LED

In this experiment, a variable output LED was used to find the design’s limit of detection, or LOD, in mW/cm². This was done by exposing the photodiode sensor to a variable output LED. The variable output LED circuit is shown in Figure 13 below.

![Circuit diagram for the variable LED circuit used in validation testing.](image)

The input voltage was calculated such that the current across the resistor increase by 2mA for each reading. The voltage values corresponding to this increase were measured using a digital multimeter, or DMM, and were thus used in all following experiments. Voltage across the diode was also measured using a DMM and recorded in the Table 9. Calculated values for resistance
across the led, current across the led, power consumed by the LED, and light intensity emitted are also shown.

Table 9: Numerical and calculated values for the variable LED circuit.

<table>
<thead>
<tr>
<th>I R (mA)</th>
<th>Vin (V)</th>
<th>V LED (V)</th>
<th>R LED (Ω)</th>
<th>I LED (mA)</th>
<th>P (mW)</th>
<th>LI (mW/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1.9</td>
<td>1.83</td>
<td>52286</td>
<td>0.035</td>
<td>0.064</td>
<td>0.256</td>
</tr>
<tr>
<td>4</td>
<td>2.1</td>
<td>1.895</td>
<td>18488</td>
<td>0.103</td>
<td>0.194</td>
<td>0.777</td>
</tr>
<tr>
<td>6</td>
<td>2.4</td>
<td>1.957</td>
<td>8835</td>
<td>0.222</td>
<td>0.433</td>
<td>1.734</td>
</tr>
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<td>8</td>
<td>2.6</td>
<td>1.99</td>
<td>6525</td>
<td>0.305</td>
<td>0.607</td>
<td>2.428</td>
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</tr>
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<td>12</td>
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<td>1.071</td>
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</tr>
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<td>14</td>
<td>3.4</td>
<td>2.1</td>
<td>3231</td>
<td>0.650</td>
<td>1.365</td>
<td>5.460</td>
</tr>
<tr>
<td>16</td>
<td>3.6</td>
<td>2.125</td>
<td>2881</td>
<td>0.738</td>
<td>1.567</td>
<td>6.269</td>
</tr>
<tr>
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<td>3.9</td>
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<td>2488</td>
<td>0.869</td>
<td>1.879</td>
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<tr>
<td>20</td>
<td>4.1</td>
<td>2.186</td>
<td>2284</td>
<td>0.957</td>
<td>2.092</td>
<td>8.368</td>
</tr>
<tr>
<td>22</td>
<td>4.4</td>
<td>2.222</td>
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<td>1.089</td>
<td>2.420</td>
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<tr>
<td>24</td>
<td>4.6</td>
<td>2.245</td>
<td>1907</td>
<td>1.178</td>
<td>2.643</td>
<td>10.574</td>
</tr>
<tr>
<td>26</td>
<td>4.9</td>
<td>2.28</td>
<td>1740</td>
<td>1.310</td>
<td>2.987</td>
<td>11.947</td>
</tr>
<tr>
<td>28</td>
<td>5.1</td>
<td>2.301</td>
<td>1644</td>
<td>1.400</td>
<td>3.220</td>
<td>12.881</td>
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<td>30</td>
<td>5.4</td>
<td>2.335</td>
<td>1524</td>
<td>1.533</td>
<td>3.578</td>
<td>14.314</td>
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<td>32</td>
<td>5.6</td>
<td>2.358</td>
<td>1455</td>
<td>1.621</td>
<td>3.822</td>
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<tr>
<td>34</td>
<td>5.9</td>
<td>2.392</td>
<td>1364</td>
<td>1.754</td>
<td>4.196</td>
<td>16.782</td>
</tr>
</tbody>
</table>

Here, the resistance across the LED was calculated using the following equation:

\[
R_{LED} = \frac{R \cdot V_{LED}}{V_{in} - V_{LED}}
\]

Equation 3

This value was used to calculate the current across the LED, which was calculated using the following equation:

\[
I_{LED} = \frac{V_{LED}}{R_{LED}}
\]

Equation 4
Then, the current across the LED was used to calculate the power across the LED, which was done using the following equation:

\[ P_{LED} = I_{LED} \times V_{LED} \] 

Equation 5

Finally, the power across the LED was used to calculate the light intensity of the emission of the LED. This was done using the following equation:

\[ \text{Light Intensity} = \frac{P \times \text{Percent Efficiency}}{\text{Area}} \] 

Equation 6

where the power was calculated using Equation 5, a power efficiency of 20% was used for the led based upon the data sheet, and the Area corresponds to the surface area of the sensor. In this case, the surface area of the sensor was 0.05cm\(^2\).

Upon exposure to the light emission, the sensor’s resulting output voltage and output in relative light units was recorded. Relative light units for this system were based upon the amplification system used in both the circuit and microprocessor resolution. The resulting values can be seen in Table 10.
Table 10: Recorded output voltages of the photodiode circuit shown in Figure 13, along with corresponding RLU values, for each of the three design components.

<table>
<thead>
<tr>
<th>Without Concentrator Lens</th>
<th>With Concentrator Lens</th>
<th>Without reflective film</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vout</td>
<td>RLU</td>
<td>Vout</td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>0.01</td>
<td>0</td>
<td>0.01</td>
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<tr>
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<td>117965</td>
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<tr>
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<td>2457600</td>
<td>1.10</td>
</tr>
<tr>
<td>1.39</td>
<td>2732851</td>
<td>1.22</td>
</tr>
<tr>
<td>1.47</td>
<td>2890138</td>
<td>1.28</td>
</tr>
<tr>
<td>1.57</td>
<td>3086746</td>
<td>1.40</td>
</tr>
<tr>
<td>1.65</td>
<td>3244032</td>
<td>1.46</td>
</tr>
<tr>
<td>1.76</td>
<td>3460301</td>
<td>1.56</td>
</tr>
</tbody>
</table>

Baseline 0 V | Baseline 0 V | Baseline 0.08 V

A plot of the data shown in Table 10 can be seen in Figures 14 and 15, below – displaying output voltage and relative light units, respectively, as a function of emitted light intensity from the variable output LED circuit shown in Figure 13.
In Figure 14 above, it can be seen that the design alternative containing the polycarbonate reflective interior coating but not the concentrator lens was the most efficient design.

In Figure 15 above, it is again verified that the design alternative containing the polycarbonate reflective interior coating by not the concentrator lens was the most efficient design.
It was determined visually that the concentrator lens was inhibiting the efficacy of the design. In order to quantify how much, an average of the percent decrease from the most efficient design combination, without lens but with PC reflective interior was calculated. It was determined that, on average, 10% of light was being absorbed by the lens – which could not be compensated for by the amount of light concentrated onto the sensor.

6.2.2 Biological Validation

As previously stated, the team also performed a series of biological validation testing, using the luciferase-luciferin reaction kit along with the Charm PocketSwabs®, in order to determine if our design was indeed as sensitive as the Charm NovaLum™ system.

First, a limit of detection test was performed using the Charm PocketSwab® as the means of creating the bioluminescent reaction along with pure ATP, in accordance with the Materials and Methods section of this chapter. It was found that the lower LOD was 800,000 femtomoles of ATP, corresponding to an output voltage of 0.00488 V and an RLU value of 9594. A graph of the resulting ATP LOD data can be seen in Figure 16 below.

![Figure 16: Line chart showing a comparison between the limit of detections, upper and lower, of our design and the Charm NovaLum™ system.](image)

Femtomoles of rat blood, rat feces, and rat urine as detected by the Charm NovaLum™ were calculated using the following equation found in Turner et. al., 2010:

\[ RLU = 379.84 \times (\text{fmol ATP}) + 908.68 \]

Equation 7
As can be seen in the figure above, Urine, Feces, Blood, and epithelial cells all fall within the range of 100-100,000 femtomoles. Unfortunately, as our system was unable to detect less than 800,000 femtomoles, we were also unable to detect any biologics, regardless of dilution factor, in our testing.

6.2.3 Smartphone Application

To verify that the Android™ application was working properly, the application was run both in a Genymotion emulator and on a Motorola Droid Max Smartphone. Within the emulator, the phone was run both on API level 10, 14, and 17, working best on API 14 and up. Figure 17, below, shows the Genymotion emulator menu, from which the Ambulops application is accessible.

Figure 17: The Ambulops application for data processing was successfully installed on the Genymotion emulator device.

After installation, the app could be opened, and the user was prompted with a Login screen, as seen in Figure 18, which allows the user to register with a specific organization or login if already successfully registered.
Figure 18: The user can select an organization to be associated with and register a unique username/password. 

Once logged in, the user can select which ambulance they are collecting a sample from, where in the ambulance the sample was collected from, and note any important information about either the dispatch or cleaning (e.g. type of call, if a new cleaning solution was used, timing of cleaning). Figure 19, below, shows the steps the user would take for these inputs.
Users can now select and note A) ambulance, B) where the sample was collected, and C) any notable information about either the cleaning or dispatch.

After the proper inputs are selected, the user clicks “Read” to run a reading. At this point, the Droid Max was used to verify that the Bluetooth™ collection between the Arduino™ and the application was working correctly. Connection was successful, and data was transferred to the phone. The circuit in Figure 20, below, was used for app troubleshooting purposes, a simpler version of a photodiode circuit. For the purposes of the team’s actual sensor testing, the blue-enhanced circuit was used.

Figure 19: Users can now select and note A) ambulance, B) where the sample was collected, and C) any notable information about either the cleaning or dispatch.

Figure 20: A simpler photodiode circuit was used for troubleshooting purposes (Outside Science, 2012).
To ensure that other aspects of the app were working and to help accustom users to the graphical interface, the team also has the option to simulate a reading, in which case the data is not saved. Figure 21, below, shows the results from a reading as well as the settings menu.

Figure 21: Simulated readings could also be created with the application.

All data taken from the application was pushed to Parse.com for storage, later to be retrieved by a web application. Figure 22 shows the parse database prior to a series of readings.
Figure 22: A parse.com database was used for data collection.

Once all the user inputs had been set, whether in a simulated or actual data collection, a reading could then be initiating. From left to right, Figure 23 below shows the prompt initiated during a reading, the dialog instructing the EMS personnel how to interpret the results, and the min and max values collected.

Figure 23: From left to right, the user is prompted with A) an alert notifying that the sample is being collected, B) instructions and notification on the cleaning results, and C) the min and maximum readings.
Upon collected and analysis, users are given one of three dialog prompts as seen in Table 11. These prompts were designs to be an easy to understand review of the ATP test results, put into language that would be relevant to EMS personnel.

Table 11: Dialog Prompts for Cleaning Results

<table>
<thead>
<tr>
<th>Cleaning is sufficient! Good to GO!</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Results unclear… Please test again.</td>
<td></td>
</tr>
<tr>
<td>Warning! Unsafe level of bacteria detected… Clean again, and repeat testing…</td>
<td></td>
</tr>
</tbody>
</table>

Once the data from the reading is successfully collected, the reading data is saved as an array batch and pushed to parse. A new set of readings can be seen posted in Figure 24 below.

Figure 24: After collection, data is successfully pushed to the Cloud for later retrieval.
Upon being pushed to the Cloud, the data uploaded can be retrieved and manipulated in excel in order to create a trend analysis.

### 6.2.4 Cloud Application

To take this design a step beyond the Charm® system, a web app was designed, as mentioned for organizational and monitoring purposes. Figure 25 shows the home screen for this app. The app was run in Mozilla Firefox with a debugger as the application was developed.

![Image of the home screen of the Ambulops Administration app](image)

**Figure 25:** The home screen gives the user a number of options, including access to device troubleshooting and the option to create an organization.

The login tab on this panel allows the user to either sign up for an account or log in, as seen in Figure 26. Organization IDs would be given with the device at time of purchase.
Figure 26: For security reasons, the user must sign up to create an account before being allowed to access and edit data.

The organizations tab then brings up a list of organizations. For the purpose of proof of concept, some filler as well as some real organizations were added to the database as seen in Figure 27.

Figure 27: The “Organizations” tab brings up a list of organizations with basic information about each.
From here, the user can successfully select an organization to view more closely and/or to edit as seen in Figure 28. Login and security credentials should be assigned to each user to dictate access levels.

![Image of UMass Medical organization details](image)

**Figure 28:** Selection of a particular organization allows the view to access details about said organization as well as associated vehicles and devices.

From this page, the user can even select a vehicle to view and/or edit as seen in Figure 29. This can allow organization administrators to keep track of other vehicle information and usage/service patterns in addition to the accumulated cleaning information, thus, possibly saving the organization money. Please note that the support email is not yet, at the date of publication, a working email address.
As shown in Figure 30, editable text boxes were used for easy updates on organization and vehicle information, in case of error and proper record keeping. For the organization, this feature can be used in the event of change of address, and for vehicles, this was considered an important feature in order to add any necessary notations about wear and service of the vehicle.

During the initial admin setup and in the event of a new vehicle purchase, organization heads also have the option to add a vehicle, as seen in Figure 31. A similar form is to be used for organization creation.
Unfortunately, the most critical functionality of this application, the graphing and tracking functions, were not able to be ironed out completely. However, this development sets up a good platform for further development and organization of collected data.
Chapter 7: Discussion

7.1 Interpretation of Results

As can be seen in Figures 14 and 15, it was determined that the concentrator lens significantly decreased the efficacy of our design – by around 10%. This could be due to several factors. One factor contributing to the inefficiency of the concentrator lens was that the lens was aligned simply by site. Ideally, a series of lasers would be used in order to calibrate the position of the lens in order to ensure that the output area was directly in line the input area of the sensor. As the concentrator lens was specifically chosen due to its dimensions – that is, the input area is equal to the output area of the luciferase-assay vial on the PocketSwab Plus®, and the output area is equal to the input area of the sensor – the lens does need to be properly aligned in order to achieve maximum efficacy. The 10% loss seen could possibly be due to a 10% displacement between the ideal location and the actual location of the lens.

While the lens was found to be an unnecessary cost without further calibration, the polycarbonate reflective coating was shown to improve the efficacy of the design by 84%. Several factors contributed to this improved efficacy. For one, the interior coating keeps out ambient light, improving the baseline of the tests. As can be seen in Table 10, the baseline of the design that contains the reflective interior film is zero – which shows that no ambient light is getting into the chamber. However, once the reflective interior film is removed, the baseline is increased to 0.08V, or approximately 157,000 RLU. With this much light getting through, it would be impossible to detect light below 1 mW/cm² accurately.
7.2 Discussion of Innovation and Significance

7.3.1 Economic Impact

Despite issues with the lower limit of detection, the design was deemed successful due to the incorporation of the Android™ and Cloud applications, as well as the fact that our device could detect an ATP-luciferase reaction for less than $300. That being said, the team has successfully produced an ATP detection device that is affordable for the ambulance industry. This could significantly impact that economics of the industry. EMTs could spend less time cleaning what they have validated to be thoroughly cleaned, and more time sterilizing high risk ambulances – improving the safety of the health care industry at a cost that motivates officials to do so.

7.3.2 Manufacturability

For purposes of prototyping, the team created the 3D SolidWorks® design for the housing using 3D printing, which cost around $70. However, the team would ideally like to injection mold the sample housing in order to further reduce cost and improve durability. Injection molding would also offer a broader range of materials and would produce a product much faster than 3D printing, improving manufacturability.

7.3.3 Societal Impact

This product has great potential for societal impact as its use will encourage proper cleaning and ease the minds of EMS patients, knowing that there is a measure in place to identify breakdowns in current cleaning protocol, improve overall efficiency of the ambulatory environment, and reduce risk of pathogenic infection.

7.3.4 Ethical Impact

While reducing pathogenic risk and improving health of EMS personnel and patients, this device can only help to better ensure a good and satisfying life. The more any patient’s time
within the confines of an ambulance and/or hospital is reduced, the more time this patient has to lead their life to its fullest capacity. If EMS personnel can optimize their cleaning and operating procedures through the use of this device, this will also lead to a more satisfying career and life as they may have more peace of mind and be subjected to fewer potential lawsuits regarding wrongful illness and injury from transport.

7.3.5 Health and Safety Impact
This device is operated remotely via Bluetooth™ serial connection, thus minimizing transfer of potential pathogens from the swab chamber to the EMT operating sample analysis. Overall, this device seeks to reduce pathogenic risk as a whole, thus increasing health and safety of both EMS personnel and patients. The device presents momentary potential exposure during sample collection. However, the overall exposure will be reduced the sooner a significant risk has been identified and addressed. This device has also been designed to be as user friendly and ergonomic as possible, thus reducing any potential strain on the user.

7.3.6 Environmental Impact
The team’s device is completely reusable, as the only disposable components are the swabs that are used with our device. As a result, this device leaves negligible, if any, negative impacts on the natural environment. The swabs themselves are somewhat bulky in their current design, but the swabs are constructed from completely recyclable plastic. If the swabs are redesigned, this device will leave an even smaller footprint on the environment, particularly in comparison to agar plates which have much greater surface areas than the collection swabs. If used appropriately, this device may actually minimize cleaning that needs to be done, as cleaning efficiency and efficacy are increased. By minimizing cleaning, this reduces the volume of hazardous cleaners that must be used, thus resulting in a positive impact on the environment.
7.5.7 Sustainability

Currently, the device runs off minimal energy consumption, requiring only 5 V to power the op-amp amplifying the output signal from the sensor. With such a low voltage requirement, the device uses significantly less energy than comparable products on the market which require 2 kV for the PMT. The device is currently intended to operate off of a 9V battery. However, the circuit could be easily modified so that the device could run off of a solar powered battery. The Solarpod Polaris® 5K is capable of powering 5V USB which would be sufficient for our device. The pod also has a 230V outlet, which would be capable of powering a design modification requiring more voltage as will be discussed in the future recommendations of our product. This battery also makes the possibility of third world applications of our device more feasible.

7.5.8 Political Impact

The low power consumption and low cost of our product makes it feasible for use in third world scenarios. Currently, ATP detection is too costly to implement in areas of fiscal need. With our system, anyone with minimal training can assess cleaning efficacy in any environment – reducing the risk of hospital and ambulance acquired infections around the globe. And with the use of the Smartphone, as long as the phone is charged there is no limitation in terms of where the device can be used. While the phone is in a Wi-Fi accessible area, the Cloud can be reached. However, our design also offers fast results in real time, allowing for quick results and enhances responsivity.
Chapter 8: Conclusions and Recommendations

In conclusion, the team has successfully created a Smartphone enabled device for under $300 capable of detecting ATP, in reaction with a luciferase assay. The design is easy to use, inexpensive, and environmentally friendly. However, the team found that the super-blue enhanced photodiode used as the sensor for bioluminescence simply wasn’t sensitive enough. As every other component of the design was successful, the team felt that the issue of sensitivity could be resolved several ways.

One way to improve the sensitivity of the detection is to use a different sensor. Based upon the team’s limited budget, only a $100 photodiode could be afforded. However, as the team verified that the expensive concentrator lens was no longer required, more money could be spent on a different sensor with improve sensitivity. One such sensor that the team would recommend is an avalanche photodiode. Avalanche photodiodes are capable of detecting less than 1mW/cm$^2$ of light, well within the required range of detection of an ATP reaction. Some are actually capable of counting individuals photons, similar to a photomultiplier tube. However, unlike photomultiplier tubes, sensitive avalanche photodiodes can be purchased for around $300, as opposed to $1000 - $2000 for PMTs. A budget analysis of the new design containing an avalanche photodiode can be seen in Figure 32.
Figure 32: Budget comparison between the current design containing a super-blue enhanced photodiode and the recommended design containing the avalanche photodiode.

The disadvantage of using an avalanche photodiode versus a photomultiplier tube is that they are thermally less stable – in that their responsivity linearity decreases significantly beyond 30°C. This issue can be resolved by storing the device in an air conditioned environment – which is already required for the storage of most ambulances in a depot.

Another way to improve the sensitivity of the detection is to use an instrumentation amplifier in the photodiode circuit, as opposed to the op-amp used in our design. An instrumentation amplifier is a type of differential amplifier that uses several op-amps and high value resistors in order to eliminate the need for input impedance matching. This makes the instrumentation amplifier suitable for low intensity measurements, due to low DC offset, low drift, low noise, and high open loop gain.

The AD620 is acceptable for photodiode usage, and is the industry standard for low cost (Kitchin & Counts, 2006). A full table of recommended instrumentation amplifiers, including those for photodiode sensors, has also been included in Appendix F.

<table>
<thead>
<tr>
<th>Item</th>
<th>$B \text{ PD}</th>
<th>\text{Avalanche}</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Electronics</strong></td>
<td><strong>$124.00</strong></td>
<td><strong>$282.00</strong></td>
</tr>
<tr>
<td>Photodiode</td>
<td>$99.00</td>
<td>$256.00</td>
</tr>
<tr>
<td>Amplifier</td>
<td>$5.00</td>
<td>$6.00</td>
</tr>
<tr>
<td>Circuit: microprocessor, capacitors, resistors, etc.</td>
<td>$20.00</td>
<td>$20.00</td>
</tr>
<tr>
<td><strong>Biologics Per Test</strong></td>
<td><strong>$2.50</strong></td>
<td></td>
</tr>
<tr>
<td>Sample Swabs</td>
<td>$2.50</td>
<td>w/ bio amp</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td><strong>$171.05</strong></td>
<td><strong>$123.08</strong></td>
</tr>
<tr>
<td>Lens</td>
<td>$90.00</td>
<td>-</td>
</tr>
<tr>
<td>3D Printing &amp; assembly</td>
<td>$74.08</td>
<td>$74.08</td>
</tr>
<tr>
<td>PC film with Reflective Coating</td>
<td>$1.00</td>
<td>$1.00</td>
</tr>
<tr>
<td>Battery</td>
<td>$5.97</td>
<td>$48.00</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>$297.55</strong></td>
<td><strong>$405.08</strong></td>
</tr>
</tbody>
</table>
The third and final recommendation to improve the efficacy of our inexpensive, Smartphone enabled contaminant detection device is to enhance the luciferase-assay itself. This should be done for several reasons – both because it would enhance the reaction to the visible region, making the reaction easier to detect, and also because if a team created a different assay system than the PocketSwab Plus® used for our validation, the team would no longer be reliant on Charm Sciences, Inc. for supplies.

Another avenue that should be investigated is improving upon the biologics of the ATP-luciferin/luciferase reaction itself. By optimizing the reaction, the emissions can be made bright enough to be easily seen with the naked eye, thus also making the reaction easily detectable by the team’s current device. As seen in Figure 33 below, modification significantly amplifies the reaction so that emissions are bright enough to easily be viewed with the naked eye. The two circles on the right represent modified reactions, which are as bright as a LED.

![Figure 33: Biological modifications brighten the luciferin/luciferase reaction (Haenish et al., 2011)](image)

There are a few ways of potentially enhancing the reaction largely by either addressing the reaction of the ATP and the means of extraction of ATP from the bacteria and cells of samples. The first means of enhancement is through the incorporation of luciferase-regenerating enzyme (LRE). This enzyme cycles luciferase back into the reaction so that ATP, and not the assay components, is the limiting factor of the reaction. Thus, the ATP contained in the sample is completely reacted, thus ensuring that all ATP within the sample has been taken into account.
This is important, because in the scenario of contamination, a false positive is generally more preferable to a false negative reading on a sample containing pathogenic material.

Another means of enhancement that is currently under investigation is through the use of a highly purified fusion protein consisting of polyphosphate kinase and adenosine kinase. The purification process requires use of polymerase chain reaction (PCR) to amplify the protein chains. After this process is complete, the fusion protein is combined with adenosine monophosphate (AMP) and a commercially available luciferase assay (such as the Promega® Enlighten) to amplify the ATP reaction. This amplification process relies on ATP recycling to convert AMP back to ATP, thus amplifying the overall luminescence of the reaction. Figure 34, below, from Satoh et al. illuminates the chemical breakdown of this amplification process (Satoh et al., 2004; Chen et al., 2009).

This amplification process increases the luminescence of the reaction by nearly 40 fold for the highest level of colony forming units (CFU) of bacteria. Furthermore, Satoh et al., with this methodology, were able to detect as low as 1 CFU of *E.coli*. This was with a table-top illuminometer. However, Satoh et al. considers their range of detection to be in the $10^{-6}$ picomole range.

![Reaction diagram](image-url)

*Figure 34: “ADK, PPK, AMP, and excess [polyphosphate (polyP)] were prepared in the reaction mixture. ATP amplification started when ATP was added to the reaction mixture and ended when endogenous AMP was converted to ATP.” (Satoh et al., 2004)*
range, four orders of magnitude beyond the limit of detection prior to ATP amplification ($10^{-14}$). Using a similar means of bioluminescence amplification, this methodology would bring the team’s current device into the detectable range where it needs to be, thus able to consistently detect urine, feces, and blood cells (Satoh et al., 2004).

Finally, another way to modify the biological reaction would be to alter the means by which ATP is extracted from the cells. Current means of extraction, including the Charm PocketSwab®, rely on a lysis buffer. The Charm® system’s buffer is proprietary information; however, the type of buffer that is typically used is made from a combination of acids and bases, and this can interfere with the reaction. Yang et al. suggest that boiling DI water can be used as an alternative means of extraction as this inhibits the enzymes that break down ATP (ATPases). Thus, this means of extraction helps maximize detection of ATP and prevents interference with the reaction (Yang et al., 2002).

Regarding the application and coding development, there are a few bugs in the Smartphone application that need to be fleshed out. For example, the placeholder text should probably be shortened so that it is easier to replace. This was more of an issue in the emulator, however, than on an actual phone. Test number should also be updated to track with the actual number assignment to the collection series for the organization. This number should count upwards instead of displaying “419” each time.

The web application also could use improvements. Unfortunately, the bugs with the graphing features on this interface need to be debugged. The team highly recommends, however, looking into Highcharts javascript open source coding. They have sample code for running parallel series over time as well as interactive charts which would allow users to highlight and
select different ambulances and sample locations to compare over time. A sample chart can be seen in Figure 35 below.

Figure 35: Highcharts is strongly recommended as a useful tool for future expansion (Highcharts, 2013).

Login and security credentials, as mentioned, should be added so that only credible and appropriate personnel have access to records. Whether the data falls in public domain would be at the discretion of individual organizations and/or the local health and safety departments. Furthermore, the device troubleshooting blog should be created before actually pushing this device and these applications to market. Some formatting also should be cleaned up so that aesthetics are optimal on several different operating systems.
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ISBN: 1-933013-54-0


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Virox, technologies inc. “Cleaning and Disinfection protocol for emergency services fire, ambulance, police, search & Rescue (Canada).

## Appendices

### Appendix A: Summary of current ATP tests on the market

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Hygiena™</th>
<th>Charm™</th>
<th>Charm™</th>
<th>Biotrace™</th>
<th>BioControl™</th>
<th>Neogen™</th>
<th>Marche®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of ATP Sample/Collection Device</td>
<td>Ultrasnap</td>
<td>PocketSwab Plus</td>
<td>PocketSwab Plus</td>
<td>Clean-Trace</td>
<td>Lightning MVP Swab</td>
<td>AccuClean</td>
<td>Hy-Lite Rinse Pen</td>
</tr>
<tr>
<td>Price/Per Test</td>
<td>$1.65 - $1.90</td>
<td>$2.45 - $2.75</td>
<td>$2.45 - $2.75</td>
<td>$2.45 - $2.75</td>
<td>$2.59</td>
<td>$2.50</td>
<td></td>
</tr>
<tr>
<td>Single Use Test</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Total Test Time</td>
<td>45 seconds</td>
<td>65 seconds</td>
<td>65 seconds</td>
<td>25 seconds</td>
<td>25 seconds</td>
<td>30 seconds</td>
<td>45 seconds</td>
</tr>
<tr>
<td>Shelf Life</td>
<td>12 months</td>
<td>12 months</td>
<td>12 months</td>
<td>6 months</td>
<td>6 months</td>
<td>12 months</td>
<td></td>
</tr>
<tr>
<td>Write On Label</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Chemistry</td>
<td>Liquid-Stable</td>
<td>Freeze Dried</td>
<td>Freeze Dried</td>
<td>Liquid Stable</td>
<td>Freeze Dried</td>
<td>Freeze Dried</td>
<td>Freeze Dried</td>
</tr>
<tr>
<td>Name of Instrument</td>
<td>systemSURE II</td>
<td>Luminator-T</td>
<td>NovaLum</td>
<td>Unilite NG</td>
<td>Lightning MVP</td>
<td>Acquapoint</td>
<td>HY-Lite 2</td>
</tr>
<tr>
<td>Price</td>
<td>$800 - $1,200</td>
<td>$3,000 - $3,500</td>
<td>$4,000+</td>
<td>$3,000 - $4,000</td>
<td>$3,000</td>
<td>$1,495</td>
<td>$4,000</td>
</tr>
<tr>
<td>Sensitivity (Femtomoles of ATP)</td>
<td>.97 - 9,999</td>
<td>1.73 - 100,000</td>
<td>N/A</td>
<td>1.53 - 100,000</td>
<td>.2 - 10,000</td>
<td>2.6 - 999</td>
<td>N/A</td>
</tr>
<tr>
<td>Reproducibility</td>
<td>8% - 18%</td>
<td>9% - 28%</td>
<td>N/A</td>
<td>8% - 20%</td>
<td>11% - 56%</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Instrument Read Time</td>
<td>15 seconds</td>
<td>5 seconds</td>
<td>5 seconds</td>
<td>10 seconds</td>
<td>10 seconds</td>
<td>10 seconds</td>
<td></td>
</tr>
<tr>
<td>Self Calibration</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>How Instrument Displays Results</td>
<td>RLU - 1 minute to 1 RLU relationship</td>
<td>RLU</td>
<td>RLU</td>
<td>Log Scale &amp; RLU</td>
<td>RLU</td>
<td>RLU</td>
<td></td>
</tr>
<tr>
<td>Assay Steps</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

| Data Storage (# of test results stored before deletion) | 500 | 4,800 | 6,000 | 5,000 | 10,000 | 9 | 2,000 |
| # of Programmable Sampling Sites | 100 | 300 | 400 | 400 | 1,000 | 99 | 50 |
| Power Supply | 2 AA Alkaline batteries | Internal rechargeable NiCd | Internal rechargeable NiCd | Internal rechargeable lithium ion battery | Internal rechargeable lithium ion battery | Internal NiMH rechargeable battery | Alkaline batteries |
| Battery Run Time | 3 - 6 months | 8 - 12 hours | 16 - 20 hours | 24 hours | 8 hours | 8 hours | N/A |
| Battery Charge time | No charge time required | 2 - 3 hours | 2 - 3 hours | 3 hours | 2 - 3 hours | 2 - 3 hours | N/A |
| Battery Replacement Cost | < $2.00 | > $100.00 | > $100.00 | > $100.00 | > $100.00 | > $100.00 | < $10.00 |
| Size of Instrument (H x W x D) | 18.5 x 7 x 3 cm | 22.5 x 13 x 8 cm | 18.1 x 9.8 x 6 cm | 15 x 10 x 20 cm | 20.5 x 10 x 6.5 cm | 14 x 8.5 x 4.5 cm | 11 x 13 x 2.8 cm |
| Can Operate in One Hand | Yes | No | Yes | Yes | No | Yes | No |
| Weight | .67 lbs | 2.5 lbs | 1.38 lbs | 1080g | 1.5 lbs | .8 lbs | 2.5 lbs |
| Warranty | 16 months | 12 months | 12 months | 12 months | 12 months | 12 months |
| Rent/Lease Program | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Trade-Up Program | Yes | No | No | No | No | No | No |
| Data Analysis Software | DataSURE II | Charm Link | Charm Link | Biotrack | Trax | Data Manager | N/A |
| Excel Compatible | Yes | Yes | Yes | N/A | Yes | N/A | N/A |

## Appendix B: Pairwise Comparison Chart

<table>
<thead>
<tr>
<th></th>
<th>Non-Toxic</th>
<th>No residue</th>
<th>Disposable</th>
<th>Meets EPA</th>
<th>Detects Contaminants</th>
<th>Detects Pathogens</th>
<th>Consistent</th>
<th>Accurate</th>
<th>Easy to Use</th>
<th>Low Cost</th>
<th>TOTAL</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Toxic</td>
<td></td>
<td>0.5</td>
<td>1</td>
<td>0.5</td>
<td>0</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>No residue</td>
<td>0.5</td>
<td></td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Disposable</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Meets EPA</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Detects contaminants</td>
<td>1</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Detects Pathogens</td>
<td>1</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Consistent</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Accurate</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Easy to Use</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Low Cost</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
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<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>
Appendix C: Rating Assessment

Units: Rating assessment of ease of disposal 0 (worst) to 100 (best) in minimal waste produced:
- No trash, completely reusable 100
- Minimal trash produced, less than an agar plate 75
- Some trash produced, equivalent to an agar plate 50
- Lots of trash produced, more than an agar plate 25
- Excessive amounts of waste, equal to/ greater than a biohazards bag 0

Units: Rating assessment of ease of use 0 (worst) to 100 (best):
- No training required intuitive 100
- Minimal training required beyond EMS protocols 75
- Some extra training needed in collecting biological samples 50
- Some microbiology training needed, far outside EMS scope 25
- Excessive training necessary, outside of scope of EMS and nursing 0

Units: Rating assessment of ability to detect contaminants 0 (worst) to 100 (best):
- Detects all contaminants at any amount 100
- Minimal trash produced, less than an agar plate 75
- Some trash produced, equivalent to an agar plate 50
- Lots of trash produced, more than an agar plate 25
- Excessive amounts of waste, equal to/ greater than a biohazards bag 0

Units: Rating assessment of portability (table top/handheld) 0 (worst) to 100 (best):
- Completely handheld/portable, can be held or placed on table 100
- Completely portable, handheld only, difficult to use when on table 75
- Reasonably portable, can be moved but mostly stationary 50
- Not very portable, can be moved but difficult/heavy 25
- Fixed device, requires extra equipment not available in hanger 0

Units: Rating assessment of cost 0 (worst) to 100 (best):
- Very cost efficient, cheap materials, minimal burden on budget 100
- Reasonably cost efficient, cheap materials, some burden on budget depending on number of tests 75
- Larger initial investment, relatively low cost per use 50
- Costly initial investment, extra labor/upkeep involved 25
- Cost completely unreasonable for depot budget 0

Units: Rating assessment of ability to meet regulations 0 (worst) to 100 (best):
- Meets all EPA, FDA, and other appropriate regulations 100
- Meets most regulations 75
- In line with technology currently being considered for approval 50
- Unclear as to whether regulations are met 25
- Does not meet appropriate regulations 0

Units: Rating assessment of short testing time 0 (worst) to 100 (best):
- Test time < 5 minutes 100
- Test time 5-20 minutes 75
- Test time 20 minutes to an hour 50
- Test time > 1 hour 25
- Testing takes multiple hours 0
Appendix D: Types of QA Devices Currently on the Market

**ATP Monitor**

**Advantages**
- Quick Results (<2 min)
- Low concentration required
- Easy to read digital indicator

**Disadvantages**
- Expensive initial investment:
  - Device ~$4000,
  - Swab ~ $2.50
- Limited detection of gram-negative bacteria

**Airborne**

**Advantages**
- Can detect airborne bacteria

**Disadvantages**
- Expensive:
  - Device: ~$900
- Agar:
  - Difficult to use
- Long culture time (~3 days)

**Rapid Antigen**

**Advantages**
- Quick Results (<10 min)
- Cheap overall (~$20)
- Easy to read dye indicator

**Disadvantages**
- Higher concentration required
- Affected by chemical cleaners
- Can only detect specific antibodies
Appendix E: Arduino™ Code

#define inPin0 0

void setup(void) {

    Serial.begin(9600);
    Serial.println();
}

void loop(void) {

    int pinRead0 = analogRead(inPin0);
    float pVolt0 = pinRead0 / 1024.0 * 5.0;
    Serial.print(pVolt0);
    Serial.println();
    delay(100);
}

### Appendix F: Instrumentation Amplifier Recommendations

<table>
<thead>
<tr>
<th>Transducer Type</th>
<th>Type of Output</th>
<th>Output Z</th>
<th>Recommended ADI In-Amp/Diff Amp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermistor</td>
<td>Resistance changes with temperature (−TC), 4%/°C @ +25°C, high nonlinear output, single-supply</td>
<td>50 Ω to 1 MΩ @ +25°C</td>
<td>AD620, AD621, AD623, AD627, AD629, AD8221, AD8225</td>
</tr>
<tr>
<td>Thermocouple</td>
<td>Low source Z, 10 μV/°C to 100 μV/°C, mV output level @ +25°C single-supply</td>
<td>20 Ω to 20 kΩ (10 Ω typ)</td>
<td>AD620, AD621, AD623, AD627, AD8221, AD8222, AD8230</td>
</tr>
<tr>
<td>Resistance Temperature Detector (RTD) (In Bridge Circuit)</td>
<td>Low source Z with temperature (+TC), 0.1%/°C to 0.6%/°C, single- or dual-supply</td>
<td>20 Ω to 20 kΩ @ 0°C</td>
<td>AD620, AD621, AD623, AD627, AD8221, AD8225, AD8230, AD8250, AD8251, AD8555, AD8556</td>
</tr>
<tr>
<td>Level Sensors Thermal Types Float Types</td>
<td>Thermistor output (low), variable resistance, output of mV to several volts, single-supply</td>
<td>500 Ω to 2 kΩ 100 Ω to 2 kΩ</td>
<td>AD626, AD628, AD629, AD8225, AD8553</td>
</tr>
<tr>
<td>Load Cell (Strain Gage Bridge) (Weight Measurement)</td>
<td>Variable resistance, 2 mV/V of excitation, 0.1% typical full-scale change, single- or dual-supply</td>
<td>120 Ω to 1 kΩ</td>
<td>AD620, AD621, AD8221, AD8222, AD8225, AD8230, AD8555, AD8556</td>
</tr>
<tr>
<td>Current Sense (Shunt)</td>
<td>Low value resistor output, high common-mode voltage</td>
<td>A few ohms (or less)</td>
<td>AD626, AD628, AD629, AD8202, AD8205</td>
</tr>
<tr>
<td>EKG Monitors (Single-Supply Bridge Configuration)</td>
<td>Low level differential, output voltage, 5 mV output typical, single- or dual-supply</td>
<td>500 kΩ</td>
<td>AD620, AD621, AD623, AD627, AD8220, AD8221, AD8222, AD8225, AD8553</td>
</tr>
<tr>
<td>Photodiode Sensor</td>
<td>Current increases with light intensity, 1 pA to 1 μA output, single-supply</td>
<td>10⁶ Ω</td>
<td>AD620, AD621, AD623, AD627, AD8220, AD8221, AD8222, AD8553</td>
</tr>
<tr>
<td>Hall Effect Magnetic</td>
<td>5 mV/kg to 120 mV/kg</td>
<td>1 Ω to 1 kΩ</td>
<td>AD620, AD621, AD623, AD627, AD8221, AD8222, AD8230, AD8250, AD8251</td>
</tr>
</tbody>
</table>