Fluorescent marker and measurement system for quantitatively determining hand hygiene proficiency with use of alcohol-based hand sanitizer

Final Design Report
December 12th, 2008

Design Team
Emily Andrews, *BSAC*
Allie Finney, *BWIG*
Rachel Mosher*, Team Lead*
Susie Samreth, *Communicator*

Client
Dr. Christopher Crnich MD MS
Section of Infectious Diseases
University of Wisconsin-Madison

Advisor
Professor William Murphy
Department of Biomedical Engineering
University of Wisconsin-Madison

Table of Contents

Abstract …… 3

I. Introduction & Background …… 3

II. Design Requirements …… 4
- Fluorescent Marker …… 4
- Measurement System …… 5

III. Design …… 6
- Fluorescent Marker …… 6
  - Application of Marker …… 7
  - Candidates for Marker …… 7
  - Optimization Consideration …… 8
- Measurement System …… 8
  - Spectrofluorometer …… 8
  - Hand Holder …… 8

IV. Prototype and Testing …… 9
- Competing Products …… 9
- Spectrometry as a method for evaluating hand hygiene …… 10
- Testing of Fluorescent Markers: Methods, Results and Discussion …… 12
- Translating 2D into 3D Data …… 13
- Results: Comparison of Tested Fluorescent Markers …… 16

V. Ethics and Safety …… 17

VI. Future Work & Conclusion …… 18

Appendices
- A. References …… 20
- B. Product Design Specifications …… 21
- C. Testing Protocol …… 24
- D. Fluorescent spectra changes of biological compounds …… 26
- E. Fluorescent spectra changes of existing products …… 30

Abstract

Thousands of deaths in hospitals are caused by improper hand hygiene practices of the healthcare worker. By not practicing hand hygiene effectively or at all, infection rates continue to rise. Hospitals, in attempts to curb this negligence, have implemented several different monitoring and teaching tools. However, no existing product demonstrates the effectiveness of a user's hand hygiene practices when using alcohol-based hand sanitizer. The approach taken to meet this need was to identify a fluorescent marker to represent the sanitizer coverage on the hand. Ideally, the marker would undergo a fluorescent spectra change with exposure to alcohol. Additionally, need for a portable spectrofluorometer and measurement accessories for research queries were requested. After extensive testing, diethylamino methyl coumarin has been selected as the preliminary marker. Also, a fiber-optic spectrofluorometer has been selected for purchase and proof of concept for standardizing testing has been developed. Further investigation and testing will continue into the upcoming semester to optimize both the fluorescent marker application as well as the data collection techniques.

I. Introduction & Background

Every year, nearly 99,000 deaths are caused by hospital-associated infections (HAIs)(Wallace, 2007). These are communicable diseases contracted in the hospital and, frequently, transmitted to patients by their clinicians who have not followed proper hand hygiene protocol. According to the Centers for Disease Control and Prevention (CDC), approximately a third of these infections could be prevented by the implementation of a hand hygiene program, but hand hygiene compliance in clinical environments is currently reported to be only 20-50% on average. The overarching goal of this project, therefore, is to reduce the number of HAIs through improvements in hand hygiene training and monitoring.

Hospitals, with the recommendation of the CDC, have already taken some initiative to monitor hand hygiene compliancy among their staff. Methods vary from examining the volume of hand sanitizer dispensed through in a set period of time to the more extreme, video-taping of clinicians to record who performs hand hygiene they should and who does not. The problem with these approaches is that none of them monitor exactly how effectively health care workers are actually applying the sanitizer practicing hand hygiene.

There exist teaching methods that demonstrate proficiency, however. For example, a commercially available product, GloGerm™, is commonly used to teach and demonstrate the proper hand hygiene routine. Typically a white powder or lotion, this product is nearly invisible to the eye after application, but glows brightly under ultraviolet light and is intended to simulate bacterial contamination (GloGerm, 2008). In the training environment, clinicians apply the product to their hands, view initial fluorescence, perform hand hygiene, and then visually observe remaining fluorescence in areas of the hand that have not been properly cleaned. If participants have adequately performed hand hygiene, their hands will not glow at all, as the product has been removed via soap and water in combination with mechanical removal due to friction.

Additionally, GloGerm™ can be used to effectively demonstrate transmission of bacteria with contact between surfaces, hands, and patients (GloGerm, 2008). Surfaces within a training environment which are tagged with this product teach participants how clinically relevant procedures result in bacterial contamination. A trainer may apply the product to their own hands or training environment surfaces unbeknownst to the clinicians, inducing transmission upon a handshake or performance of routine tasks which require contact with tagged surfaces.

GloGerm™ and other comparable products are successful tools because they provide immediate visual feedback to clinicians and have various applications with significant clinical relevance. However, within the past few years, hand hygiene protocol has deviated from traditional soap-and-water hand washing to use of alcohol-based hand sanitizers, which provide a quicker and more convenient method for performing hand hygiene in the hospital. While proven effective for removing bacteria, use of hand sanitizer eliminates the relevance of GloGerm™ teaching tools since the application of the alcohol-based gel does not remove the fluorescence and only spreads the glowing product across the surface of the hand. Moreover, this qualitative observation in fluorescence is not sufficient to ensure compliance with hand hygiene protocols; instead, a more accurate quantitative measurement at common problem areas including fingertips and the thenar space is necessary (Infection, 2006).

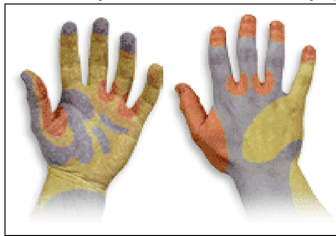


Figure 1: Areas commonly missed when performing hand hygiene are highlighted in orange. Areas that are not missed are highlighted in yellow. (BBC, 2008)

As determined by these motivations, the objectives of this project are: first, to identify a molecule with intrinsic fluorescence to act as a marker to indicate hand sanitizer coverage and, second, to quantitatively measure marker fluorescence associated with improper hand hygiene.

II. Design Requirements

In order to achieve a successful design, the prototype, consisting of these two main components—the fluorescent marker and the marker fluorescence measurement system—must meet particular essential requirements. Each component has its own firm constraints as well as more fluid guidelines that offer progression for next semester. As the semester began with primary focus on the former component, dictation of these requirements will be explained first.

Fluorescent Marker

The fluorescent marker is the crux of the project. Identifying this marker shapes the specifications of the measurement system, which is why researching this component first was essential. When defining this component, three main conditions were declared. The first and foremost requirement of the fluorescent marker is that it must be safe for human contact as it

will be applied directly to the skin. Ideally, the molecule should be approved by the FDA as a topical cosmetic, but at minimum, be accepted by the medical community as a precedent. There are few fluorescent molecules currently approved by the FDA, however it is likely many molecules have simply not been submitted to be approved (FDA, 2008). Motions to make the marker a FDA-approved compound could be taken once identified as the optimal molecule.

Second, since the method for determining hand hygiene proficiency is to analyze the fluorescence of the marker directly on the skin, it is crucial that the molecule selected has a distinctly different fluorescent spectra or intensity peak than that of the intrinsic fluorescence of the skin. Having a clear difference between spectras allows the ability to distinguish the actual coverage of the marker without the misidentification of spectrums. Taking this concept into account raises the issue of determining the spectra of human skin which appears, after much research, to be a little navigated field of spectrometry. On top of this issue, the question is raised about whether hand dryness affects the spectra significantly. These last two issues will need further exploration next semester when a three-dimensional spectra can be collected. For now, use of precedent products, such as GloGerm™, will serve as a guideline until skin spectra data can be collected next semester.

Finally, the third key requirement for the fluorescent marker to meet is that it must be compatible for use with alcohol-based sanitizer. Depending on the method selected for application, this can be accomplished in one of two ways. The marker molecule must either be able to be stably mixed into the sanitizer thus withstanding the alcohol interaction, or the molecule's fluorescence must be attenuated with the exposure to the alcohol in the sanitizer. Further explanation of this requirement will be explained in Section III of this paper.

For the future, more fluid requirements exist pertaining to the application of the fluorescent marker to the hands. Possibilities of containing the marker in a solution, similar to how GloGerm™ works, requires the defining of solvent viscosity for easy coverage of the marker on the hand. A second future requirement, also pertaining to the application, involves the development of creating a "stealth" way of transmitting the marker to hands without the testing subject knowing. Examples of solutions for requirement involve developing a special sponge on an ink pen that is emits the marker. These remaining two requirements will be more clearly defined in the upcoming semester.

Measurement System

This second component of the design concerning the collection of data and standardization of testing has been pursued in the last weeks of the semester and is still very preliminary. The primary requirement for this component is to provide clinicians with immediate feedback regarding their hand hygiene proficiency while in the clinical environment. Therefore, it is necessary that the device used for marker measurement functions independently and is portable for use throughout the hospital. The measurement device must provide real-time, quantitative measurements on the three-dimensional surface of the hand. Finally, the measurement device and measurement process must return consistent, accurate data at standardized sample locations which focus on the common problem areas of the hand. Together, these product requirements for marker measurement have guided the design process to successfully achieve the goals outlined in the problem statement.

III. Design

With the product requirements defined, concentration can be brought to development. As stated earlier, the fluorescent marker was the first objective pursued for two main reasons: it needed to be proved that there is validity for investing in a spectrofluorometer for hand hygiene detection, and the spectrofluorometer needs to be purchased with customized settings that depend on the fluorescent marker it would be detecting. Following the fluorescent marker research and testing, means for quantitatively measuring fluorescence was then investigated and developed.

Fluorescent Marker

As a reminder, the fluorescent marker is intended to show where hand hygiene was not carried out properly by either its absence/presence or the intensity level of the marker on the hand. There are two possible methods for application of the fluorescent marker to the hand, which in principle, are of the same concept. The general basis of each is that the marker after being applied to the skin will show the coverage, or lack of coverage, of the hand sanitizer. In both methods, it is ideal that the clinician is unaware of the testing as to avoid the Hawthorne Effect—a phenomenon where the individual would alter their typical routine because they know they're being observed (Adair, 1984). This allows the study to have controlled, real data for constructive analysis.

Application of Marker

The first method, similar to the existing product explanation in Section I of the paper, is to apply the marker to the clinician's hands prior to hand hygiene through either a marker-containing lotion or via the "germ" method, in which inanimate objects would be tagged with a powder form of the marker. With the marker on the skin, the clinician will then perform hand hygiene. How this method differs from the currently marketed product is that the mechanical removal of the marker is eliminated. Now, ideally, the marker fluorescence is to be attenuated by the alcohol exposure. Using ultra violet light for detection, the fluorescence spectra change would then be at a noticeable difference from the initial state. This method offers the challenge of finding not only a fluorescent molecule that attenuates with alcohol exposure, but of identifying a reaction that transpires nearly instantaneously. Another design problem with this choice of application is the question of what insures that the marker covers every part of the hand initially. If the marker is applied as a lotion, wouldn't it only cover the parts of the hands typically covered by hand hygiene anyway since the application methods are similar? Yet an advantage of this method is that it allows for more dynamic testing since it could be used to tag objects to illustrate "germ" transport and the importance of proper hand hygiene.

The second method of application, and the simpler of the two, involves essentially spiking the hand sanitizer itself with the fluorescent marker. In this approach, after clinicians perform hand hygiene with this altered sanitizer, the marker would then exist on the parts of the hand where they covered proficiently with the sanitizer. The marker will then fluoresce revealing the missed, less fluorescent areas. The significant issue with this method includes the concern of the stability of mixing the fluorescent marker in this alcohol solvent.

With either approach, the numerical fluorescence data can be collected and analyzed to reveal the extent of the clinician's hand hygiene proficiency.

Candidates for Marker

Various fluorescent molecules were identified for use with the first method with the intent that these compounds would undergo some sort of fluorescence attenuation with exposure to alcohol. The basis for marker selection was to select compounds with not only known intrinsic fluorescence but for them to be biological compounds that would not be harmful for skin application. The first immediate compounds tested where phenylalanine, tyrosine and tryptophan—all amino acids found in the human body. Following these, chlorophyll and B12 vitamin were tested. In addition, the currently used products were also analyzed to ensure there was reason for identifying a new compound. Products tested include GloGerm™, Glitterbug™ and Visirub®. Further explanation of the fluorescent marker and in-depth explanation of the testing and analysis will be discussed in the Prototype and Testing portion of the paper. Table 1 offers a quick summary of the results.

Table 1. Explanation of Fluorescent Molecules Tested

Marker	Peak Emission	FDA Approval	Cost Per Ounce	% Intensity Change	Reason for Testing	Reason for Not Pursuing
Visirub®	495nm	No	$18.00	74.49	Existing clinical use, low pigment residue	This molecule will be pursued further.
GloGerm™	350nm	No	$2.10	73.83	Current product	Toxicity of coumarin derivative
D&C Red	350nm	Yes	$2.29	52.75	FDA Approved	Pigment residue, medium change in intensity
DayGlo	480nm	No	N/A	47.85	Current product	Toxicity of coumarin derivative
Glitterbug™	450nm	No	$8.30	6.8	Current product	Toxicity of coumarin derivative
Chlorophyll	350nm	No	$1.45	32	Biological compound	Pigment residue, weak overall emission spectra
Phenylalanine	350nm	No	$15.00	3.19	Biological compound.	Small change in intensity
Tyrosine	350nm	No	$9.40	3.75	Biological compound	Small change in intensity
Tryptophan	350nm	No	$9.40	45.72	Biological compound, Emission spectrum highly sensitive to local environment	Small change in intensity

Optimization Consideration

Preliminary consideration and testing has begun for developing a solvent for containing the fluorescent marker and aiding in its application. Initial requirements examined include viscosity, the non-reactivity of the ingredients, and the ability to keep the marker equally suspended. An approach that is being considered is to develop a solvent similar to hand sanitizer, except not including the alcohol. Also, glycerol has been identified as the key ingredient in lotions thus was considered in depth for optimization. The results from this exploratory testing are further explained in Section IV.

Measurement System

Spectrofluorometer

After considering the need-based requirements for a measurement system intended to measure the fluorescent changes of the marker directly on the skin, an excellent spectrofluorometer candidate was discovered. The Ocean Optics Jaz spectrofluorometer is a field-portable, handheld instrument with onboard microprocessor with capabilities of delivering real-time spectra data immediately following analysis (Ocean Optics, 2008). This fits the requirement specifications precisely. Additionally, the Jaz is a stackable, modular device allowing the ability to customize and change components and settings easily. An accessory that the project demands the addition of is a fiber-optic bundle allowing for the collection of spectra off of a three-dimensional surface—the skin. The bundle accessory would transmit ultra-violet light through the excitation monochromator to the skin and then collect the fluorescence emission through the emission monochromator to bring to the detector (Ocean Optics, 2008). The fluorescence data then collected will be displayed on the digital screen of the spectrofluorometer displaying the intensity values and corresponding spectrographs. Data is saved to an SD card allowing for easy transfer to another computer for further research and analysis. The Jaz also allows for Ethernet connection to send data over a server. This can allow for a development of a database where progress can be tracked. The Jaz offers so many configurable aspects that could be useful in the future after testing results are collected. For example, the Jaz allows expansion up to three channels for simultaneous, multi-point measurement. And finally, the spectrofluorometer can be purchased with an optional battery pack which would further aid in ease of use for field studies.

Figure 2: Jaz Spectrofluorometer
Source: www.oceanoptics.com

Hand Positioner

The collection of this data is only one half of the measurement system. The remaining requirement for this component is the need for developing a standardized method of the testing a data collection. The hand positioning device is an additional component being developed to hold one's hand in a stationary position while fluorescence is being measured by a spectrofluorometer. To optimize reproducibility between test subjects, it is imperative that all subjects position their hand at the same distance and angle with respect to the fiber optic head

of the spectrofluorometer. It is also important that the same areas of each test subject's hand are measured, despite the differences in hand sizes between subjects.

The areas of the hand that are of most interest are the fingertips and the skin between the thumb and index finger, as these are the parts of the hand that are frequently not covered with enough hand sanitizer (Infections, 2008). In addition, these are areas of the hand most likely to make contact with a patient and are, therefore, very likely to aid in disease transmission between the clinician and the patient. To get the most meaningful data with the minimum amount of testing, the hand positioning device was designed to be used to test fingertips, the skin between the thumb and index finger, and the back of the hand. The back of the hand was chosen as a control area since most people tend to cover this area sufficiently with hand sanitizer.

A preliminary, proof-of-concept design for the hand positioning device has been developed. This device is composed of two wooden sides, and a wooden top with a hole drilled in it. When one's hand is placed underneath the hole, a fiber optic head above the device will be able to detect fluorescence on the skin through the hole. A concave area was carved on the top of the device surrounding the hole to allow the fiber optic head to be positioned at an angle with respect to the skin if needed to obtain the best data. An image of the hand positioning device is below.

Figure 3: Proof-of-concept: Hand Positioning Device.

The cylinder, with a spring inside, rotates about an axis several inches away. This permits the cylinder to be at two different positions needed to test the three areas of interest on the hand. In one position, the cylinder is placed directly below the viewing hole. Then the cylinder is pushed down, and the hand is placed on the top of the cylinder so that the top of the cylinder is centered in the palm of the hand. When the cylinder is released, the center of the back of the hand will be seen through the hole in the top of the device.

To view the area of the hand between the thumb and the index finger, the cylinder is pushed back until it reaches a stop. When the testing subject wraps their hand around the cylinder (as if they were holding a cup), and ensures that they hold the cylinder as far up as possible, the target area will be viewed through the viewing hole. When the cylinder is in this position, the one or more fingertips can be measured as well.

Considerable testing and redesign will be done with this device in the upcoming semester to develop a system most effective for testing.

IV. Prototype and Testing

Competing Products

GloGerm™ and Glitterbug™

GloGerm™ and Glitterbug™ are very similar products designed for the purpose of hand hygiene training. Both are topical agents designed to simulate the spread and distribution of germs. The composition of the faux-germs consisted of fluorophore-based topical agents

coated on plastic beads, which promotes adherence to skin. These products require the use of black lights to detect “simulated” germs on hands and surfaces.

GloGerm™ is available in a variety of forms, namely powders, liquids and gels (GloGerm™, 2008). One type (and the type tested in during the course of this study) is composed of 85% Mineral oil and 15% GloGerm™ powder (the identity of which is unknown). Glitterbug™ is available only in lotion and powder form. The ingredients of the lotion product include water, invisible blue pigment (identified to be a Coumarin derivative), mineral oil, glycerin, glyceryl stearate, PEG-100 Stearate, Cetyl Alcohol, Stearic Acid, Triethanolamine, DMDM Hydantoin, Iodopropynyl Butylcarbamate (Glitterbug™, 2008). It was determined that the fluorescent tracer in Glitterbug™ to be of a coumarin derivative.

In addition, the MSDS for GloGerm™ stated that exposure to a large dose or repeated small doses of mineral oil by inhalation, aspiration, or ingestion of GloGerm™ can lead to lipio pneumonia or lipio granuloma, low-grade, persistent, localized tissue reactions. Though this condition has not been proven to be fatal, there exists ambiguitiy in the classification of this molecule as a carcinogen due to lack of adequate human and animal testing (GloGerm™, 2008).

MSDS for GloGerm™ and Glitterbug™ lotion do not indicate immediate health risks, though the MSDS for the Glitterbug™ powder form does touch on the potential cancer hazard of the formaldehyde ingredient (it has 0.1% formaldehyde content). Further investigation of the specific coumarin derivative would be imperative for characterizing the potential carcinogenic properties of the product. Neither the coumarin derivative nor any of the aforementioned products have undergone FDA approval. The potential risks associated with the aforementioned product indicate a need for an alternative approach to hand hygiene evaluation.

Potential Harm of UV-Based Approach

GloGerm™ and Glitterbug™ observe functional dependency on UV light. The suggested UV light range is that of long-wave, UV-A ultraviolet light. These wavelengths range from 3500 to 3800 angstrom units and elicit little to no harm to skin or eyes. Exposure to UV lights should still be limited, and individuals should not look directly at the black light. The use of black lights does add another dimension of potential harm. In conjunction with the ambiguity of the safety of the chemical components, this sheds light on the need for a safer, more sensitive method of hand hygiene evaluation.

Spectrometry as a method for evaluating hand hygiene

At the core of the design specifications for this semester was to determine whether one could detect fluorescence intensity attenuation by alcohol based hand sanitizer via spectrofluorometric techniques. The underlying basis for this premise was that the current state of the art hand hygiene evaluation technique of examining fluorescence distribution under UV light was highly qualitative and exhibited low sensitivity. To reiterate, the current state of the art conditions works only with the soap and water hand hygiene technique. Researchers and clinicians have found little to no consistent results when altering the system to a alcohol-based hand sanitizer approach of hand hygiene. Thus, a variety of resolutions were brainstormed, one of which entailed the use of a spectrofluorometer to characterize fluorescence spectra of a variety of samples.

Background on Fluorescence and Spectrofluorometers

Spectrometry is a powerful, high sensitivity method capable of evaluating various parameters of a sample. Its versatility, historical use and efficacy provide ample advantages for the application of evaluating hand hygiene efficacy. In particular, spectrophotometers are commonly utilized to characterize absorbance and fluorescence spectra of samples to which they are exposed. Reflectance fluorescence spectrometry is an imaging modality used to quantify emission spectra of a sample of interest. Reflectance mode is characterized by a light source transmitting light to the sample, allowing for the sample to interact with the light source and emit back scattering light. This emission returns the fluorescence emission to the detector, and sophisticated computer processing produces an output of fluorescence spectra (Lerner, 2008). Fluorophores are proteins of complex molecular structure and properties, which are able to exhibit fluorescence upon excitation by a specific wavelength. Each fluorophore has a characteristic excitation and emission set of wavelengths or spectra. For the purposes of our design, the assumption was drawn that chemical interaction of the hand sanitizer solvent would interact and alter the fluorescence of fluorophores.

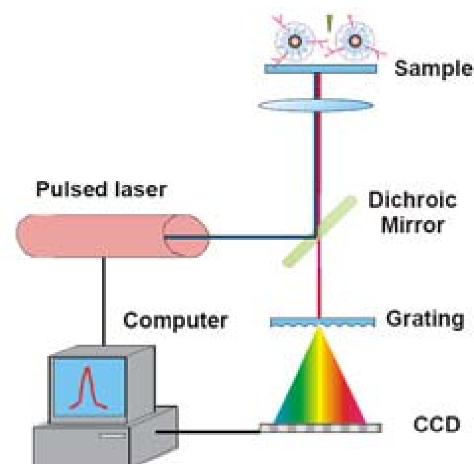


Figure 4. Diagraph of Spectrometry
Source: *www.nanohealthalliance.org*

Goals of Characterizing Fluorescence Spectra of Samples

The current UV-based hand hygiene evaluation technique provides only qualitative data. The main disadvantage of this method is low sensitivity and lack of standardization in measuring the problem areas often missed in hand cleansing techniques. The main goal of testing was to determine if one could evaluate hand hygiene by a more quantitative, specific and sensitive method. Specifically, we sought to answer the question of whether spectrofluorometry could be utilized to detect spectral changes in fluorescence intensity, and provide a basis for which to evaluate hand hygiene efficacy. The long-term goal is to characterize the spectra of various fluorescent markers exposed to alcohol-based hand sanitizer on the surface of skin.

The team sought to explore a variety of options for testing in-vitro. Testing was performed under the assumptions that: 1) alcohol was the active ingredient which might elicit a change in fluorescence spectra (though it might be the case that another active ingredient in the hand rub would elicit a change in spectra), 2) The desired 'change' in spectra is characterized by attenuation in fluorescence intensity following addition of ethanol, though it was later realized that any change in fluorescence intensity could act as a viable indicator of hand sanitizer application.

Testing of Fluorescent Markers: Methods, Results and Discussion

Preliminary Testing of Fluorescent Markers

The first phase of testing consisted of identifying potential fluorescent markers that might exhibit significant spectral change in the presence of ethanol. Identified markers were prepared in triplicates in cuvettes, first diluted in deionized water and then with the addition of alcohol to those same samples. The spectrofluorometer utilized to analyze samples was the Spex FluoroLog-Tau. Spectral emission data was obtained for each respective sample. Spectral data was obtained within a 300-650nm range, 2.5nm slit length and 350nm excitation wavelength. Integrals of spectral plots were obtained via FluorEssence computer software. An integration function was applied to the data to calculate average intensity areas. Each average area were subsequently graphed and compared for each marker. The testing protocol is further enumerated in Appendix C. In Graph 1, data are presented with standard error bars representing 95% confidence interval. Statistical significances were calculated by the unpaired t-test with an error probability, p. Differences were considered as statistically significant only for $p <= 0.05$.

Statistical Analysis of Average Fluorescent Intensities

Table 3 displays a comparison of differences in average intensities of samples before and following addition of ethanol indicate that Visirub® exhibited one of the highest attenuations (approximately 75% decrease). The next step was to perform statistical analysis to discern whether these results were statistically significant. Comparison of all of the markers tested across the board is shown in Graph 1. The error bars on this graph represent 95% confidence intervals, and further inspection of these trends along with the p-values indicates that we cannot conclude that our results are statistically significant. The p-values are the result of a two-sample t-test (based on a 95% confidence) against the null hypothesis that no difference in intensity occurs following the addition of ethanol to the markers. Though p-values were well below the alpha value of .05, the caveat in the results is that *all* of the markers observed p-values that met this trend. Thus, one could not conclude that a particular marker experienced significantly more attenuation than another. This issue could be resolved by increasing the sample size. One should also bear in mind that the decision of which marker to use should not be solely based on statistical analysis alone.

Graph 1. Results from all compounds tested

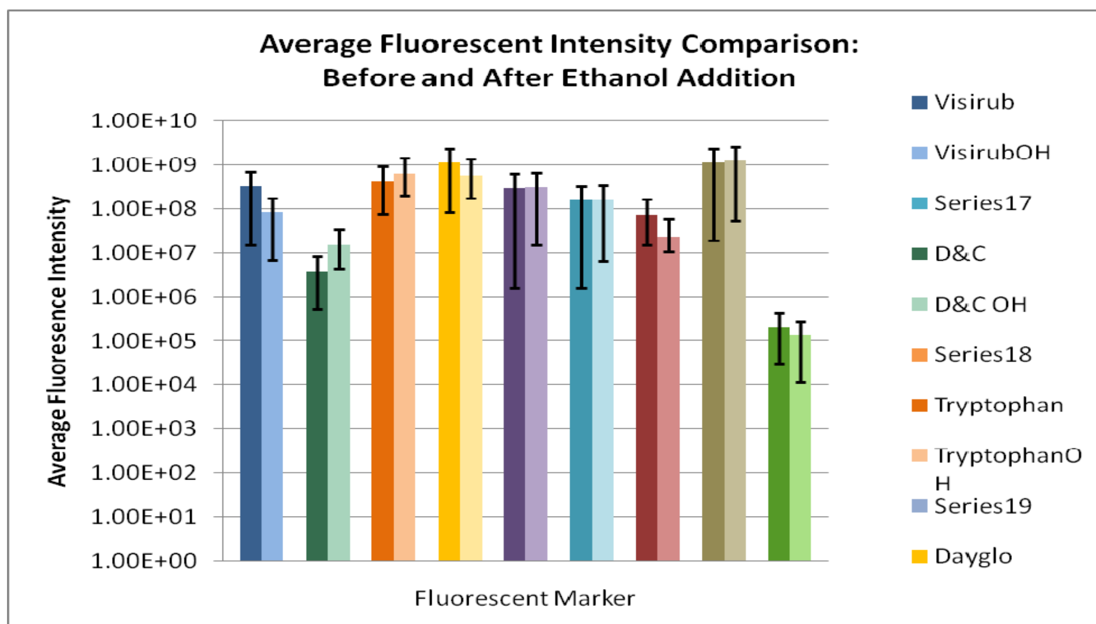


Table 2: P-values obtained from alcohol-attenuation testing.

Marker	P-Value
Visirub®	4.55E-07
GloGerm™	6.49E-03
D&C Red	7.11E-03
DayGlo	1.44E-02
Glitterbug™	1.60E-02
Chlorophyll	3.06E-02
Phenylanaline	9.46E-02
Tyrosine	1.20E-01
Tryptophan	1.71E-01

Table 3: Fluorescence intensity change of molecules following exposure with alcohol.

Marker	%Change in Intensity
Visirub®	74.49%
GloGerm™	73.83%
D&C Red	52.75%
Dayglo	47.58%
Glitterbug™	6.80%
Chlorophyll	32.00%
Phenylalanine	3.19%
Tyrosine	3.75%
Tryptophan	45.72%

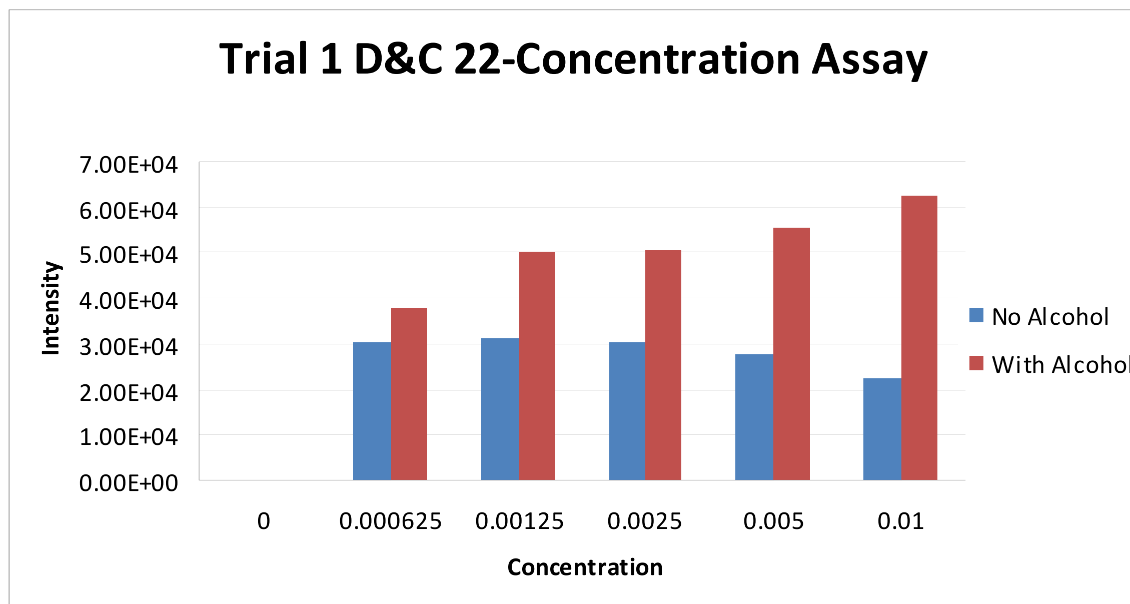
Translating 2D into 3D Data

Preliminary Studies via Fluorescent Analysis of Plate Reader

Optimizing concentration served to ensure fluorescence within the detection limits of the detector with little to no evidence of marker residue. This would alleviate the confounding variables associated with the Hawthorne affect, in which the user might temporary improve their hand hygiene performance as a result marker visibility on their hands (Adair, 1984). Optimizing viscosity served the purpose of ensuring adequate marker coverage. Coverage,

represented by an even distribution of the fluorophore on the hand, is of absolute importance in that it establishes a standard reference point from which to evaluate hand hygiene. For example, in the case that the user does not apply the topical agent adequately and misses important problem areas, one might obtain misleading results since the spectrofluorometer might detect no change in fluorescence intensity. This would confound the study since one would not be able to survey the efficacy of hand hygiene on the crucial areas of the hand.

Plate reader testing was utilized to optimize marker concentrations and allow us to characterize data in a 3-D manner. A preliminary concentration assay was performed for D&C Red by subjecting the fully concentrated solution to serial dilutions. The results of the assay are represented in Graph 2 below. One should note that the fluorescent intensities are of a different order of magnitude than those obtained under the cuvette system. The stipulation, however, lied in the fact that the two systems (cuvette-based versus plate reader-based spectrofluorometer) differed fundamentally in their sample analysis.



Graph 2. Test of ethanol concentration effect on fluorescence attenuation.

In traditional spectrofluorometers, samples are read laterally and the optical pathlength is fixed by the physical dimensions of the cuvette, see Figure 5. In micro-plate spectrofluorometers, samples are read vertically, thus optical pathlength is variable since it is dependent on the volume of fluid in the well and the degree of curvature of the meniscus (McGown, 1999). The variable light path renders difficulty in comparing results obtained in microplate readers and spectrofluorometers. To maintain consistency in systems, data acquisition of plate reader concentration optimization assay was discontinued in order to pursue other endeavors.

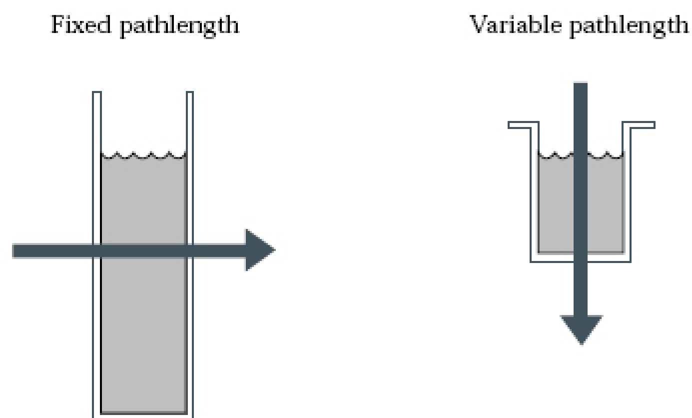


Figure 5: Optical lightpath in cuvette and in microplate
Source: (McGown, 1999)

Preliminary testing to optimize viscosity was performed via the addition of glycerol to a variety of the marker samples. This strategy did not indicate an appropriate level of viscosity and thickness for optimal coverage. Testing was discontinued based on the fact that only qualitative analysis of viscosity could be obtained; testing would prove to be much more feasible with the use of the portable spectrofluorometer described. Ideally the product should display a consistency that affords the same coverage as provided by various lotions and/or hand sanitizer solutions. Thus, the proposed surfactants include but are not limited to: carbomer, tocopheral isopropylacetate, and a variety of occlusive and humectant moisturizers and/or emmolients. Occlusives act as moisturizers for the skin; examples of which include bath oils, lotions and creams. Humectants hydrate and soften the skin; examples of which include glycerin, urea and alpha hydroxy acids (Duffil, 2008).

Skin and Skin-Substitute Materials

Several options for skin or skin-like substrates were considered for the purpose of mimicking the morphological and chemical environment in vitro. The requirements for the skin substrate include the ability to mimic similar absorption phenomenon of alcohol as actual skin, resist degradation or significant alteration of material after application of hand sanitizer solvent and/or fluorophore-based topical agent, and other physical parameters such as a similar intrinsic fluorescence and structural morphology to that of actual skin. The options that were identified over the course of the semester include cadaver skin, reconstructed human epidermis, pigskin/nude mouse skin and nylon wool dressings. A table demonstrating the advantages and disadvantages are included in Table 4. Ultimately testing was not pursued due to a limited timeline and the need for IRB approval for certain skin substrates (e.g. cadaver and animal skin). Such testing will be pursued with much emphasis in the future semester.

Table 4. Advantages and Disadvantages of Skin and Skin-Substitute Materials

Substrate	Advantages	Disadvantages
Cadaver Skin	Best mimic of actual skin morphology Inexpensive	Preservatives (e.g. formaldehyde) might alter response to hand rub solvent or fluorophore Requires IRB Approval (time-consuming)
Reconstructed Human Epidermis	Mimics epidermis Substrate is biologically active and viable	Inconsistent absorption pattern with real skin Expensive
Pigskin/Nude Mouse Skin	Available resource through Veterinary School of Medicine	Inherent morphological and chemical differences between animal and human skin
Nylon Wool Dressings	Provides only a surface on which a reaction can occur Inexpensive	Synthetic Offers no similarities with skin

Results: Comparison of Tested Fluorescent Markers

Evaluation of the fluorescent marker candidates is seen in summary in Table 1, shown earlier in the paper. Majority of the biological compounds were eliminated from consideration because of minimal attenuation subsequent to alcohol addition. The graphs depicting the data can be seen in Appendix D. While Visirub® was selected to contain the best marker, options will remain open for the research to come, especially with the advent of a new spectrofluorometer system.

Visirub® as the optimal molecule of choice

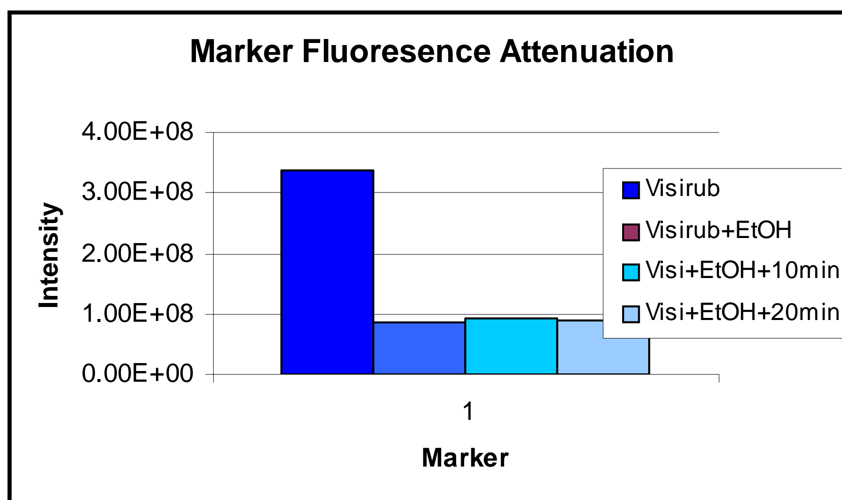
As just stated, Visirub® was selected as the molecule of choice due to its high difference in fluorescence attenuation as a result of alcohol addition, high inherent intensity and near colorless solution that would allow for ease of concentration optimization. Testing of these molecules in the recommended dosage of 10mL Visirub® to 500mL solvent (or 1:50 dilution), and its established use in clinical setting and acceptance by the medical community (Hygiene, 2005).

The active ingredient which displayed fluorescent properties in Visirub® was Diethylamino methyl coumarin (DEAC). Although a coumarin derivative, MSDS do not indicate harmful effects of DEAC to skin. Thus the safety and ethical consideration of the molecule can be ensured, though further testing and research would need to be explored to verify this finding.

Time Dependency of Fluorescence

Further testing was done to determine whether the fluorophore attenuated over time. Samples were prepared in the same manner as specified earlier; the only difference was the added dimension of time. Following the addition of ethanol, spectral data of each sample was

acquired after ten and twenty minutes respectively. The results in Graph 3 depict that a significant amount of average intensity attenuation did occur following the addition of ethanol. However, the data does not allow us to infer that fluorescence intensity was dependent on time, though our time durations were limited. It might be the case that more extended time intervals would need to be evaluated to draw statistically sound conclusions.



Graph 3: How time effects fluorescence Attenuation of Visirub®.

V. Ethics and Safety

Considerable ethical consideration was taken into account throughout the development of the prototype, especially in selecting a fluorescent molecule. Even in initial stages of the semester, safety was of utmost concern. Discarding immediate threats like Green Fluorescent Protein, the options were quickly slimmed down. Testing was carried out with only biological molecules, or FDA-approved compounds. However, testing was also done with the existing products and dramatic change in fluorescence with the exposure to alcohol was seen in GloGerm™ (see Appendix E). As GloGerm™ was then pursued further, discovery of the active ingredient—a coumarin derivative—led to ethical troubles. Reading the Materials Safety Data Sheet on coumarin revealed that it may be only slightly hazardous as an irritant when exposed to skin, but extremely hazardous when ingested (Coumarin, 2008). Coumarin is also listed to have possible carcinogenic effects and could cause the decrease of overall health by its accumulation in organs with extensive, continuous use. The greatest risk with this compound is that it may cause liver damage (Coumarin). With this information, the decision was made to end the pursuit of this compound as a potential candidate for the fluorescent marker.

However, as testing continued there was little to no success with the other compounds. Attention then shifted to the existing product, Visirub®, as it also showed significant fluorescent intensity change with alcohol exposure. Yet this product also contained a coumarin derivative—diethyl amino methyl coumarin. The MSDS for this product did not list the ingredients as anything more than a minor skin irritant. Additionally, this product is used frequently for testing at the University of Wisconsin Hospital. While remaining slightly skeptical

as to the actual safety of any coumarin-containing product, pursuit of this derivative will be continued next semester. The general acceptance of this product in the medical community is a huge reason as to why testing was not completely halted. While this compound has been chosen as the primary candidate for the fluorescent marker, continual research for a new compound will not end with this semester.

A final consideration for safety of the user concerns the mechanism for restraining the hand during testing. As designing the hand positioning component is very much still in preliminary development, acknowledgement of safety parameters has already been made. While it may be crucial that as many variables are removed from testing as possible, the hand must never be restrained in such a way that could cause harm to the user.

VI. Future Work and Conclusion

With the next semester to come, optimizing the product components will be of primary concern. The immediate work to be accomplished with this involves the spectrofluorometer. This entails determining the appropriate parameters and specifications for exclusively the Jaz spectrofluorometer. This will be done by taking the data from the selected fluorescent marker as to make certain the most effective and accurate customizable settings and accessories will be purchased with the spectrofluorometer.

After obtaining the spectrofluorometer, extensive testing will be conducted with the Jaz to collect three-dimensional spectra data of the skin as well as new data of the fluorescent marker. Spectras from the problem areas with and without fluorescent marker present will have to be documented for they will be used in optimizing testing techniques and application procedures.

From data collected in the aforementioned testing, optimal marker concentrations will be determined as to provide for the most accurate coverage detection. Variables in play include keeping molecules evenly distributed in the sanitizer solution and also how molecules collect on the hand when applied to the skin. This analysis may lead to several possible avenues for optimization. Ideas to develop "stealth" marker transmission techniques have already been discussed, for example an ink pen that dispenses the marker through its pores. Also deliberated was the possibility of creating a solution for the marker to reside in, separate from the alcohol-based sanitizer.

Additionally, with reference to the spectrofluorometer, the distance between the fiber-optic head and the device, and the angle between the fiber-optic head and the skin will be optimized to get the most accurate results. The device itself will then be tested in conjunction with the Jaz from Ocean Optics. People with hands of different sizes will be tested with the device after covering their hands with a solution containing a fluorescent molecule. If differences in data exist due solely to differences in quality of coverage, the device will not need any modifications other than permanently attaching the fiber optic head to the device. If errors exist in the data due to the design of the hand positioning device, it will be modified accordingly.

The final element that would be left to be accomplished next semester will be to determine the standards for hand hygiene. Extensive testing of the actual application

technique and data collection process will be needed to establish these standards. The idea is to obtain spectra values from numerous individuals to discover the range of expected values for all three trouble areas as well as determine the optimum values of proficient hand hygiene. After these values and ranges are determined, a rubric or key will be developed as to allow users of the product to immediately interpret the quantitative spectra data into more qualitative data of the hand hygiene effectiveness. For example, ranges of proficiency will be enumerated from 'Excellent' to 'Poor' with associated spectra values. Also, means for taking individual's variant intrinsic fluorescent skin spectra into account for this rubric to be most accurate.

Much headway has been made with identification of a potential fluorescent marker and the selection of a spectrofluorometer. Substantial progress has even been made in developing ways to standardize and control testing procedures. In the coming semester, final strides will be made to push the prototype to completion. With so many components considered all ready, the time to optimize and enhance each feature comes next.

Appendix A

References

Adair, G. The Hawthorne effect: A reconsideration of the methodological artifact. Journal of Appl. Psychology **69** (2), 334-345.

Duffil, Mark. “Emollients and Moisterizers”. 2008. DermNet NZ. <http://www.dermnetnz.org/treatments/emollients.html.>

FDA/Center for Food Safety & Applied Nutrition, ed. "Color Additives and Cosmetics." US FDA/CFSAN - Color Additives and Cosmetics. US Food and Drug Administration. 2008 <http://www.cfsan.fda.gov/~dms/cos-col.html>.

Glitterbug™ MSDS. 2008.< http://www.brevis.com/files/MSDS.GLITTERBUG™.POWDER.html.>

GloGerm™ MSDS. 2008. <http://www.GloGerm™.com/msds.html.>

Coumarin MSDS. 2008. <http://www.sciencelab.com/xMSDS-Coumarin-9923563.>

“Hygiene Training for Correct Hand Disinfection*”.* 2005. Bode, Bode Chemie Hamburg. ,http://www.bode.sk/dokumenty/produkty/Visirub®.pdf.. Translated from Google.

Infection Control. "Handwashing for Staff." Mar. 2006. Mercy University Hospital. <http://www.muh.ie/hospitalinformation/info/hai/staffhandwashing.pdf>.

Lerner, J. The Optics of Spectroscopy - A TUTORIAL. 2008. Horiba Jobin Ivon. <http://www.jobinyvon.com/SiteResources/Data/Templates/1divisional.asp?DocID=616&v1ID= &lang=#11.>

McGown, Evelyn. “UV Absorbance Measurements in SpectraMax Microplate Spectrophotometers MaxLine Application Note #32.” *Molecular Devices Corporation.* 1999.

“Pupils shown play on food danger.” BBC News. 2 December 2008. < news.bbc.co.uk/1/hi/england/berkshire/3728163.stm>

Ocean Optics, comp. "Jaz." Ocean Optics. 2008. <http://www.oceanoptics.com/products/jaz.asp>.

Wallace, Kara. "Ecolab Hand Hygiene Compliance Monitoring Program Helps Hospitals Reduce Infection." 25 June 2007. Ecolab Inc. <http://investor.ecolab.com/releasedetail.cfm?releaseid=250848>.

Appendix B

Product Design Specification

Last updated: *November 28th, 2008*

Title: *Ethanol Degradable Indicator of Hand Hygiene Quality, Team Hand Hygiene*
Client: *Dr. Christopher J. Crnich*
Team Members: *Rachel Mosher (Leader)*
Susie Samreth (Comm's)
Allie Finney (BWIG)
Emily Andrews (BSAC)

Function:
A topical compound containing a marker characterized by fluorescent properties that will be altered upon contact with ethanol will serve as an indicator of hand hygiene quality. Following development of this compound, a portable fiber-optic spectroflourometer and hand positioner device will be designed to quantitatively measure the effectiveness of hand washing technique utilizing waterless alcohol-based sanitizer.

Client requirements:

- •Eliminate current dependency on soap and water as only modality for fluorophore attenuation via GloGerm™ and blacklight
- •Topical agent exhibiting intrinsic fluorescence which must be attenuated or eliminated when exposed to alcohol-based hand cleansers
- •Determine if fluorescence spectroscopy can successfully detect attenuation of fluorophore due to alcohol solvent
- •Construct rubric to translate quantitative data into qualitative data
- •Construct a mobile spectrofluorometer intended for research and clinical setting
- •Standardize hand hygiene evaluation method to optimize reproducibility and precision in quantitative data analysis

Design requirements:

1. **Physical and Operational Characteristics**
    a. *Performance requirements*: The overarching goal is for the product to serve as a teaching aid for alcohol-based hand cleansing with the target audience of health care professionals. The elected fluorescent marker will simulate bacteria and should be invisible throughout the visible light spectrum and fluoresce with exposure to UV light. This fluorescence should be attenuated or eliminated following hand cleansing. The product will also include a standardized mechanism for collecting quantitative data.
    b. *Safety*: The fluorescent marker must be safe for application to human skin and should only include substances approved by the U.S. Food and Drug Administration (FDA). The spectrofluorometer and measurement accessories should not put the user at risk at any time.

c. *Accuracy and Reliability*: As a didactic tool, the marker is required to have a visible change in fluorescence following hand cleansing. However, to ensure a qualitative change, measurable data must be collected to prove a quantitative change in both fluorescence and amount. Every application of this product must reliably change in fluorescence following hand cleansing to successfully achieve its performance requirements. Spectrofluorometer measurement accessories should decrease variables and increase accuracy with standardized data collection points.
d. *Life in Service*: The shelf life of this product should, at minimum, be comparable to other basic hospital products including alcohol-based hand cleansers and lotions. While fluorescent molecules will exhibit a decay factor, the alteration in fluorescence after hand cleansing must last approximately one hour.
e. *Shelf Life*: The shelf life of the fluorescent marker will be dependent upon the chemical and physical properties of the solution. Ideally, the chemicals should have a shelf life of at least one year. The spectrofluorometer and measurement accessories should have a life span of at least 15 years expecting proper care and maintenance.
f. *Operating Environment*: The initial product will have to be restricted to classroom studies. For testing on the travel of "germs" originating from stationary objects in the hospital setting, FDA approval will be required for patient safety.
g. *Ergonomics*: The marker must be safe for human contact and easily disposable after use. The spectrofluorometer unit should provide means to be easily lifted and moved.
h. *Size*: The marker must be small and not visible without special diction, i.e. spectrofluorometer. The size should mimic the size of typical contaminants that could be found on the hand—approximately 5 microns. The spectrofluorometer and accessories must be small enough to be moved easily and frequently.
i. *Weight*: Weight of the marker should be negligible. The spectrofluorometer should be no greater than 20 lbs.
j. *Materials*: The marker should adhere to FDA regulations and standards, and should not include any hazardous substances.
k. *Aesthetics, Appearance, and Finish*: The marker should blend with the skin tone to minimize its appearance as much as possible. The measurement device should have a professional appearance and match the hospital aesthetic.

2. Production Characteristics

a. *Quantity*: The amount needed for testing should suffice, though mass-production of the device should be considered.
b. *Target Product Cost*: Cost of the identified agent should be minimized to ensure affordable mass-production expenses in the future. Current budget is $10,000, which includes the purchasing of a spectrofluorometer.

3. Miscellaneous

a. *Standards and Specifications*: FDA approval is required if the fluorescent marker or other chemicals used in the design project have not been submitted to the FDA and approved for use on the skin.

b. *Customer*: The fluorescent marker solution should be easy to apply to the hands, non-toxic, have no long-term effects on the skin, have little or no color, and have little or no unpleasant odor. The solution should also have a smooth consistency and not cause any drying of the skin, if possible.
c. *Patient-related concerns*: Since alcohol will be applied to the hands, there is minimal risk of bacterial or viral contamination. The spectrofluorometer measurement accessories will have to be cleaned between patients to keep testing standardized. After testing, patients should be instructed to wash hands again with soap and water to remove the remaining fluorescent marker. Safeguarding of patient data is not necessary.
d. *Competition*: A comprehensive literature and patent search has been done to eliminate the possibility of similar products on the market.

Appendix C

Spectromoter Protocol
Updated 11/20/2008

Turn on spectrofluorometer:

1. Switch for lamp and power, must start at least 30 minutes before running tests.
2. Ensure that spectrofluorometer is set to right angle mode (nob on top of spectrofluorometer).
3. Open up fluoroview software
4. Open up project (C:/Data/Hand_Hygiene_Project)

Data Preparation:

1. If necessary, grind solid substances with mortar and pestle.

Solid Samples:

2. Weigh out 0.025 g per solid sample per cuvette. (repeat for multiple cuvettes)
3. Add 1mL of DI H20 to cuvette.
4. Add solid to cuvette and mix thoroughly with pipette.

Liquid samples:

1. Add 1mL of liquid to cuvette (less for substances which we are running low on…e.g. Visirub®)

Data Collection:

2. Input cuvette into the spec, add magnetic stir bar if necessary and turn on magnetic stir.
3. Start with measurement of emission spectra for standards (DI H20 and Fluoroscein for now…)
4. Known excitation/absorbance wavelengths

5. Known emission ranges:

6. Click 'run' to run samples, then once data is collected rename output graph AND output data (double click on data to get there).

7. Once finished with non-alcohol samples, repeat all data collection with addition of 100uL of EtOH to each sample. Mix well with pipette or vortex.

Optional Titration Method (to be applied after preliminary data analysis)

1. Titrate with alcohol until a notable change is seen. Need to decide what exactly will constitute a 'significant' change. (Need some stat input)
8. Once validated sample affected by alcohol, apply more specific protocol (e.g. 3 different alcohol concentrations, 5 times)
9. Data Analysis-account for noise, error, efficacy

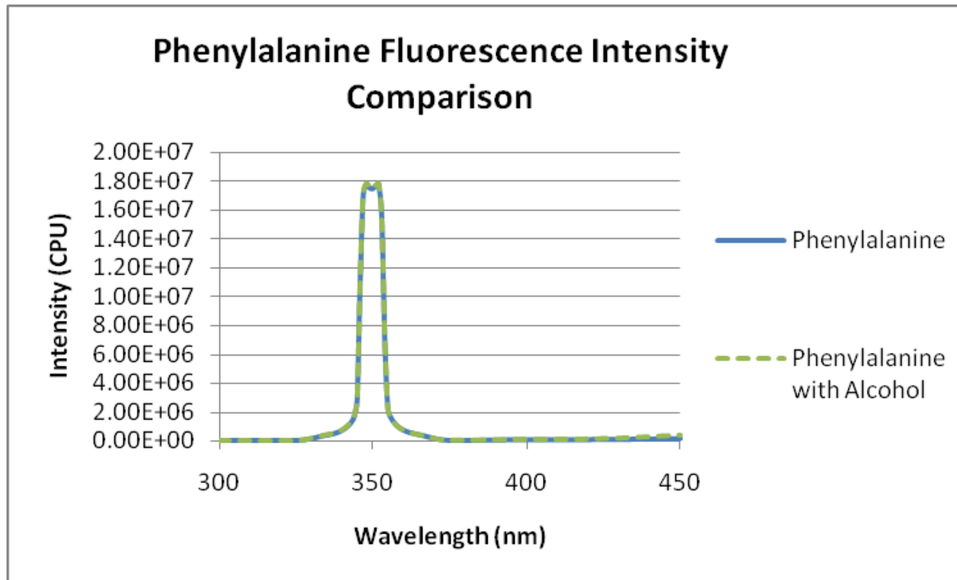
Data Analysis:

1. Integration function in software.

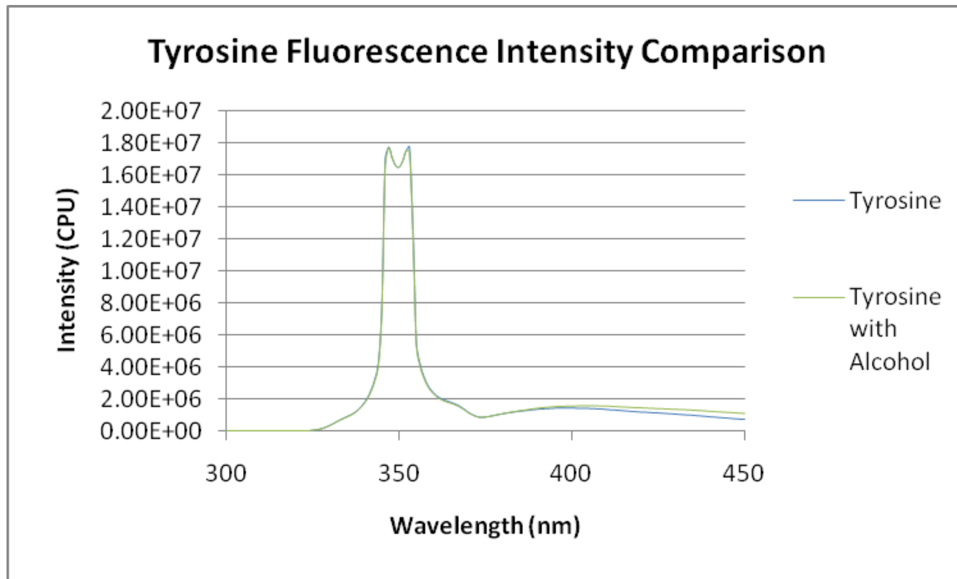
2. Overlay non-alcohol data with alcohol data to see the ‘significant change’

Appendix D: Fluorescent spectra changes of existing products

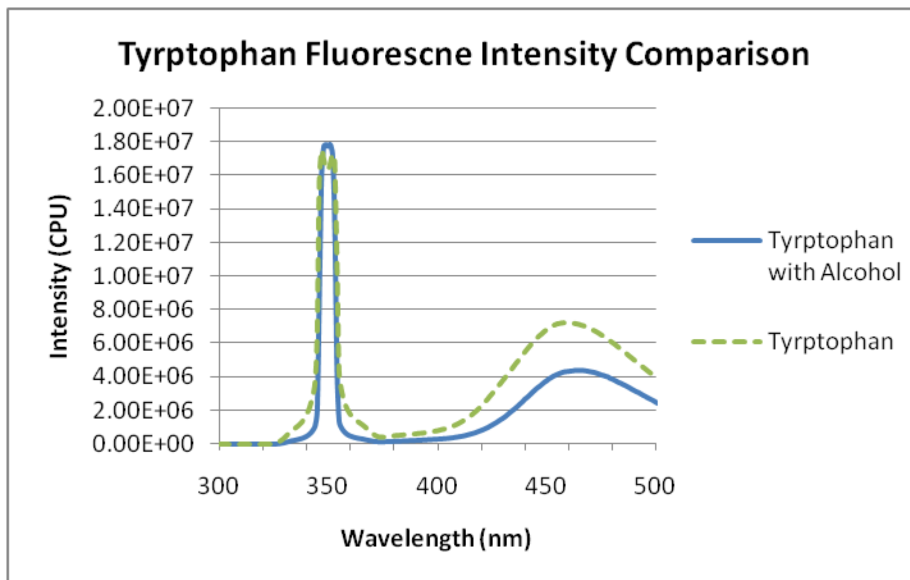
Graph I. Phenylalanine



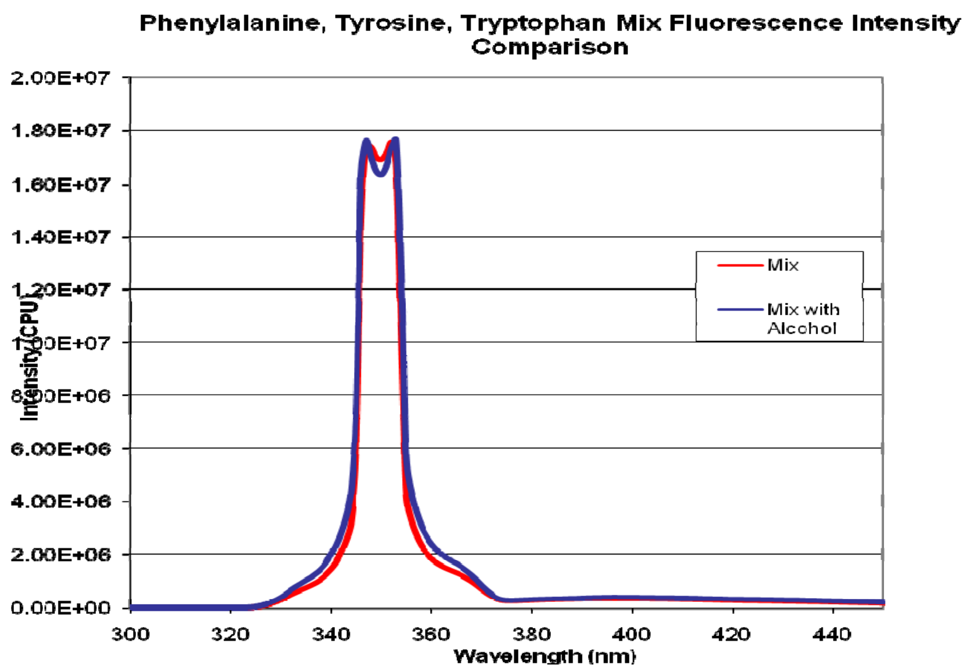
Graph II. Tyrosine



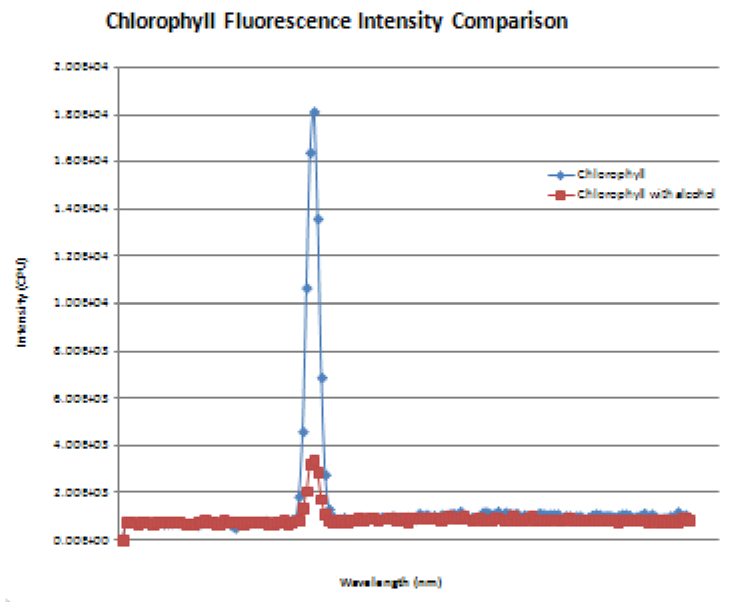
Graph III. Tryptophan



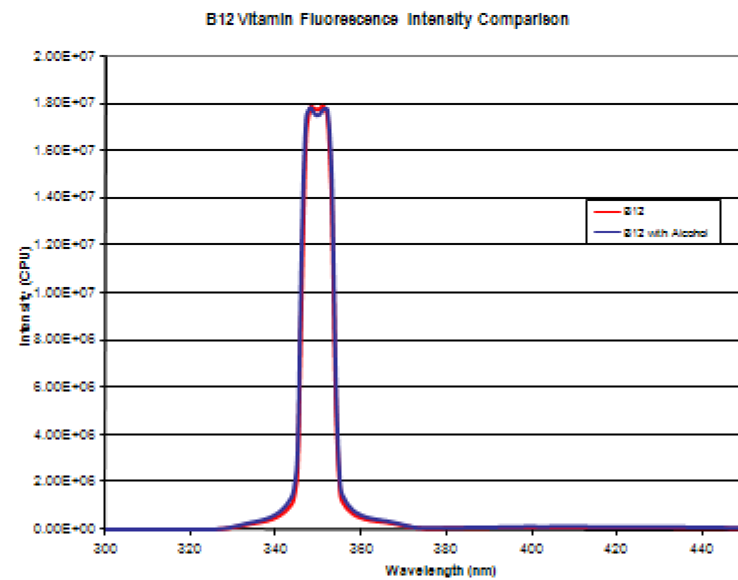
Graph IV. Mix of Phenylalanine, Tyrosine, and Tryptophan



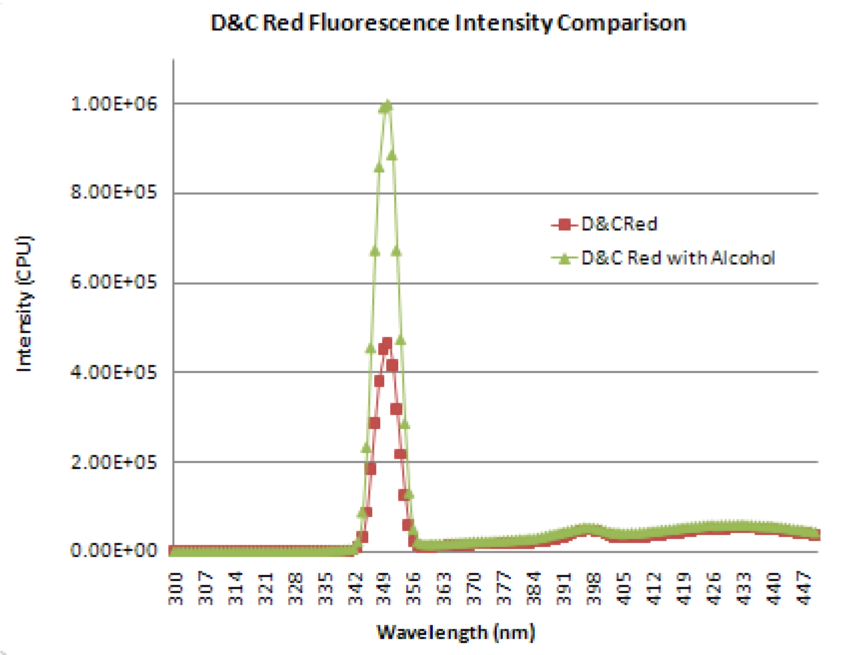
Graph V. Chlorophyll



Graph VI. B12 Vitamin

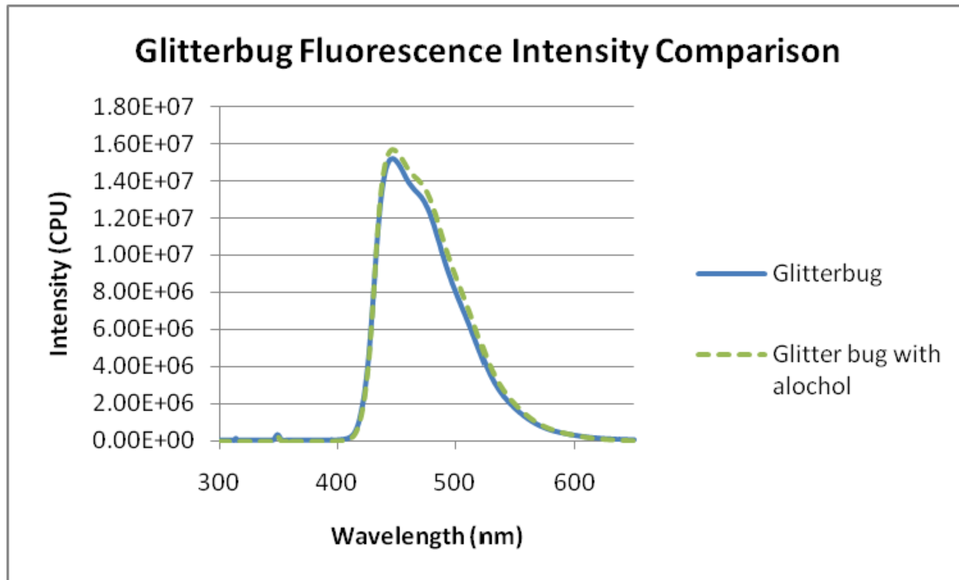


Graph VII. D&C

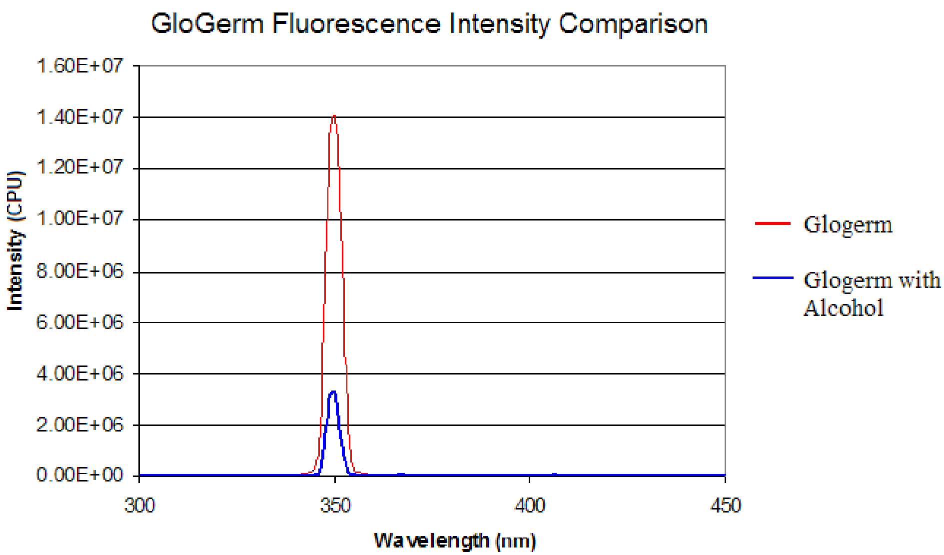


Appendix E: Fluorescent spectra changes of existing products

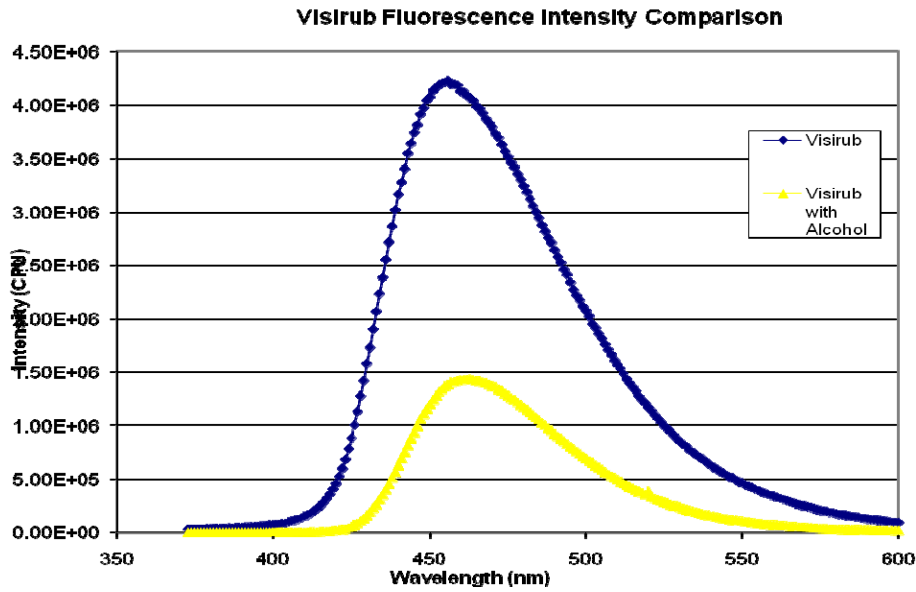
Graph I. Glitterbug™



Graph II. GloGerm™



Graph III. Visirub®



Graph IV. DayGlo