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Characterization of Pleural Effusion using the Multivariable Bedside Test

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Abstract

Pleural effusion is excess fluid that accumulates in the fluid-filled space between the lungs and chest cavity. The condition is diagnosed approximately one million times each year in the United States; however, the ability to characterize the fluid as transudative or exudative in a quick and concise way still remains a challenge. We have created a method that allows for the differentiation of the two types of pleural effusions to occur in a timely manner at the patient's bedside. Our team considered varying methods and received client input to accomplish this goal. The methods we determined to differentiate between transudative and exudative fluid include pH, glucose, specific gravity, total protein, and catalase. We evaluated the options to create the most feasible and efficient design. Successful completion of the design decreases the diagnostic time and increases the convenience of the test. Furthermore, the Multivariable Bedside Test (MBT) can be produced at a low cost, improving health care for those in the military and developing counties.

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BACKGROUND

MOTIVATION

Approximately one million pleural effusions are diagnosed in the United States each year [1]. Depending on the cause, the excess fluid may be either transudative or exudative. These two categories help physicians determine the cause of the pleural effusion. Most effusions are caused by congestive heart failure, malignancy, infections, and pulmonary emboli [2]. Normal pleural space contains approximately 1 mL of fluid due to a balance between hydrostatic and oncotic forces in the visceral and parietal pleural vessels. In contrast, pleural effusions can exceed 1500 mL in volume. When the fluid reaches around 500 mL, it can restrict breathing by limiting the expansion of the lungs during inspiration [3]. The severity of the effusion depends on the primary cause, which can be determined by the fluid type, making it imperative for a quick, easy, and reliable test for the type of fluid.

This device also can be used in many settings. Potential applications include use in emergency situations, military, third world countries, rural clinics, and veterinary hospitals. The rapid diagnosis provided by this device is useful in emergency situations, where immediate diagnosis is crucial in order to determine the appropriate treatment method to ensure patient survival. Furthermore, the device is applicable in military situations where access to appropriate health care facilities is limited, such as in combat overseas. Conditions in the military include poor resources and a rigorous environment. Thus the ideal device would be compact and lightweight to accommodate frequent relocation. This is similar to the conditions of a third world country, where funding for health care may be limited. Rural clinics and veterinary hospitals do not always have the resources available on site to analyze the samples. Often times the fluid is sent to a laboratory at a different location, which could take days to get results. Our device would provide an easy, inexpensive solution to these problems.

PLEURAL EFFUSION

Pleural effusion is excess fluid that accumulates in the fluid-filled space between the lungs and chest cavity. There are two types of pleural effusions, transudative and exudative, which can be identified by the contents of the fluid. Currently, the most common criterion used to distinguish between the two is known as Light's criteria. According to Light's criteria, a pleural effusion is said to be exudative if at least one of the following is true [4]:

- The ratio of pleural fluid protein to serum protein (total amount of protein in the blood) is greater than 0.5
- The ratio of pleural fluid lactate dehydrogenase (LDH) to serum LDH (total amount of LDH in the blood) is greater than 0.6
- Pleural fluid LDH is greater than two-thirds the upper limit of normal for serum LDH level

The type of pleural effusion, transudate or exudate, is dependent on the cause. Transudative effusions often result from a hydrostatic pressure imbalance within the lungs, which is commonly caused by heart failure, pulmonary embolism, and cirrhosis. Furthermore, exudative effusions often are the result of pneumonia, cancer, kidney disease, and inflammatory

disease [3]. Most patients with effusions do not present symptoms until an excess of 500 mL of fluid has built up in the lungs. Symptoms may include chest pain, dry coughing, and difficulty breathing.

Pleural effusion can be diagnosed using varying methods including chest x-ray (Figure 1), computerized tomography (CT) of the chest, and ultrasound of the chest. After diagnosis, the patient may undergo an invasive procedure called thoracentesis (Figure 2). During this process, a needle is inserted between the ribs to remove a sample of fluid. The fluid is then taken to a lab and analyzed for levels of different metabolites to characterize the effusion [5].

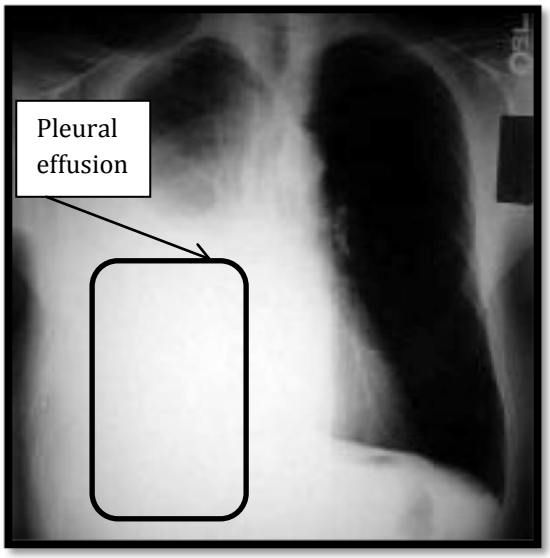

Figure 1. Chest x-ray of the chest showing a right-sided pleural effusion [6].

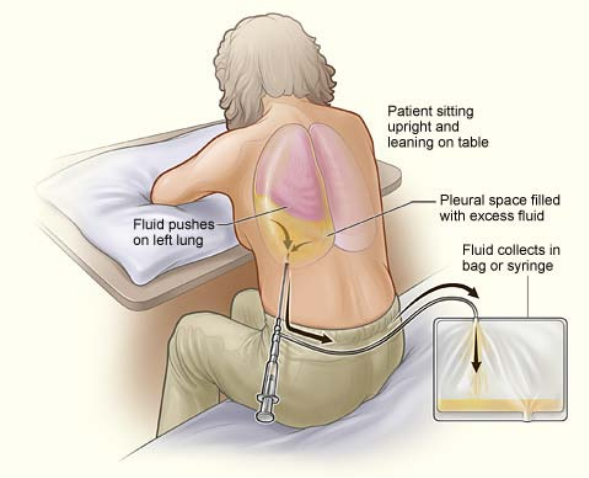

Figure 2. The illustration shows a person having thoracentesis. The person sits upright and leans on a table. Excess fluid from the pleural space is drained into a bag [7].

CLIENT INFORMATION

Our client is Dr. Steven Yale, the director of clinical research at Marshfield Clinic**.** He specializes in internal medicine and is interested in improving the diagnostic process of pleural effusion.

PROBLEM STATEMENT

Dr. Steven Yale has requested a clinical method that is rapid, cost efficient, and convenient, for the characterization of fluid properties to determine the transudative or exudative nature of the fluid.

Current methods for the determination between the two types of fluids are expensive, time consuming (>24 hours), and complicated [8]. The simple design of the model allows any clinician to efficiently use it at the bedside. In addition, the model's small size increases its portability and application to a variety of healthcare fields.

COMPETITION

Our team came across three methods competing designs: magnetic resonance spectroscopy (MRS), ultrasounds of the chest, and pleural fluid laboratory analysis. MRS is a non-invasive technique that is used to measure concentrations of different metabolites in body tissue. Unlike Magnetic Resonance Imaging (MRI), MRS detects the chemical composition of the scanned tissue [9]. Generally, an MRS is used in combination with an MRI. The information given by an MRS is displayed graphically (see Figure 3). An MRS gives information such as metabolite concentrations of the pleural fluid to differentiate transudative from exudative effusions; however, it is neither cost effective nor convenient to perform at the patient's bedside.

Our client is looking for a clinical method that is inexpensive and simple to use. Magnetic resonance spectroscopy can cost $2,000 or more. Our device, in total, only costs $38.37 (Table 2). Also, our device is easily transportable and convenient. It has the potential to be used in third world countries and in the military where technologies are limited. In these situations, an MRI or MRS machine may not be easily accessible.

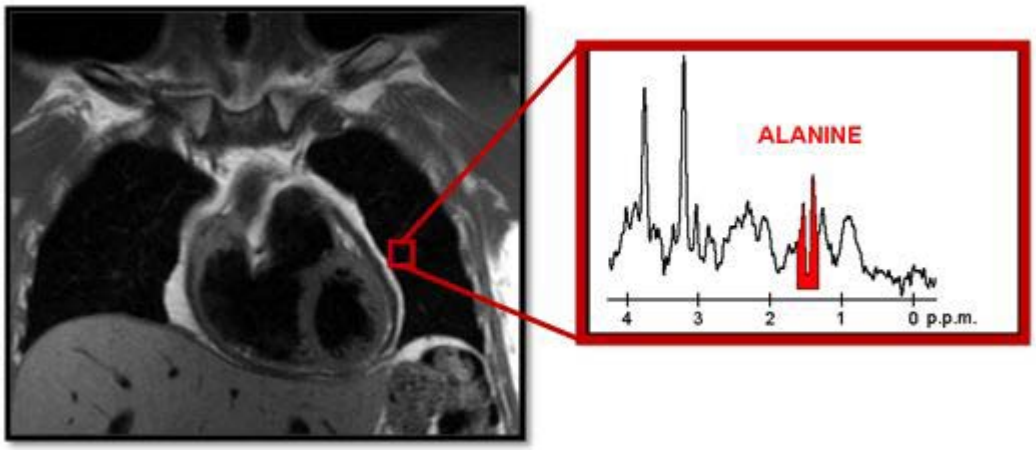

Figure 3. Magnetic resonance imaging scan of patient's chest with graphically displayed information from an MRS [10,11].

The next competing method is an ultrasound of the chest. This allows for visualization of the fluid location and volume, and helps determine where to insert the thoracentesis needle. This method does not provide any useful information regarding fluid content, which is necessary for differentiating between exudative and transudative pleural effusions.

The final competing method is a laboratory analysis of pleural fluid. First, thoracentesis is used to acquire a sample of the pleural fluid. This fluid is sent to a lab to be examined for malignant cells, cellular makeup, chemical content, disease causing organisms, and Light's criteria [5]. Although this method accurately differentiates exudative and transudative effusions, the results are rarely obtained in less than 24 hours. Rapid characterization of pleural fluid within a few minutes is one of our major design requirements.

DESIGN ALTERNATIVES

Due to the broad range of possible designs and interests our client presented to us at the beginning of the semester, we had numerous options to consider for this project. Dr. Yale was interested in both locating the pleural effusion fluid and analyzing it to differentiate between exudative and transudative effusions. After through research and a significant amount of brainstorming we came up with four different design options. One possibility was an electrical impedance tomography device (EIT Belt). This device can be used to evaluate where the fluid is located. Other options including a FastEEM Probe, a rapid protein test, and a multi-variable bedside test were consider for their ability to evaluate the biological properties of the fluid.

EIT BELT

Electrical impedance tomography (EIT) is a non-invasive imaging technique that creates images using the impedance distribution of electrical conductivity. Alternating electrical currents are applied to the body through electrodes on the skin, and measurements of the surface voltage are simultaneously taken. Since biological tissues have varying electrical properties, the voltages create a distribution that produces an image [12]. This method can be used in the form of an EIT belt to evaluate pleural fluid location. In order to analyze the surface voltages, a system such as the CardioInspect PulmoTracePro can be used to produce an image similar to Figure 4. Although this method is able to locate the fluid, there are concerns regarding its accuracy and functionality. In the study, the minimal volume of pleural fluid assessed was 300 mL, smaller volumes were not analyzed. Another problem with this method is that the measurement is a global average of lung resistivity and does not allow analysis of separate thoracic compartments. Bioimpedance techniques are also unable to differentiate between pleural effusion and pulmonary edema, decreasing its reliability [13]. Specific to our project, there is the concern of characterizing the fluid. The bioimpedance method is unable to differentiate between transudative and exudative fluids due to the fact that it does not characterize the fluid, it merely detects it [12]. Despite its downfalls, the EIT belt provides a design that is lightweight, portable and noninvasive.

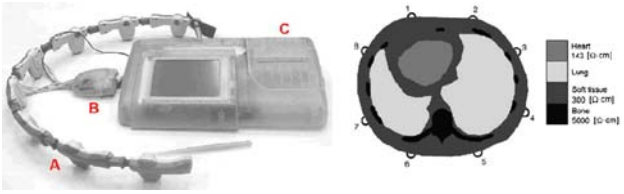

Figure 4: EIT belt(left) and image(right) created in PulmoTrace Pro in order to determine the location and amount of fluid in the pleural space [14].

MULTI-VARIABLE BEDSIDE TEST

After evaluating several articles about singular tests for the differentiation of pleural fluid, we brainstormed the idea of combining these tests into one rapid bedside test. By combining the tests, we could improve the specificity and sensitivity of exudate and transudate diagnoses. Currently, in order to quantify the protein levels the sample is sent to lab [8]. Although lab tests such as protein characterization yield accurate diagnosis using Light's criteria,

the process requires a minimum of 24 hours for results. A rapid bedside test would shorten the time between the thoracentesis and the clinician's diagnosis so that treatment could begin more rapidly. The main downside of this design is that a sample would still have to be obtained using thoracentesis. Despite this, the rapid bedside test is portable, inexpensive, easy to use, and can analyze pleural fluid in a short amount of time.

FASTEEM PROBE

One way to determine the fluid characteristics in vivo is to use the FastEEM approach. A probe with a fiber optic tip is inserted into the body, and a sequence of ten laser pulses and two white pulses are emitted. This probe also collects the reflectance and fluorescence, which is delivered to the diffraction grating and dispersed onto a CCD detector. This data acquisition process is completed in approximately 0.3 seconds. The data is then analyzed using LabView 7.0 and a custom software program. The schematic in Figure 5 outlines the important components of the device. By analyzing the reflectance, the presence of proteins, ions, and other metabolites can be determined [15]. It is less invasive than the standard thoracentesis needle and has accurate results; however, the design has several drawbacks. The FastEEM equipment is very expensive and the results are complicated to interpret. This design also utilizes cutting edge research making it difficult to undertake in a semester.

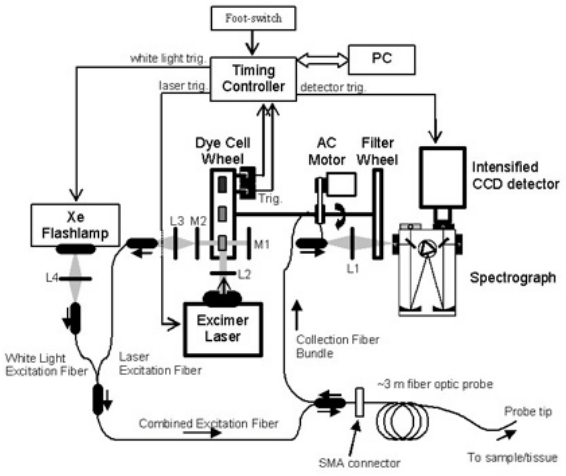

Figure 5: Schematic layout of FastEEM probe [15]

RAPID PROTEIN TEST

After thoracentesis, the current protocol is to send the pleural fluid specimen to lab for chemical tests, microscopic evaluation, and infectious disease tests. Included are tests for glucose, LDH, amylase, triglycerides, total cell counts, tumor markers, gram stain and protein [5]. The protein and LDH concentrations are then used in conjunction with Light's criteria to determine if the pleural fluid is exudative or transudative. A rapid protein test would minimize the time between thoracentesis and diagnosis, and thus treatment. Several methods were researched. The Biuret test was the fastest and most accurate [16]. The Biuret test involves combining the biuret reagent with the pleural fluid and evaluating the results with spectroscopy. The reagent is made from a combination of sodium hydroxide and copper sulfate. In the presence of protein the blue reagent turns into a violet mixture. When combined with short-

chain polypeptides, it turns pink [17]. Unfortunately, a colometric analyzer required for the analysis of results of the Biuret Test is not as portable as desired.

DESIGN MATRIX

In order to evaluate the different design options, a design matrix (Table 1) was used to determine the best design. The design matrix allowed us to choose a design quantitatively with minimal subjective bias. The four design alternatives were ranked in terms of the following criteria. Ultimately, the Multivariable Bedside Test was chosen due to it having the highest value.

Sensitivity to fluid type was weighted the highest due to the need for an accurate test. Sensitivity indicates the proportion of actual positives that are identified as such. Thus in this case, the number of exudative fluids that are given a positive test result. High specificity, or the number of transudative fluids that receive a negative test result, is also essential to limit false diagnoses. The EIT belt was given the lowest score in this category due to its inability to differentiate between exudative and transudative fluids. The other designs received higher scores based on their ability to differentiate between the fluids.

The second highest rated category was ease of use. It is important that health care professionals can use our design efficiently and effectively. This includes convenient instructions, minimal time to conduct test, and clear, easy to interpret results. The multi-variable bedside test and the bedside protein analysis would have the highest performance in this category.

Time is another important aspect, although not as crucial as the aforementioned criteria. With only a semester to complete the design, consideration was given to the amount of time necessary to create a working prototype. The FastEEM probe is very technical and would take longer than a semester to design and create. The other three tests provide a design that would be able to be produced in a semester's time.

Size is another design aspect that was considered. The device should be no larger than 16x11 cm to be included in a thoracentesis kit in order to increase the practicality of the design. The only two options that could realistically fit into the kit are the Multi-variable Bedside Test and the bedside protein analysis

The level of invasiveness is a key aspect of any medical procedure and should be minimized when possible. Ideally the device should be noninvasive, similar to the EIT belt. The MBT and the Bedside Protein Analysis are considered minimally invasive, as they both require the removal of pleural fluid through a thoracentesis. However, the needle used for thoracentesis is small enough to reduce significant complications and the thoracentesis procedure has been deemed safe [18].

Cost was the last design aspect considered. It was weighted the lowest due to the lack of limitations in the provided budget from the client. However, we still included cost in the design matrix since a lower cost allows greater marketability.

	Weight	Fast EEM/Ramen Probe	EIT Belt	Multi-Variable Bedside Test	Bedside Protein Analysis
Sensitivity	1	4	1	**4**	3
Ease of Use	0.75	4	4	**5**	5
Timeline	0.5	2	4	**5**	4
Size	0.5	3	2	**5**	4
Invasiveness	0.5	4	5	**2**	2
Cost	0.25	2	3	**4**	5
Total	3.5	12	10.25	**14.75**	13

Table 1. Design Matrix. The winning design was the Multi-Variable Bedside Test with a score of 14.75.

DESIGN OPTIONS

After choosing to pursue the Multi-Variable Bedside Test, there were many options that still needed to be considered, including the tests that would be included and the layout of the final design.

TESTS

In order to determine which tests to include in the rapid bedside test, we researched and brainstormed properties that were different between transudative and exudative effusions. The list of different properties includes cholesterol, albumin, protein, pH, lactase dehydrogenase, specific gravity, amylase, lymphocytes, bilirubin, and sialic acid. There are also a couple tests used to differentiate between exudates and transudates including the catylase test and Rivalta's test. Since the catalase test often gives false positives when blood is present in the sample, an easy way to test for blood in the sample also had to be established. Although this list is large, many possibilities were eliminated for different reasons including inaccuracy, sensitivity, specificity, or the need for comparison to blood serum. This list was reduced to the evaluation of protein, pH, specific gravity, glucose and catalase. These tests will be explained and analyzed in the final design section, pg 13.

Although cholesterol has been proven to be as sensitive as Lights criteria, it is less specific. Further research is needed to determine how cholesterol passes into the pleural space and the exact cut off that should be used for determining exudates from transudates [19]. Additionally, we decided not to include this test because the quantification instrument, CardioCheck, is neither portable nor disposable [20]. Therefore, the cholesterol test does not fit the design requirements.

Albumin is any protein that is water-soluble. They are produced in the liver [21]. Albumin is found in the urine so there are urine dipstick tests to measure its concentration [22]. The difference in albumin concentrations correlates with those of total protein concentrations as a whole. Therefore, we decided to test total protein concentration instead of the specific albumin concentration. Albumin is also measured using pleural fluid to serum ratio, which we will not be able to get without testing the blood as well.

Lactate dehydrogenase (LDH) is important in the energy synthesis in cells because it catalyzes the conversion of lactate into pyruvate. When cells die the LDH makes its way into the blood stream [23]. The pleural effusion fluid to blood serum concentration ratio is used in Light's criteria to differentiate between transudates and exudates [4]. Again this test was not used because we chose not to test the blood.

Amylase is normally found in very small amount in the body, but is secreted if the pancreas becomes damaged or blocked [24]. Amylase is present in pleural effusions that are caused by pancreatic disease, malignancy, or esophageal rupture [25]. There are urinary dipsticks to test the concentration of the amylase enzyme however there is not a standardized value for how much amylase indicates the previously stated causes [26].

Lymphocytes are cells that are responsible for immune responses. There are two main types of cells B cells and T cells. Lymphocytes secrete products that are found near inflammation in the body [27]. If more than fifty percent of cells in a sample of pleural effusion are lymphocytes this indicates inflammation and an exudative effusion. The patient is believed to have malignancy, tuberculosis, or coronary artery bypass surgery complications [25]. Unfortunately there are not rapid tests available to test lymphocyte concentrations.

Bilirubin has been proved to be statistically equivalent to Light's criteria. A pleural fluid to blood serum ratio of over 0.6 indicates an exudative effusion [28]. Bilirubin was not used because blood is needed to test the ratio. Although there is an easy was to detect levels using a test strip, we choose not to include a blood sample test [29].

Sialic acid has also been proven to exist in different levels in malignant pleural effusions. The sialic acid is tested in both the pleural fluid and blood serum to determine the ratio. The sensistivity and specificity of this ratio, with a cut off of .7 for malignant effusions, is 76.67% and 20%. Our design does not test the patient's blood; therefore, we are unable to use this test in our design [30].

Rivalta's test is a reaction that takes place between the pleural effusion fluid and acetic acid. One drop of acetic acid is dissolved in 5 mL of deionized water and 1 drop of effusion fluid is added. If the drop disappears and the resulting solution is clear, the test is negative indicating a transudative effusion. If the drop retains shape and creates a cloudy precipitate, then the test is positive signifying an exudative effusion [31]. Although the Rivalta's test is straight forward there are three main reasons we did not proceed to include it in our final design. The Rivalta's test is used frequently with felines and has been researched in the veterinary field, but lacks statistical evidence in human trials [31]. There is also a lack of standardization with this procedure; there are no set standards which would cause a problem when trying to integrate the test into our product. Lastly, the reason for a positive precipitate for the reaction has yet to be investigated [32]. Do to the lack of research on this test we decided to not include in our

final design as it may not be very accurate and also may be a duplicate test of a substance in the fluid.

DESIGN LAYOUT

Three design layouts were created (Figures 6, 7, and 8) and evaluated on simplicity, organization, and usability. It is essential that our design can be quickly read and understood by health care professionals. For this reason we consulted health care professionals to get their input on the design. The feedback we received indicated that design alternative 3 was the best option. It was selected because the spectrum could be ready easily left to right, and also the reader's eye was directed easily down the base in sequential order of the tests. The simplicity was also an important factor because it would be the easiest to implement, even with staff that have minimal knowledge of the procedure [33,34,35].

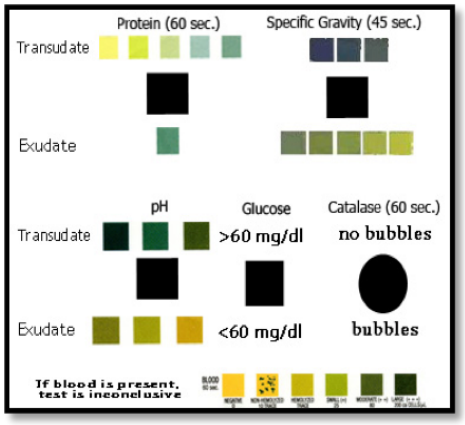

Figure 6. Design Alternative 1

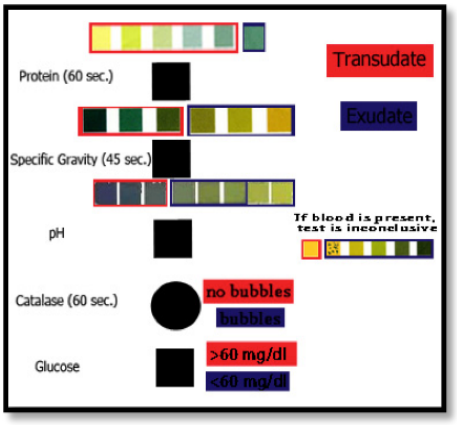

Figure 7. Design Alternative 2

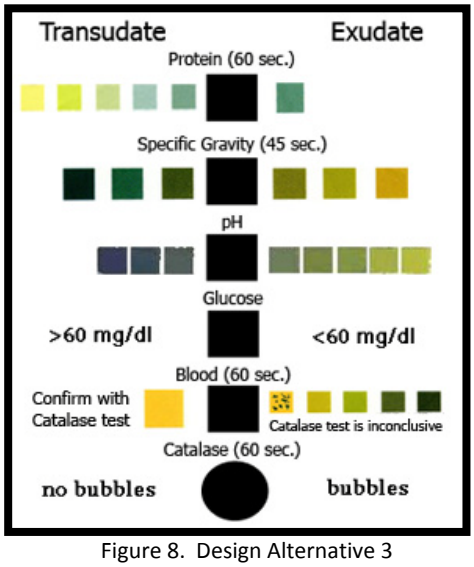

Figure 8. Design Alternative 3

FINAL DESIGN

The final design chosen to characterize a patient's pleural fluid was the Multi-Variable Bedside Test (MBT). Furthermore, Design Alternative 3 was chosen as the final layout for the MBT. Input from Dr. Yale and other health professionals were taken into consideration in the selection of the final design. Advantages of this design include the ease of implementation and simplicity of analysis, which is apparent in the instruction manual (Appendix B).

The MBT test utilizes five biological markers found in pleural fluid to differentiate between transudative and exudative pleural fluid. By combining the five methods, it is our intention to improve upon the sensitivity and specificity of each test. The tests will be conducted to identify the total protein content, specific gravity, pH, glucose level, and the presence of blood and catalase. The classification requirements for each of these tests are listed in Table 2.

Test	Transudative	Exudative
Protein	<2.9 g/dL	>2.9 g/dL
Specific Gravity	<1.012	>1.02
pH	> 7.3	< 7.3
Glucose	> 60 mg/dL	< 60 mg/dL
Blood	If present catalase test will bubble (false positive)	Will not affect catalase test
Catalase	No bubbles	Bubbles

Table 2. Criteria used to analyze results of tests [4,36,37,38]

One of the most significant markers of differentiating between transutative and exudative pleural fluid is the protein content of the fluid [4]. In an exudative pleural effusion, inflammation is often times present resulting in an increase in capillary permeability [39]. Thus, allowing the movement of fluid, including proteins, across the visceral pleura. Fluid with a total protein concentration greater than 2.9 g/dL is considered exudates while fluid with a total protein concentration less than 2.9 g/dL is transudative.

The pH of the fluid can be used as an indicator for differentiating between types of pleural fluid. Fluid classified as transudative has a pH of greater than 7.3, while fluid with a pH of less than 7.3 is considered exudative [36]. Explanations of pleural fluid acidosis have not been precisely defined. However, possible mechanisms include a combination of acid production by pleural fluid or an inadequate buffering capacity of the fluid. In the case of empyemas, increased acid generation may be due to the presence of leukocytes and bacteria found within the fluid. This is a result of the metabolism of glucose to its end products, CO_2 and lactate. For malignant effusions, low pH may be due to an increase in acid production by

malignant cells. Another explanation is the impaired efflux of H^+ as a result of pleural thickening due to a tumor [36]. Determination of fluid pH will be conducted with a diagnostic test strip secured to the bottom of a well. A colorimetric analysis of the diagnostic strip will be used to determine the pH of the fluid.

An additional biological marker that can differentiate between exudative and transudative fluid is glucose. Glucose levels lower than 60 mg/dL are correlated with exudative pleural fluid while glucose levels great than 60 mg/dl are associated with transudative fluid [4]. In the Mulitvariable Bedside Test, a glucose meter is used to identify the level of glucose in the fluid. A well was created on the base plate to contain fluid intended to be used to determine the level of glucose. After inserting a test strip into the glucose meter, the strip can be inserted into the pleural fluid. Once the strip touches the fluid, it can be removed. Next, the results of the glucose test will be displayed digitally on the meter. These results can then be compared to the description found on the labeled base plate to determine which type of pleural fluid the patient has.

Specific gravity is a measure used to compare the density of various fluids. Exudative fluids have a much higher specific gravity due to the increased amount of biological materials found in the fluid [37]. Fluid with a specific gravity >1.02 is considered exudative while fluid with a specific gravity <1.012 is transudative [37].

Furthermore, the level of catalase activity within the fluid can be used to characterize the type of fluid. A unique characteristic of exudative fluid is increased catalase activity [38]. Due to the ability of catalase to speed the decomposition of hydrogen peroxide to water and oxygen [41] a simple bedside test using hydrogen peroxide can be done to verify the presence of catalase. Therefore, the fluid can be classified as either transudative or exudative. If profuse bubbling occurs within one minute of the addition of fluid to hydrogen peroxide, it signifies exudative fluid. The bubbling occurs as a result of the decomposition reaction. When hydrogen peroxide is added to transudative fluid, bubbling is not observed. The vial using hydrogen peroxide to characterize pleural fluid will contain 200 μL of 30% hydrogen peroxide. To identify the presence of catalase, 10 μL of pleural fluid, approximately one drop from a syringe, will be needed. Once the pleural fluid is added to the hydrogen peroxide, profuse bubbling should be seen within one minute. The sensitivity and specificity (Table 3) of this test is believed to be equivalent to the widely used Light's criteria, see Table 3 [38].

If blood is present in the pleural fluid, the catalase test will result in a false positive for transudative fluid as exudative [38]. Therefore in order to determine whether or not the results of the catalase tests are accurate, a reagent strip is used to confirm or deny the presence of blood. If blood is present in the fluid, the catalase test cannot be used to characterize the type of fluid and must be disregarded.

Previous studies have reported values for the sensitivity and specificity of the protein pH and catalase test (Table 3). It was our intention that the sensitivity and specificity of the MBT would be greater than any of the individual tests alone. However, in order to determine the overall sensitivity and specificity of the MBT, extensive testing must be conducted to access its accuracy.

	Sensitivity	Specificity	Citation
Protein	93.1%	50%	[42]
pH	36%	-	[43]
Catalase	98%	91.3%	[38,4]

Table 3. Literature values of sensitivity and specificity for the tests included in the MBT.

DESIGN SPECIFICATIONS

One unique aspect of the Multivariable Bed Side Test is its ability to combine a number of tests to improve the diagnostic accuracy for characterizing pleural fluid. In order to improve upon the uniformity and continuity of the design, a base plate will be created to collectively house all of the tests. Overall dimensions of the design will be 10.2x12.7x1.9cm, with a total weight of 231 grams. Reagent strips used to analyze protein, specific gravity, pH, and blood will all be housed within separate wells (0.95cm diameter and 0.64cm deep). The test to verify the presence of catalase will be conducted in a vial containing the required 200 μL of hydrogen peroxide. In order to improve the stability of the vial, a hole (1.27cm diameter) was drilled to offer support. A label will be placed on the surface of the base plate indicating which well corresponds to each test. In addition, the label also displays the possible results of each test and whether or not those results correspond to transudative or exudatvie fluid. Upon completion of the reaction between the pleural fluid and the reagent strip, the color of the reagent strip can be compared to the colors found on the label (Figure 8). For the protein, specific gravity, and pH tests, if the color of the reagent strip matches a color on the right, the results indicate exudative fluid. If the reagent strip color is matched to a color to the left of the well it is housed in, that test results in a transudative fluid. This analysis process can be applied to only the protein, specific gravity, and pH tests. If the results of the glucose and catalase test match the descriptions found to the right of their respective wells, those tests indicate exudative fluid. Similarly, results matching descriptions to the left indicate transudative fluid. When blood is present in the fluid, the catalase test is deemed inconclusive. Thus, if the reagent strip testing for blood matches a color to the right of the well, indicating the presence of blood, the result of the catalase test cannot be used to conclude the type of pleural fluid. In this case, the thorocentsis should be repeated.

MATERIALS

Materials used for the fabrication of the Multivariable Bedside Test include a High Density Polyethelyne (HDPE) for the base plate. HDPE was purchased from McMaster Carr, Aurora, OH. This material was chosen due it is inert properties in order to minimize the unwanted reaction of the biological fluids with the plastic. Additionally, HDPE was chosen due to the workability of the plastic. The tools available for the fabrication of the design would be sufficient to cut and drill through HDPE. The material chosen for the vials was glass. Glass is

advantageous because it is inert. Furthermore, glass is clear to allow for visual observation of the reaction.

Additional materials include adhesive labels, reagent strips, a glucose meter, glucose test strips, and hydrogen peroxide. Reagent strips used were CLIA-10 Urine Reagent Strips, CLIAwaived, Inc. These strips had the ability to test for the presence of 10 biological markers; however, the only three applicable for our intended use were protein, specific gravity and blood. The CLIA-10 strips were chosen because they contained the desired tests. The pH test was conducted with pHydrion Vivid litmus paper, NewPage Productions, Inc. The pHydrion paper was chosen due to the sensitivity of the results. The glucose meter used was an OneTouch UltraMini, LifeScan, Johnson & Johnson. For compatibility reasons, OneTouch Ultra glucose test strips were used. The test conducted to determine the presence of catalase requires 200 μL of 30% hydrogen peroxide.

FABRICATION

In order to create wells with a flat surface for the reagent strips to rest on, a drill press with Forstner drill bits was used. The wells housing the pleural fluid are 0.95cm diameter and 0.32cm deep. The hole used to support the vial has a diameter of 1.27cm and a depth of 0.64cm. The spacing between holes is 0.95cm resulting in overall dimensions for the base plate of 10.2cm wide, 12.7cm long, and 1.9cm deep. Once the necessary holes were drilled, the base plate was cut to the appropriate size using a band saw. The edges were then finished with a belt sander.

The reagent strips chosen contained 10 reaction sites allowing for the detection of 10 different biological materials. However, only 3 of the reaction sites were applicable to pleural fluid. In order to eliminate unwanted portions of the strips, the desired regions of the strips were cut out and secured onto the bottom of the wells. The label for the base plate was designed using Adobe Photoshop CS3, and printed on white Avery adhesive labels. Finally, the required amount of fluid was pre-packaged in a clearly labeled, sealed vial.

The Multivariable Bedside Test is intended for use in conjunction with a thoracentesis kit. Therefore, the objective is to include the required parts for the test inside the thoracentesis kit. The common thoracentesis kit includes a section (10.9x16x1.9cm) originally intended for multiple tubing sets of various sizes; however, it is possible to utilize this space for the components of the Multivariable Bedside Test and eliminate the unnecessary tubing. Therefore, the required tools for withdrawing and analysis of pleural fluid will be contained within one kit.

COST ANALYSIS

The semester's design expenses can be viewed in Table 4. The total spent was $135.78. Each item purchased is being used in the final prototype. Due to our client's budget, cost was not a limiting factor. The fabrication of a single prototype required the materials listed in Table 5. We adjusted the costs to represent the actual price of one MBT.

Item	Cost

Item	Cost
Glucose Strips	$18.89
pH Strips	$17.10
Labels	$10.20
Multivariable Urine Test Strips	$35.90
Glucose Meter	$29.99
Plastic Piece	$23.70
Total	**$135.78**

Table 4. Total expenses: $135.78. Materials purchased September to December 2010

Glucose strip (1)	$0.76
pH strip (1)	$0.05
Label (1)	$0.10
Multivariable urine test strip (1)	$0.36
Glucose meter (1)	$29.99
Plastic (4x5x.75 in)	$5.93
Test tube (1)	$1.15
Hydrogen peroxide (30%) – 100 µL	$0.03
Total	**$38.37**

Table 5. Total cost of materials in single prototype: $38.37. Labor for fabrication not included.

TESTING

In order to ensure that our prototype follows design specifications, testing is critical. Ideally, testing would have included pleural fluid from an animal such as a dog or cat. However, we were unable to obtain pleural fluid after contacting over 30 veterinarians in the area. One limitation for testing was the time range for pleural fluid to maintain its biological properties. Pleural effusion can only be stored up to four days before the properties begin to change [44]. Due to the time restrictions associated with viable pleural effusion fluid it was difficult to acquire fluid within the time range necessary. With more time, testing with actual fluid would be beneficial. To remedy the situation, solutions were prepared to simulate properties of transudative and exudative fluid.

The fluid mimicking exudative effusions consisted of half of an egg white and approximately ten drops of lemon juice. This solution yielded all properties characterized in exudative fluid that we are testing apart from the glucose concentration (Figure 9). In exudative, glucose is generally less than 60 mg/dL; however, in our solution it was 438 mg/dL. This high concentration is due to the sugars found in egg whites. When the solution was mixed with hydrogen peroxide, the solution bubbled within one minute, indicating an exudative effusion.

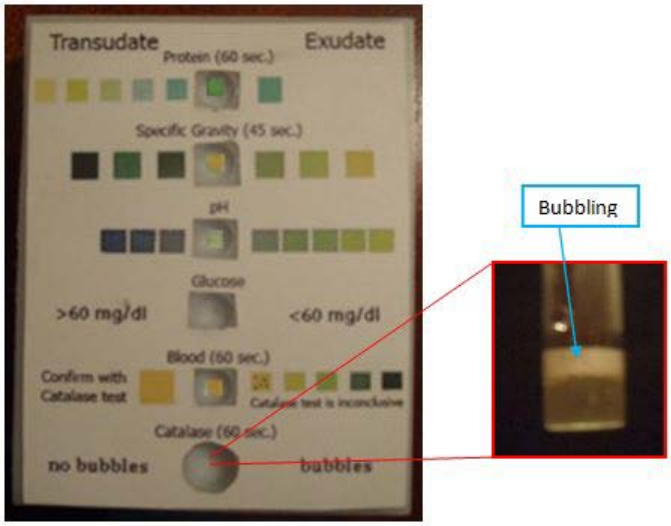

Figure 9. Exudative test results and a positive catalase test with bubbles.

The solution mimicking transudative effusions consisted of 1/8 teaspoon of baking powder, 20 mL of warm water, and ½ teaspoon of sugar. The baking powder was added to increase the basisity of the solution and the sugar was added to increase the glucose concentration. With this solution, all tested elements were fulfilled (Figure 10).

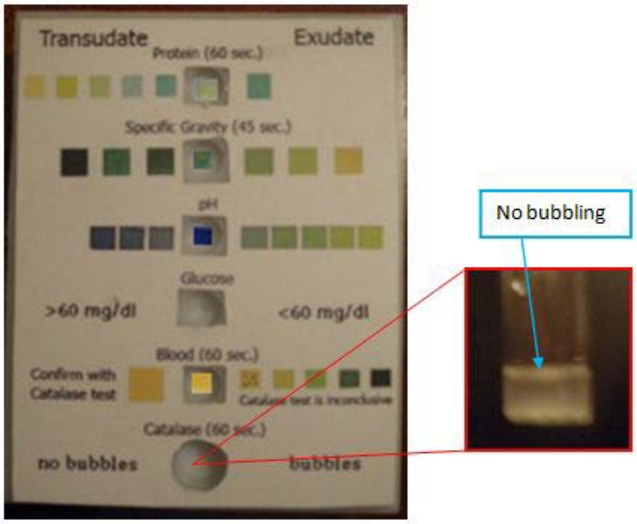

Figure 10. Transudative test and a negative catalase test with no bubbles.

We can conclude from our testing that the process of our design is efficient. Also, for different samples, our tests read the correct ranges of concentrations.

ERGONOMIC CONSIDERATIONS

Attention to human comfort and safety are essential for a successful testing device. Maintenance and operation by the user must be minimal in order to ensure satisfaction. The final testing device must also be compact for ease of handling and use. To improve ease of use, ideally all of the tests (pH, glucose, specific gravity, protein content, and blood content) would be placed on a single strip. The testing device is designed for one-time use and easy disposal for efficient clean up.

Safety considerations have been made for use of 30% hydrogen peroxide. In our design, we made sure the user would not contact the solution. Hydrogen peroxide is in a separate, sealed container, to which the sample of pleural effusion is added. This is due to the fact that hydrogen peroxide is a dangerous chemical. It may cause burning of the skin, eyes, and respiratory tract. It is harmful if swallowed or inhaled, corrosive, and contact with other material may cause fires.

ETHICAL CONSIDERATIONS

The information gathered for the presentation of this paper was done ethically. The thoughts and knowledge that were taken from previously reported studies are given credit via citations when appropriate. The authors of this paper are appreciative for the previous knowledge that allowed us to create the design proposed in this report.

Further ethical considerations should be carefully evaluated during future testing. In order to validate the sensitivity and specificity of the MBT, it is imperative that repeated testing of results should be conducted. This requires fluid to be drawn from patients to be tested with the MBT as well as the currently accepted laboratory tests. Results from the MBT would then be compared to the accepted protocol. In some patients with pleural effusion, it is not necessary to conduct a thoracentesis. Thus, some may believe that testing for the MBT may go against medical ethics. However, it has been proven that a thoracentesis is a safe procedure. Therefore it can be concluded that the proposed methods of testing are appropriate.

FUTURE WORK

The focus of future work on this design includes advancing and testing the Multivariable Bedside Test prototype. Although our design successfully incorporates several tests, additional improvements can still be made. First, the size of the design was limited by the fabrication method used. Ideally, the prototype can be made smaller by simply scaling down the dimensions, which will allow easier integration into the thoracentesis kit. As a consequence of the current large size, the prototype is unnecessarily heavy. To limit the weight of the design, the thickness of the plastic may be reduced. By using thinner plastic and possibly a molding fabrication technique, the prototype can have a more convenient weight.

Second, the layout of the design can be improved to allow more efficient mass fabrication. Instead of incorporating individual strips for each test, all five tests could be developed on one test strip. By designing a single strip with all six tests, only a single well would need to be molded into the plastic in which the strip may be placed. This modification would reduce the amount of plastic necessary for mass fabrication, consequently reducing costs. Also, by filling only one well versus five, the use of the prototype will be more convenient for physicians. Furthermore, by containing the tests in one location, the well can be sealed to preserve the test strips, which normally deteriorate within 90 days of exposure to air, according

to the CLIA-10 Urine Reagent Strips instructions. This modification will help to increase the shelf life of the prototype.

To further improve the convenience of the prototype, a comparable colorimetric test strip should replace the glucose meter. By eliminating the need for a glucose meter, the fluid analysis becomes much more consistent by using only reagent test strips. Since we were unable to find suitable glucose strips, new test strips may need to be developed with the required accuracy and concentration range required for this application. By replacing the glucose meter, the weight of the prototype will be lessened further. With only test strips in the design, the prototype will be more homogeneous and, therefore, more convenient. The importance of convenience is second only to accuracy. However, both convenience and accuracy can be increased by simplifying the interpretation of results. This may be done by incorporating a colorimetric analyzer to decrease potential error in color interpretation by the user [42].

Although the design already utilizes several tests to differentiate exudative and transudative effusions, additional tests can be incorporated to increase the accuracy and diagnostic ability of the prototype. Established criteria of the types of effusions include comparisons of the pleural concentration of the compound to the blood serum concentration. These tests include cholesterol, albumin, protein, lactase dehydrogenase, bilirubin, and sialic acid. Including additional test criteria will only improve diagnostic accuracy. However, determining this ratio requires testing the blood as well as the pleural fluid. In order to do so, additional test strips must be included in the design. Even still, determining this pleural-blood serum ratio based on colorimetric results seems a daunting task. Therefore, a method of comparison must be developed before these ratio dependent criteria can be utilized in this prototype.

Incorporating additional tests that do not depend on a ratio to blood serum could make the prototypes diagnosis more dynamic. These tests may serve to determine the cause of the effusion once the exudative or transudative nature has been verified. For example, testing for amylase concentration in the pleural fluid would allow the physician to determine if effusion is due to pancreatic dysfunction [25]. Similarly, detecting leukocyte concentration may help indicate an infectious exudative effusion [42]. This would be advantageous when determining whether or not to treat the patient with antibiotics. Often times it is imperative for rapid diagnosis of infectious effusions [45]. Thus, future work should be conducted to incorporate leucocytes in the MBT. Many urine analysis strips already have the ability to quantify the amount of leukocytes present; however, the strips used for this study did not have the appropriate cut offs.

Along with the design layout improvements, the prototype must be tested to evaluate its effectiveness as a diagnostic tool. The first step in the testing procedure will involve a small scale test of the diagnostic accuracy using previously characterized pleural fluid. By comparing the results of the prototype to the known nature of each fluid, the sensitivity and specificity of the prototype can be determined. These values will define the accuracy of the overall design and diagnostic criteria and are critical to the comparison to current diagnostic methods, including lab testing. By combining several tests into one design, the prototype should have more accuracy than any of the individual tests alone. This testing will be the most significant evaluation of our design's accuracy and, consequently, its usefulness.

Finally, although our design has been designed to fit into the thoracentesis kit, it can be further integrated into the current thoracentesis procedure. This integration into the current standard protocol will be planned in such a way as to not detract from the ease or effectiveness of the procedure. Ideally, utilizing our design in the process will improve the efficiency of the procedure. The end goal of our project is to incorporate our design into each thoracentesis kit for the convenience of physicians and others using this diagnostic tool.

CONCLUSION

This incorporative Multivariable Bedside Test allows for more rapid determination of the nature of pleural effusions. By testing several aspects of the fluid, the pleural effusion fluid can be rapidly characterized as an exudate or transudate. Consequently, this will shorten the diagnostic time and allow for treatment options to be considered with less delay by eliminating the need for lab work that may require several hours. Furthermore, by unifying several methods of effusion characterization, our design will offer increased diagnostic accuracy. With its small, straightforward design, our prototype is well suited for use in military applications, in which convenience and portability are critical. Also, the low cost of the design allows for use in 3rd world countries and rural communities that may not have the necessary lab resources. Not only will this device decrease diagnostic time, but it will also benefit hospitals and veterinary clinics around the world that may be less equipped to conduct standard diagnostic tests.

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APPENDIX

APPENDIX A.

Project Design Specifications

#22- Characterization of Pleural Fluid

September 10, 2010

Team: Carmen Coddington, Kelsey Duxstad, Bryan Jepson, Christa Wille

Client: Dr. Steven Yale

Advisor: Professor Chris Brace

Function:

Dr. Steven Yale has requested a non-invasive, clinical method to characterize the fluid properties of the pleural effusion to determine whether the pleural effusion is exudative or transudative.

Client Requirements:

Cost effective
Portable
User friendly
Accurate

Design Requirements:

1) Physical and Operational Characteristics
    a) *Performance requirements*
        i. To determine the fluid properties of the pleural effusion (pH, glucose, catalase, protein content, blood content, specific gravity)
    b) *Safety*
        i. No negative biological effects
        ii. Corrosive hydrogen peroxide needs to be well contained
        iii. Minimal exposure of body fluid
    c) *Accuracy and Reliability*
        i. Test strips accurately detect concentrations of components of fluid
        ii. Accurately differentiate between exudative and transudative effusion
    d) *Life in Service*
        i. Single use
        ii. With additional strips the glucose meter can be reused
    e) *Shelf Life*
        i. 3 months
        ii. Strips deteriorate 90 days after breaking seal
    f) *Operating Environment*
        i. Patient hospital rooms
        ii. Military zones
        iii. Veterinary hospitals
        iv. Third world countries
        v. Clinics without lab support/ equipment
    g) *Ergonomics*
        i. Easily maintained
        ii. Symmetric design
        iii. Consistent layout
        iv. Easy to read
    h) *Size*
        i. 10.2cm wide, 12.7cm long, and 1.9cm high
    i) *Weight*
        i. 231 grams
    j) *Materials*
        i. No latex
        ii. High density polyethylene
        iii. Clear plastic test tube – 8.573cm tall, 1.27cm in diameter
    k) *Aesthetics*
        i. Easy to read results
        ii. Symmetrical design
2) Production Characteristics
    a) *Quantity*
        i. One model
    b) *Target Product Cost*
        i. $38.37– with glucose meter
        ii. $8.38 – without glucose meter
3) Miscellaneous
    a) *Standards and Specifications*

i. FDA approval is required if placed in the market

b) *Customer*

i. Medical schools
ii. Hospitals
iii. Military
iv. Veterinary clinics
v. Veterinary schools

c) *Patient-related concerns*

i. If test is inconclusive, further lab work is needed

d) *Competition*

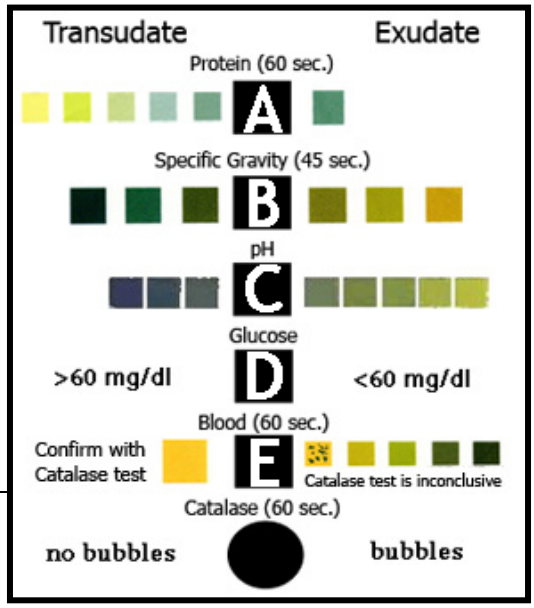
i. Magnetic resonance spectroscopy
ii. Ultrasound
iii. Protein analysis

APPENDIX B.

Instructions for implementation and analysis of the Multivariable Bedside Test

1. Fluid will be obtained through a thoracentesis
2. Extract 1 mL of fluid into a 5 mL syringe
3. In wells A, B, C, and D, add fluid drop-wise until reagent strip is covered
4. Place 3 drops of fluid in well E
5. Add 10 μL (approximately 1 drop) of pleural fluid to the vial containing 30% hydrogen peroxide*
6. Wait until the indicated amount of time has passed.
7. Circle the reference color on either side of the well that most closely matches the observed color of the reagent strip
8. Remove glucose strip from packaging and insert in glucose meter
9. Touch the glucose strip to the fluid in well D, just until fluid is drawn on to strip
10. Circle the correct description for the results of the glucose test (either <60mg/dL or > 60 mg/dL)
11. If the color of the reagent strip in well E matches the reference color on the left, results of the catalase test cannot be used to characterize the type of fluid

* Caution: Hydrogen peroxide may cause burning to skin, eyes, and respiratory tract. Harmful if swallowed or inhaled. Corrosive. Contact with other material may cause fire.



APPENDIX C. Project Schedule

Tasks	September				October					November				December	
	3	10	17	24	1	8	15	22	29	5	12	19	26	3	10
Design Process															
Research															
Brainstorm															
Prototype Design															
Fabrication															
Testing															
Meetings															
Client															
Team															
Presentations															
Mid-semester															
Final															
Deliverables															
PDS															
Peer/Self Evaluations															
Progress Reports															
Mid-semester Report															
Final Report															
Website															