Design For A Device to Supply Continuous pH Data of Embryonic Media within an Incubator

Team Members:

Sara Alford, Byoung Choe, Kristi Hinner, Steve Huffaker, Carla Maas, Gabriel J. Martínez-Díaz, Ryan Porter, Mike Swift, and Laura Wing

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Client:

Dr. Theresa Duello
Associate Professor, Obstetrics and Gynecology
University of Wisconsin – Medical School

Advisor:

Dr. Nimmi Ramanujam

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ABSTRACT: The goal of this semester was to design a system that would measure the pH of a media containing bovine embryos enclosed in a CO_2 incubator. Data needs to be stored with minimal disturbance to the environment. Background research led to three major components of the system: the probe, the stand and the data collection device. Multiple design options for each component were researched in the beginning portion of the semester. From these options, a final design solution was chosen. A micro glass bulb probe from Thermo Orion was chosen for its ability to accurately measure the pH of micro volume solutions of media to a tenth of a pH unit. A stand was designed and built with vertical and horizontal adjustments for better measurement placement. Lastly, a receiver and RS232 cable was purchased to allow for data processing at a computer located external to the incubator. The system was assembled and preliminary testing demonstrated successful transmission of pH measurement data to the computer.

1. Problem Statement:

Our objective is to design a device in order to constantly monitor the pH levels of the bathing medium for bovine embryos inside an incubator.

2. Background:

a. Embryos and development

During the embryonic stage of life, tremendous variation and development occurs in the growing organism. Hundreds of biochemical reactions must take place in order for the organism to replicate genetic material and generate cellular material properly. A slight modification of the intracellular pH (pH_i) can lead to conditions that prevent or significantly alter these reactions [3]. Because the embryonic cells are undifferentiated, a disruption in the intracellular biochemistry may lead to substantial developmental abnormalities for the organism [1]. Therefore, the maintenance of a homeostatic environment at the cellular level is of the utmost importance.

Embryonic cells do not develop in a vacuum; the ability to properly interact with the external environment is a vital necessity. Currently, researchers at the University of Wisconsin-Madison Medical School are evaluating the effects of hormonal interaction with embryonic cells during the gestation period [3]. In an effort to find a correlation between the hormonal effects and embryonic viability, researchers must quantitatively evaluate shifts in cellular characteristics such as temperature and pH_i. Changes in the pH_i can influence the tested hormonal effect, as well as the ongoing developmental reactions [2].

Due to the minute size of the embryos, a direct measurement of the pH_i is both cumbersome and time-consuming [3]. A more convenient approach is to directly measure the pH of the culture media. Measurement of the pH of the external environment—in this instance, the culture media—serves as an indicator of the cellular pH_i [3].

Our client is using pH among other factors to grow the healthiest possible embryos for shipment to Ecuador. Healthier embryos will maximize the percent of embryos that survive freezing and shipment. Once in Ecuador these embryos will be used to breed cows that are tolerant to heat and capable of producing more milk and more beef. Through many generations of cows, eventually Third World countries such as Ecuador will be able to produce more food.

b. Concept of pH

Because of its importance in many chemical reactions, pH is one of the most common laboratory measurements. A definition of pH can be summarized as the negative of the base-10 logarithm of the hydronium ion concentration [6]:

$$pH = -\log [H_3O+]$$

The pH scale extends from 1 (highly acidic) to 14 (highly basic). Because the scale is logarithmic, a change of one pH actually represents a ten-fold difference in acidity [6].

c. General Measurement Principles and Methods

Due to its importance in numerous disciplines, many methods exist to measure pH. Several examples of methods include indicator paper, pH electrodes, and the combination of light and an indicator dye. Indicator paper consists of an indicator dye adhered to paper. As the paper comes into contact with the sample, the dye changes color as a result of the sample's pH [6]. Electrodes work by measuring the changes in voltage potential caused by the changing proton concentration in the sample [5]. The results are processed and displayed by a pH meter. A final approach makes use of an indicator solution in the sample. Changes in the acidity of the solution change the color of the indicator and also cause changes in the absorption of the sample. Measuring this absorption change can be done by fiber optics or by spectrophotometric methods.

Choosing a method to find the pH of a sample ultimately depends upon the characteristics of the sample [5]. For instance, suppose an individual is attempting to determine the pH of a soil sample. The method chosen in this instance may be completely different than the method chosen to measure the pH of a blood sample. Another consideration is the type of result that is required. If only an estimate of the sample pH is desired, using indicator paper would be the best choice in terms of accuracy, ease, and cost. More accurate results would dictate using a standard pH electrode and meter.

3. Design Specifications

Several constraints make the task of designing a device to measure pH somewhat more difficult. The key considerations in finding a solution to our design problem are as follows:

- Small sample volume size
- No modification of the incubator
- Cost

First, the small volume of the sample (approximately 25-50 μl) eliminates the possibility of using standard sized probes. Probes that may be considered must offer consistent and accurate results. In addition, the probes may not inhibit or in any way impede the growth of the embryos. This inhibition could stem from the use of certain electrolyte solutions [5]. Due to the sensitive nature of the embryos, an electrolyte that is compatible with biological systems must be considered (if the probe requires such a solution).

The second constraint of not altering the incubator also determines which design solutions are feasible. Because the embryos are grown in such a controlled climate, any alteration of the incubator may have dramatic effects on the growth of the embryos. Alterations of the incubator would include drilling holes through walls, removal of the rubber gasket, or modification of the water jacket. The incubator had a volume of 3' x 2'6" x 3'.

The final constraint stressed by the client is cost. It is easy to look at different components in catalogs and lose track of the associated cost [3]. This constraint is one that will be placed on engineers at all times. In an effort to reduce costs of the final design solution, it is important to consider alternative options such as obtaining demo products for short-term use from companies, borrowing equipment from fellow faculty members and utilizing available resources to build equipment when possible (i.e. stand unit).

For a more in-depth examination of design constraints, please refer to Appendix A—Preliminary Design Specifications (PDS).

4. Alternative Design Solutions and Evaluations

When the team first evaluated this project, we realized that this project was a combination of three different components: a probe, a data collector, and a stand. Initially our team researched together, but after mid-semester, our team subdivided into a probe and data collection group, and a stand design group. Below, broken up by section, are the different designs we brainstormed and the train of thought we went through for choosing the final design.

a. Probe Component

When the group started working on this project, a major emphasis was placed on finding different techniques to measure pH. An article comparing four different techniques of pH monitoring systems in-vitro and in-vivo was a good starting point for our groups to come up with design alternatives. The fiber optic sensor and the glass bulb electrode were initially considered.

Fiber Optic Probe: The fiber optic sensor uses an indicator that free protons in the solution can bind to and cause a color change. The intensity of the color is directly proportional to the amount of free protons in solution, and thus the pH can be measured. When the fiber optic method was researched further, it was found to still be in the developmental stage. It was expensive, had difficulties with calibration, and was subject to large offsets. Due to the many limitations, it was ruled out as a probe possibility.

Glass Bulb Probe: Our group then focused on the glass bulb probe as the means of pH measurement. The glass-bulb probe is a standard pH measurement device that is on the market today. Therefore, any problems that exist with this technique have already been fixed or are well documented. The probe uses an Ag^+/AgCl electrode that is encased in a glass-like polymer to which only free protons can bind. When submerged, free protons from solution bind. The charge on the glass shell is transferred via the saturated AgCl solution through the Ag electrode and into the receiver. The receiver then uses the potential difference between the glass shell and a standard solution to determine the amount of free protons in the solution. Three probes from the Orion Micro line were prime candidates for our project due to their ability to measure the micro volumes that our client worked with. The models: 98-02, 98-03, and 98-10 were considered as possible probe choices. Figure 1 shows a complete comparison of the different specifications and characteristics of each of these models.

Orion Probe Model No.	98-02	98-03	98-10
pH range	0 – 14	0 - 14	0 - 14
Temperature range	-5°-100°C	-5°-100°C	-5°-100°C
Internal Reference	Ag/AgCl	Ag/AgCl	Ag/AgCl
Dimensions	150mm x 6 mm	83 mm x 2.5 mm	120 mm x 6 mm
Tip Diameter	2.5 mm	2.5 mm	1.3 mm
Approx. Tip Length	18 mm	48 mm	37 mm
Depth of immersion	2 mm	2 mm	1 mm
Catalog No.	9802BN	9803BN	9810BN
Adaptor Lead	BNC connector with 1 m cable	BNC connector with 1 m cable	BNC connector with 1 m cable
Minimum Sample Volume	5 µl	5 µl	0.5 µl
Cost of Probe	$329.00	$379.00	$379.00

Figure 1: Comparison of electrode attributes of the Models 98-02, 98-03, and 98-10 Micro pH Electrodes from Orion [4].

Final Probe Analysis:

Based on the information presented in figure 1, a complete comparison of the probes was easily obtained. The tip diameter, depth of immersion and sample size that was needed were compared for each of the models. With a smaller the tip diameter, the reading obtained would be better. The sample size that our client was working with was 25μl total. We needed a probe that was able to measure these small volumes with precision and accuracy. A smaller depth of immersion would also help with better readings. This means the pH probe doesn't need to penetrate into the media as much, meaning there is less chance that there will be unfavorable interactions with the embryo.

By comparing the depth of immersion and tip diameter, the Model 98-10 was our best choice. It measured the smallest sample size, with a tip diameter that was only 1.3 mm and a depth of immersion of only 1 mm. This was a big difference from the other two models that both needed a 2 mm depth of immersion and had tips of 2.5 mm diameter.

b. Stand Component

The stand component was the next part of the design solution the group examined. Ultimately, the decision to purchase or build a particular stand format was dependent upon the final probe chosen. Once the Model 98-10 probe from Orion was chosen as our final probe component, the next step was to determine what our options were for possible stands that would best suit our needs. We concluded that there were two options: purchase a stand from Orion or design a stand.

Stands exist already made through various companies such as Orion. These stands are extremely sturdy and perform all functions that the client would desire for her work. These stands included multiple features such as controls for height adjustment, horizontal placement, and a holder for the sample. They range anywhere from the mid-hundreds up to almost two thousand dollars. Due to cost restraints, this idea was no longer an option, and a group was formed to design and build a stand. The group has two designs, one of which is a modification of the first design option.

Stand Design Option #1: This stand design focused on the need to adjust the probe's position in all three dimensions. A rough sketch is shown in figure 3. The base is a rectangular sheet of a metal from which a Wells 96 plate can be attached. The plate allows for movement in all four directions through the use of smooth rods and screws. As these screws are adjusted, the wells plate will change the position along a x-y plane. Next, a clamp is included on a vertical mast that adjusts the vertical position of the probe. Markings on the side of the rod can be used to record the exact vertical position of the probe. The clamp contains teeth that would be able to firmly grasp the probe. Also included are two gearboxes that allow for the user to know the settings of the depth of the plate. These gearboxes, shown in figure 2, allow for readings taken on the front of the stand, for easier access.

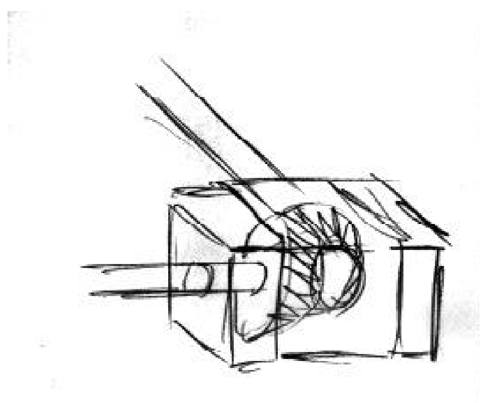

Figure 2: Rough Sketch of the gear mechanism. Two gearboxes are included in this design option. The gearboxes allow the user to see the probe and base placement looking from the front.

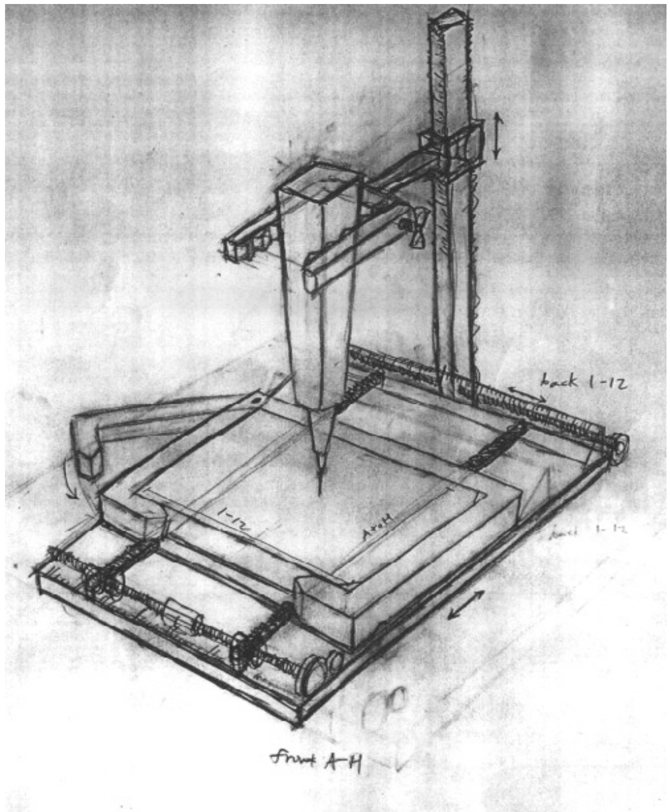

Figure 3: Sketch of the first design solution for the stand. This design's main features include movement in all three dimensions, and the ability to read the placement of the probe in the front due to gearboxes.

<u>*Stand Design Option #2 (A Modification of Design #1)*</u>: After working with the details of the first design, it became apparent that this still was too costly of a method. The gearboxes were around $200 each. Since the first design included two gearboxes, it was more costly than desired. After analyzing the necessity of the different components, the second design option was generated. This eliminated the need for gearboxes and utilized markings on the side of the base. Other than that, this design still relied on the same principles as described earlier in design option #1. A sketch is shown in figure 4.

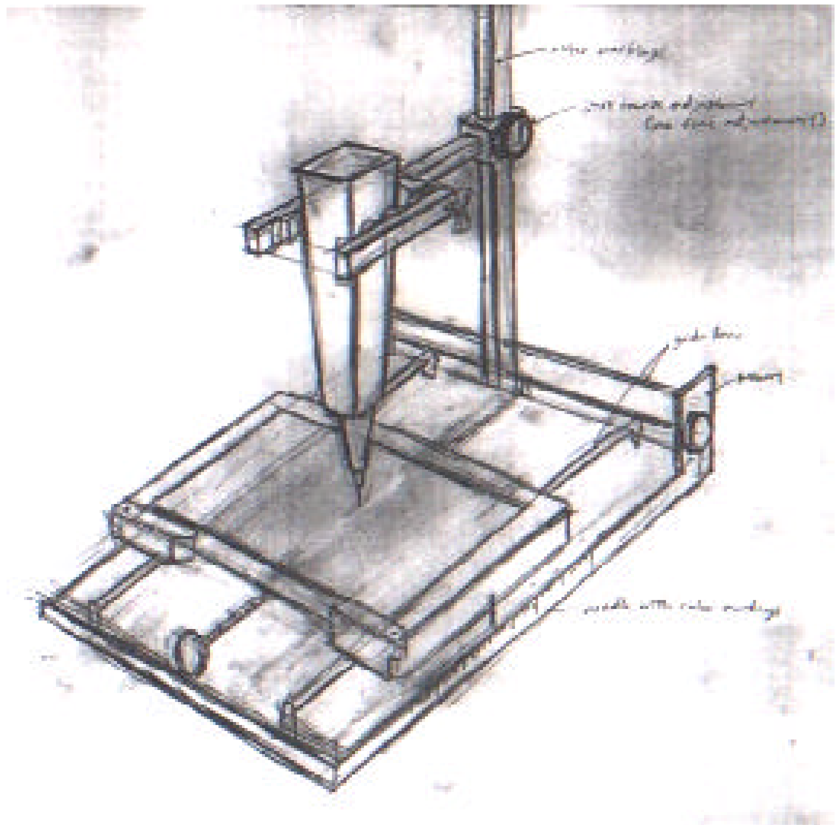

Figure 4: A rough sketch of the modified stand design. This design eliminated the need for gearboxes. It still can change the location of the probe and plate in all three dimensions. Marking devices are still on the device, though not as accessible as in the first design option.

Stand Design Analysis:

The major factor that dictated our selection for stand design was cost. Purchasing a stand would be outside of our biomedical engineering departmental budget, as well as our client's budget. Our group decided that we were capable of designing an adequate stand for the glass-bulb probe measurements. After coming up with design #1, and pricing the stand, it was approximately $800. This was still unacceptable, so the group reworked the design, eliminated expensive gearboxes that were not absolutely necessary for the stand to be functional. After this, design #2 emerged. This design still allows the client extensive movement of the probe and accurate placement. It was less costly, without giving up any features critical to the accuracy of the measurement. This stand consists of an aluminum base, a wells plate, and a mast. More in depth pictures of the stand are included in appendix D.

The base of the stand was made from two sheets of aluminum that were riveted together. The two plates are also fastened together by a U-channel. The bottom sheet is the only one that has holes for the rivets. The top sheet has slits into it to allow for the movement of nuts and bearings to pass through it, allowing movement of the wells plate. The base has a smooth and threaded rod. The smooth rod acts as a guide for the linear bearings to ride on. The threaded rod acts as the driving force to move the well plate back and forth. Nuts attach to the wells plate are threaded around the rods and are used to move the plate. The rods are held in place by aluminum bolts that restrict the rods forward and backward movement. The linear bearings will be assembled as shown in figure 5.

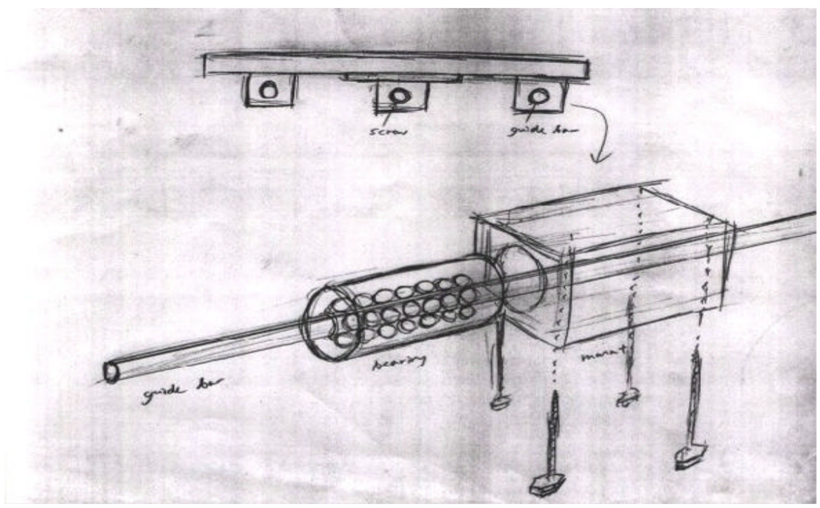

Figure 5: The linear bearings will fit into an aluminum block. This block will then be mounted to the base.

The next component of the stand is the wells plate. The plate's base is another sheet of aluminum. A region for the wells 96 plate is forged through the use of a three-angle channel system. This provides a region in which the plate can easily be slid into. A notch in this region assured that the wells plate would always be placed in the same direction due to its asymmetrical shape. There are two nuts for the drive screw mounted in blocks of aluminum on the right side of the plate. These are used to restrict movement, and also for ease of fastening to the bottom of the wells plate. Two linear bearings on the left side are also included for the same reason. Lastly, a piece of aluminum will stick out on the right side of the well plate, which will act as a pointer. Through this, the client will be able to tell which row her probe is aligned with for measurement.

The last component of the stand is the mast. The mast is also made from an aluminum block. There are two holes drilled into the block, one for a nut, and the other for a linear bearing. A rough sketch of the connection between the mast and stand base is shown in Figure 6.

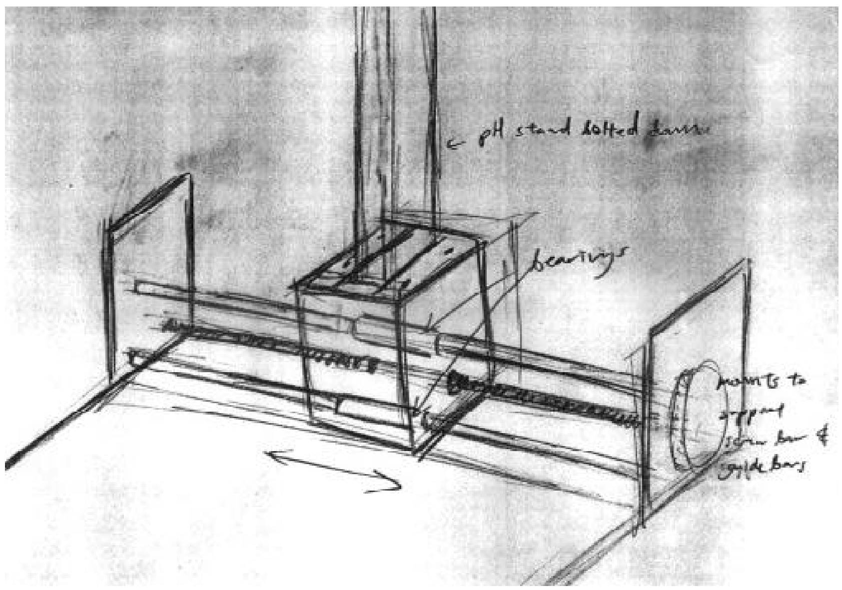

Figure 6: The stand base connects to the mast with a linear bearing rod. This is responsible for its side-to-side movement.

The nut is for a drive screw that will be responsible for the left-right movement of the probe. The linear bearing is used for structural support as well as allowing the beam to ride on a smooth rod, reducing frictional forces. A "microscope" part will be screwed on the top of this aluminum block completing the mast. The pH probe clamp and clamp holder will then be fastened to the top of this piece. The microscope part will include incremental markings so the client will know how deep the probe during measurements. Lastly, an indicator in the back of the mast will signal what column the probe is over.

c. Data Collection/Storage Component:

Although the choice of an appropriate stand was dependent on the probe, determining which data collection/storage system to use was a fairly independent decision. With the probe and stand components decided, the next step was to determine the type of receiver and data storage method that would be implemented. The main constraints with the data storage component were the physical constraints of the incubator. The receiver, if inside the incubator, needed to be able to withstand the physical conditions of the incubator. If outside the incubator, some means of transmission of data needed to be found that didn't significantly alter the incubator, thereby maintaining its controlled environmental state. Three alternatives were considered for the data collection/storage component.

- Internal data storage unit
- Rf transmitter with receiver
- Direct connection to external personal computer (PC)

Internal Data Storage Unit: Inside the incubator, there is plenty of room to include a data storage unit that could hook up directly with the probe, and store data through out the day. The unit must be battery powered and be able to be programmed to take readings of the pH throughout a designated time period. When the client opens the incubator to check on the sample, the data logger could then easily be removed. At this time, the battery could be replaced, and the data analyzed. The data collector would be subjected to the environment of the incubator, meaning it would need to work in a humid environment. This means the device would need to be sealed, or at least water resistant. A preliminary schematic diagram of this setup can be seen in Figure 7. One type of data collector mentioned was the possible use of a palm pilot.

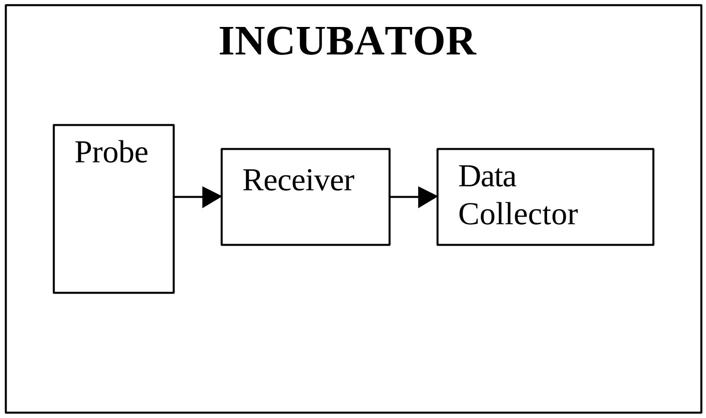

Figure 7: A schematic drawing of the flow of information in the internal data logger system. Data is transmitted to a receiver, which sends the data to a data collector. The data collector and receiver are both inside the incubator.

Rf transmitter with a receiver: In this data collection design, an Orion rf transmitter could be coupled with a receiver to transmit data to a nearby computer. The new Orion rf link enables users to break free from the constraints of cables. The Orion rf link allows you to send information being measured by instrumentation to a printer or a computer (in conjunction with Data COLLECT software using RS232 ports) for documentation. With a range of 25 feet, rf link would allow the position of the instrument to be almost anywhere within the laboratory.

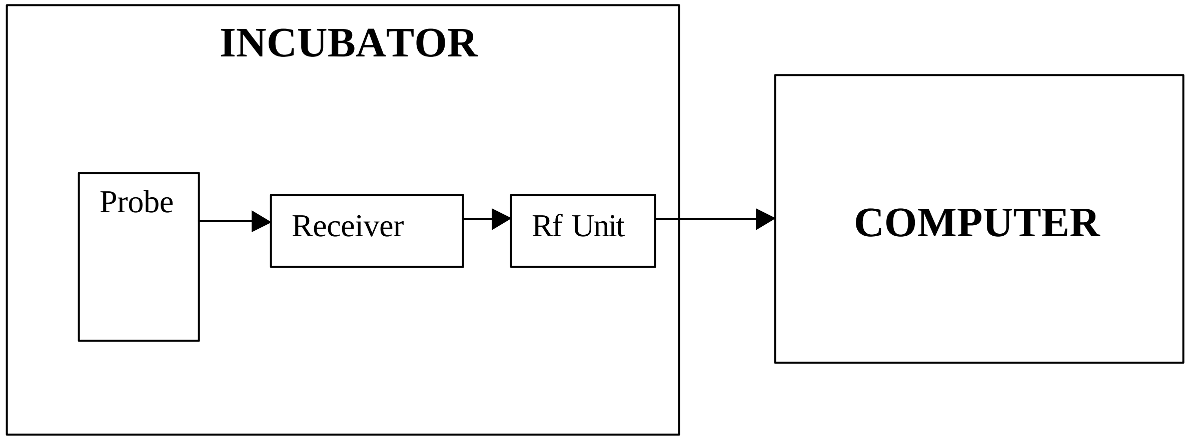

Figure 8: A schematic drawing showing the flow of information using an rf transmitter. The probe transmits data to a receiver that then send it to a rf unit. The rf unit that sends that information to a computer outside the incubator without any cords.

Direct Connection to a Computer: The probe can be connected directly to a computer that is outside the incubator. This option would require running a cord out of the incubator through the space between the door and seal. The client approved this change to the incubator after a client meeting.

A receiver would still need to be bought to change analog data to digital. The simplest and cheapest model was Model 410A. It performed all the necessary tasks for data collection the client desired, and could transmit data to a computer via a RS232 port.

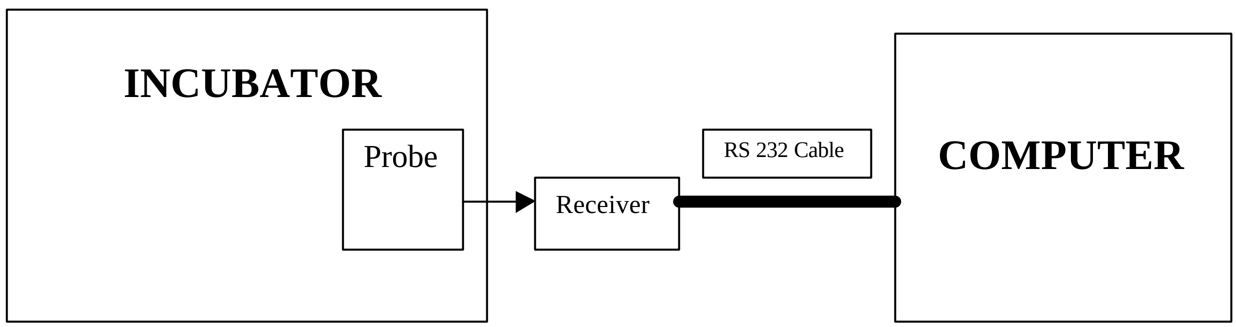

Figure 9: A schematic drawing of the flow of information in the external data collection method. The probe sends data to a receiver. The receiver then directly sends the data to a computer outside the incubator through the use of a RS 232 cable.

Data Collection Design Analysis:

The three designs were compared through the use of the following design matrix. After looking at different objectives such as the accuracy, the change to the environment, and cost of the design, the direct connection to a computer outside the incubator seemed to be the best option for our project.

	Internal Data Storage	Rf receiver	Directly to Computer
Accurate Data Measurement	+	-	+
Stable Incubator Environment	+	+	0
Data Accessibility	-	+	+
Simplicity	+	-	+
Client Preference	-	-	+
Cost	+	-	0
Total	**2**	**-2**	**4**

Figure 10: Design Matrix for the data collection devices. A + means that it met the criteria satisfactory; a 0 means it didn't meet or not meet the requirement, and a – meant that it did not accomplish this objective.

The criteria used to judge the different transmission methods were its ability for accurate measurement, maintaining a stable incubator environment, the accessibility, simplicity, client preference, and the cost. The client at first preference the rf receiver, but after talking to company representatives and two people in the department, the rf receiver was basically thrown out as an option. The ability of the rf receiver to transmit data would be inhibited by the incubator walls. Since the rf would not be able to function properly, it was ruled out as a serious possibility. The client was willing to supply us with a computer, and therefore, we strongly leaned towards the directly connection to the computer. She approved the cord leaving the incubator as an okay means of communicating our data. Also, the data is not transferred as much as in the other two methods. Therefore, there would be less apt to be any error due to transmission. Overall, the direct connection was a simpler, more practical method than either the rf transmitter or internal storage unit.

After talking with the client, she also agreed that the direct connection to an external computer would be the best option for data collection. Our group talked to a representative from Orion about what receiver would best meet there needs. They decided to order the Orion Model 410A Basic pH/Temperature benchtop meter. This was the cheapest receiver that still performed all the tasks needed of our project.

5. Final Design Solution

Our final design has five major components:

- Model 98-10 Electrode Glass Bulb Probe
- Stand
- Model 410 A Receiver
- RS 232 Cable
- Computer with software

After consulting with the client, Model 98-10 was ordered from Fischer, a company at which University of Wisconsin – Madison received a discount. The model arrived on April 17, 2001. This probe has a tip diameter of 1.3 mm and needs only a 1 mm depth of immersion.

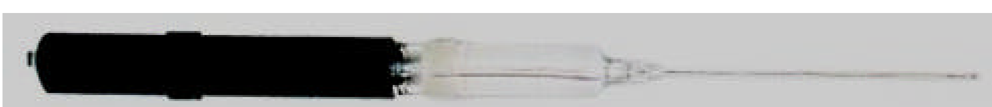

Figure 11: The Model 98-10 Micro pH electrode from Orion [4]. The 98-10 is a micro pH combination glass probe costing $379.00 and was the final choice for the probe component of our design solution. Its dimensions and specifications are stated in Table 1.

The probe is held in place in the incubator with a stand designed by members of our group. This stand, which was already explained in much detail, was constructed in the mechanical engineering machine shop. Sketches of the stand are included in appendix D.

The probe connects to the receiver by a three feet cord. If this is insufficient, a fifteen-foot extension cord can be purchased. Our group chose the Model 410 A receiver from Orion. This receiver was chosen was because it features auto calibration, automatic temperature compensation, and choice of pH resolution. The receiver digitizes the data and allows it to be transferred to a computer via a RS232 interface.

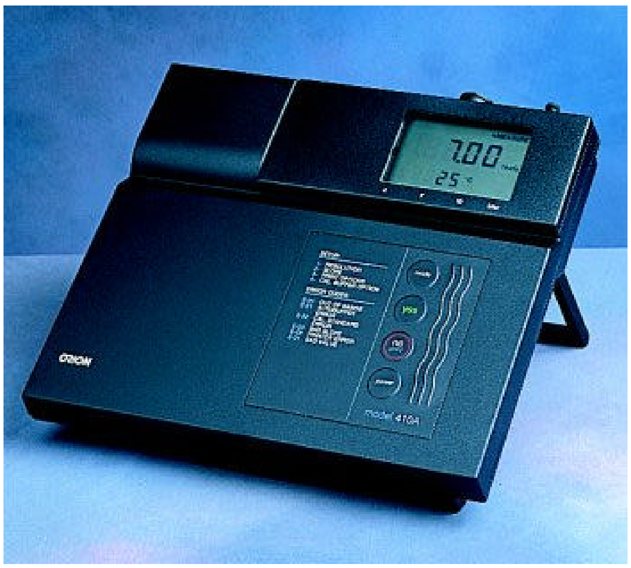

Figure 12: The Model 410 A Basic pH/Temperature Benchtop Meter from Orion [4].

Some difficulties have been presented with the RS232 interface and the available computer our client owns. The available RS232 cord typically interfaces only with a PC, not a Mac, which our client had reserved for us to use. By speaking with individuals in the Medical Electronics Shop, we were able to procure a Mac-compatible RS232 cord. However, there are no Mac-compatible software drivers available from Orion. Therefore, the client must seek out a PC if she wishes to process her data by computer. An IBM-compatible RS232 cord was ordered by our group. Upon the arrival of our products from Orion, we discovered the necessary software drivers (for PC or otherwise) were absent.

Several calls were placed to Orion to remedy the situation and as of April 29, 2001, the software drivers have been shipped.

As mentioned above, due to difficulties in computer compatibility, the 4^{th} design component has not been obtained. At this point, it appears that the client will have to obtain a PC (instead of a Mac) if she intends to process any data through the computer.

6. Conclusions

Ethical Project Constraints

Biomedical engineers are in a unique position to blend together the most advanced features of science and technology to create novel medical solutions. Artificial tissues, advanced drug design, and micro sensors are a small example of what the field is capable of creating. While these inventions appear to be boundless in their therapeutic value, it is the responsibility of the engineer to consider the possible ethical ramifications associated with all possible design solutions.

Any design problem presented to an engineer will possess a certain degree of ethical consideration. A preliminary examination of the design problem facing this group, revealed few ethical issues. However, after consulting with our client, the issue of the use of animals in research became a primary example of a topic that must not be overlooked by engineers

At this institution, any scientist seeking funding for research involving animal subjects must first submit an Animal Care and Use Protocol with the Research Animal Resources Center (RARC) [4, 7]. Furthermore, the protocol and researcher are reviewed annually in order to confirm that the proper procedures are being followed.

According to our client, because she uses bovine embryos in her research, there are few ethical issues to consider in our design process [3]. To obtain the embryos, the ovaries of slaughtered cows are harvested and inseminated with frozen sperm. Since the animal has already been sacrificed for another purpose, there is little opportunity for inhumane treatment of the animals. Further, the embryos are not chemically altered or mutated in any manner, which eliminates ethical considerations associated with genetic engineering [3]. Our client explained that when her work involves human subjects, the ethical considerations and issues are greater. Accordingly, the monitoring of her work by the appropriate organizations is more extensive.

Future Project Development

Due to several factors, the opportunity to evaluate the final design solution was not available. First, several problems occurred related to the purchased probe from Orion. Once the probe arrived, it was determined that the necessary software drivers had not been included with the product. This eliminated the possibility of testing the ability of the system to feed data to a computer. Aside from missing software, the purchase subgroup discovered that the probe was defective. Part of the glass had become dislodged from the probe leaving the probe disabled.

To remedy the situation, both the client and the purchase subgroup contacted Orion to obtain a replacement. Orion stated the Model 98-10 probe was not in stock. The group then made efforts to contact other companies, but found no replacements. Orion was contacted again, by the client, and a Model 98-03 probe was ordered and served as a suitable replacement probe. Additionally, the RS232 cable arrived and was tested using a PC-compatible laptop. The probe was successfully calibrated at a pH of 7.0 and the data exported to the computer via hyperterminal software. Continuous data acquisition was initially unsuccessful, however, a shareware program exists to solve this problem and is currently being evaluated as a potential solution. Clearly, additional testing of the probe at this point is necessary. Please refer to Appendix B for a further listing of possible experiments.

Several experiments should be performed upon the probe. Already, extenuating circumstances have prevented testing, however, it is a critical step that must not be overlooked. Examining whether the mineral oil overlay interferes with or alters results in one factor that must be examined. Additionally, the probe should be tested under a variety of pH conditions ranging from strongly acidic to strongly basic. Ultimately, testing will illustrate the shortcomings and the accuracy of the device.

Aside from probe difficulties, evaluation of the stand could not be completed due to delays in manufacturing. Due to the fact the stand group had to come up with a cheaper design, manufacturing of the stand was also delayed. The limited hours of the machine shop also impeded the progress. Despite these problems, the stand prototype will be completed by May 4, 2001. Further evaluation of the product will need to be completed thereafter, in particular the possibility of the stand developing rusty components. The materials used in construction of the stand are not stainless steel and due to the humidified environment, this is a consequence that the client must evaluate in using the stand on a long-term basis.

Additionally, the accuracy and precision of the aiming device of the stand should be quantitatively verified. In the course of the client's experiments, it will be necessary to remove the probe in order to change media and conduct evaluations on the viability of the embryos. In considering possible implementations or additions to the stand, automating the device would be a potential avenue to explore. Several commercial stands utilize an automated system where the sample wells are given a specific location. The user then chooses a location allowing the stand to move automatically.

Further evaluation must be conducted to determine the success and shortcomings of our final design solution. To accomplish this, several factors need to be tested and examined. The first of these is the effect of running a wire out of the incubator door on the internal environment of the incubator. If the device alters the temperature or carbon dioxide levels within the incubator, the embryos' growth could be hampered.

Please refer to Appendix B—Possible Experiments for an evaluation of potential experimental setups.

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Appendices

Appendix A—Preliminary Design Specification

Preliminary Design Specifications

April 15, 2001

pH Monitor for *In Vitro* Bovine Embryo Incubation Labs

Team Members:

Sara Alford, Byoung Choe, , Kristi Hinner, Steve Huffaker, Carla Maas, Gabriel J. Martínez-Díaz, Ryan Porter, Mike Swift, and Laura Wing

Client:

Dr. Theresa M. Duello
5240 Medical Sciences Center
1300 University Ave
tmduello@facstaff.wisc.edu

Design Statement: To design a device to measure the pH of approximately 20 – 50 μl of CR1 media surrounding *in vitro* cultured bovine embryos. The testing must occur within a CO_2 humidity controlled incubator. The data collection must occur without modification of the incubator.

Client Requirements:

- Does not interact with or disturb the embryos
- Does not alter the existing incubator
- Accurate to one tenth of a pH unit

Design Requirements:

Performance: The pH meter will obtain continuous readouts of bathing media surrounding *in vitro* bovine embryos. The device must not interact with or disrupt the embryos. The readings must be accurate to the tenth of a pH unit. The measured pH will also reflect the average pH of the bathing media. The device will take readings from cultures held in a CO_2 incubator and will either fit inside the incubator, or have leads that run into the incubator (without inhibiting or changing the incubator). The pH meter must be able to collect data for a minimum of 3 days.

Safety: The device will be properly insulated to avoid undesired electric discharge. The entire device, or the section in contact with the bathing media, can be sterilized to avoid contamination of the embryos.

Accuracy and Reliability: The device will continuously measure pH between 5 and 9 with accuracy to a tenth of a pH unit. Stand adjustment must be accurate enough to allow correct positioning of pH probe in order to record the desired pH.

Life in Service: The meter will be used long enough to obtain a reliable reading, and readings will be recorded at least daily during embryo growth cycle.

Shelf Life: The device will function for a comparable pH meter lifetime (~2 to 10 years).

Maintenance: The pH meter must be calibrated on a regular basis (~bimonthly). Calibration includes testing and resetting the meter in correspondence with the pH of known solutions (i.e. 5, 7, and 9). The cables and connections of the meter will be checked regularly (~annually). The device must be sterilized between each use (~ethanol wash, disposable sections, autoclave, etc.).

Operating Environment: The operating environment is a lab setting. The incubator is approx. 3’ x 2’6” x 3’. The incubator is held constant at 5% CO_2 and 35 degrees C.

Ergonomics: Controls for the pH probe stand should be user-friendly for adjustments.

Size: The pH meter and stand need to fit inside the incubator (dimensions given above). The pH probe tip must be small enough to measure the microvolumes of media.

Materials: The section of the device in direct contact with the media will be composed of materials that will not alter the bathing media or interact with the embryos. All components within the incubator must be able to withstand the environmental conditions.

Quantity: The client requires only one device for the time being.

Target Product Cost: Costs over the BME budget must be approved by client. Estimating around $1,000 for all project components.

Standards and Specifications: The device must be approved by Dr. Duello and must be tested extensively as to determine if there is any interaction with the media or the embryos.

Customer: The client (Dr. Duello) suggested the idea of a small microprobe in measuring the pH levels. She stated that the ideal meter would read the pH within the embryo, but the pH in the surrounding media would be sufficient. She would like the meter to make the reading within the incubator as to avoid moving and possibly traumatizing the embryos. The type of dish that the embryos are cultured in is variable and the client even suggested that another shape of culture dish might prove easier to make a measurement in. She initially specified that the device must not alter the incubator's function/performance in any way. Later she approved a cord exiting the incubator running under the bottom of the door.

Competition: There are many existing methods for measuring pH levels in solution. There are devices commercially available for these methods.

Appendix B—Possible Experiments

Experiment 1: Determine the effect of running a wire out of the incubator door on the internal environment of the incubator.

- Setup: obtain 5 wires that vary in diameter; setup a wire as if it were the cord exiting the incubator, going to the computer; close door
- Monitor the following variables at specific time intervals over the course of 48 hours:
    - Temperature—using a thermometer
    - Percent of CO_2—using the Fryrite device
    - Humidity—using ???
- Repeat experiment for each wire to determine if the size of the diameter of the wire causes changes in the incubator environment.

Experiment 2: Verify the precision and accuracy of the aiming device of the stand.

Experiment 3: Determine the effect the mineral oil overlay has (if any) on the probe tip and pH measurements.

- Setup: using stand and probe setup, prepare 5 volumes of 30μl of CR1 media (A group) and 5 volumes of 30μl CR1 media with a mineral oil overlay (B group), the amount used will be the same amount that client will use during embryo experimentation; calibrate probe, if necessary
- Take pH measurements from each sample in the A group; record results; take pH measurements from each sample in the B group; record results
- Compare percent difference

Experiment 4: Verify that the pH probe does not interfere with the ability of the embryos to develop normally.

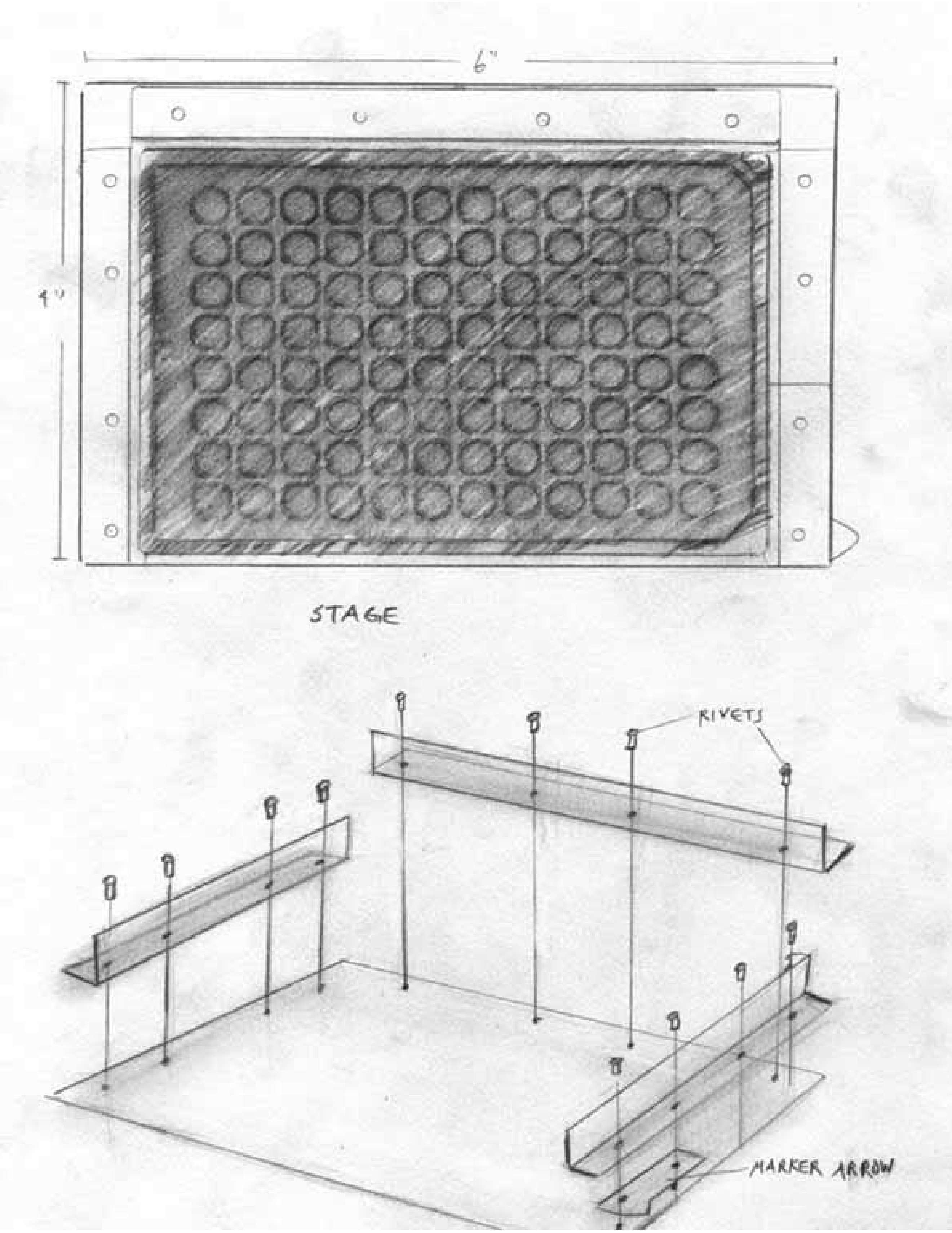
Experiment 5: Examine the capabilities of the pH probe/meter to function under a wide variety of pH conditions (strongly acidic to strongly basic).

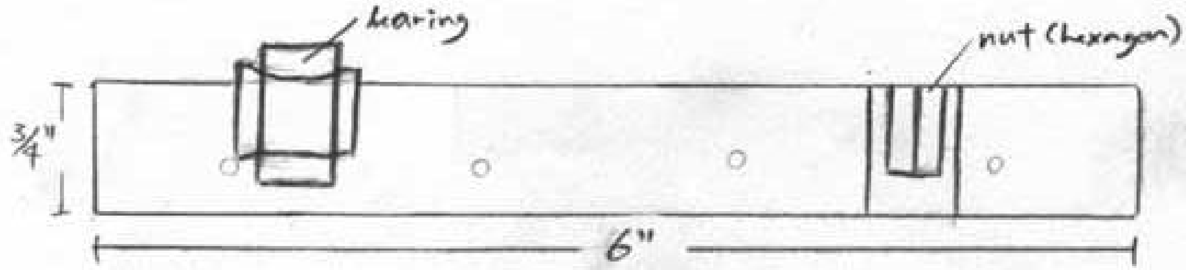
- Conduct these experiments using large volumes initially, followed by an identical situation using microvolumes.

Appendix C: Cost of Materials

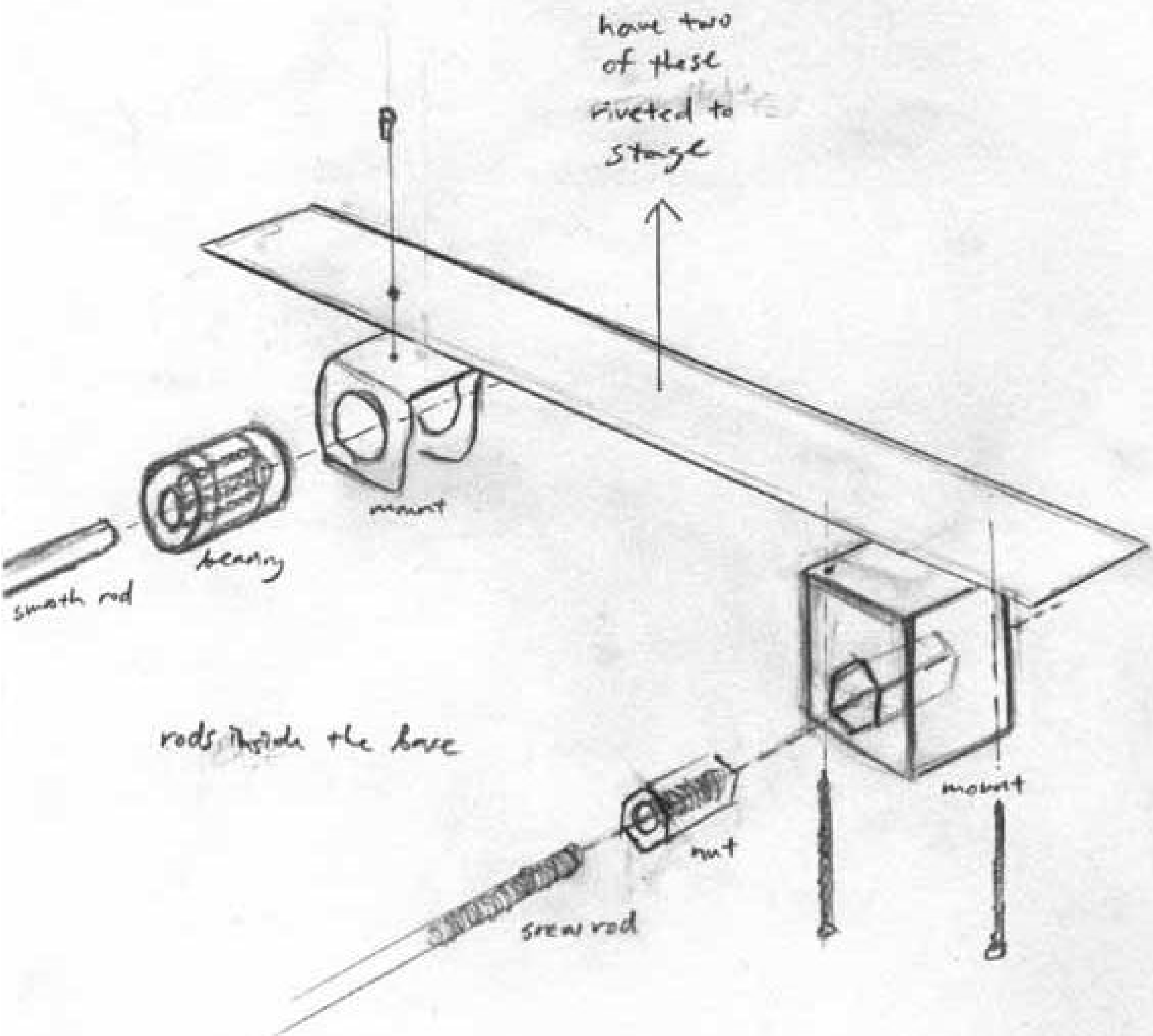
Design Component	Manufacturer	Cost of Item	BME Budget	Client's Account
Model 98-03 pH electrode	Orion	$303.20		XX
Model 410A Receiver	Orion	$345.60		XX
RS232 Cable/Software	Orion	$148.00		XX
Mac-compatible RS232 cable	UW Medical Electronics	Free		
Stand: aluminum rods, sheet metal, nuts, bolts, bar holders, U-channel, angle channel	Menard's	$50	XX	
Aluminum block (back mast)	UW Machine Shop (ME)	$12	XX	
Linear bearings	Mcmaster.com	$24	XX	
Probe clamp & clamp holder	Fishersci.com	$24		XX
Microscope part of the mast	Edmondoptics.com	$80	XX	
TOTAL COST		**$986.80**	**$166.00**	**$820.80**

Appendix D: Stand Design Plans



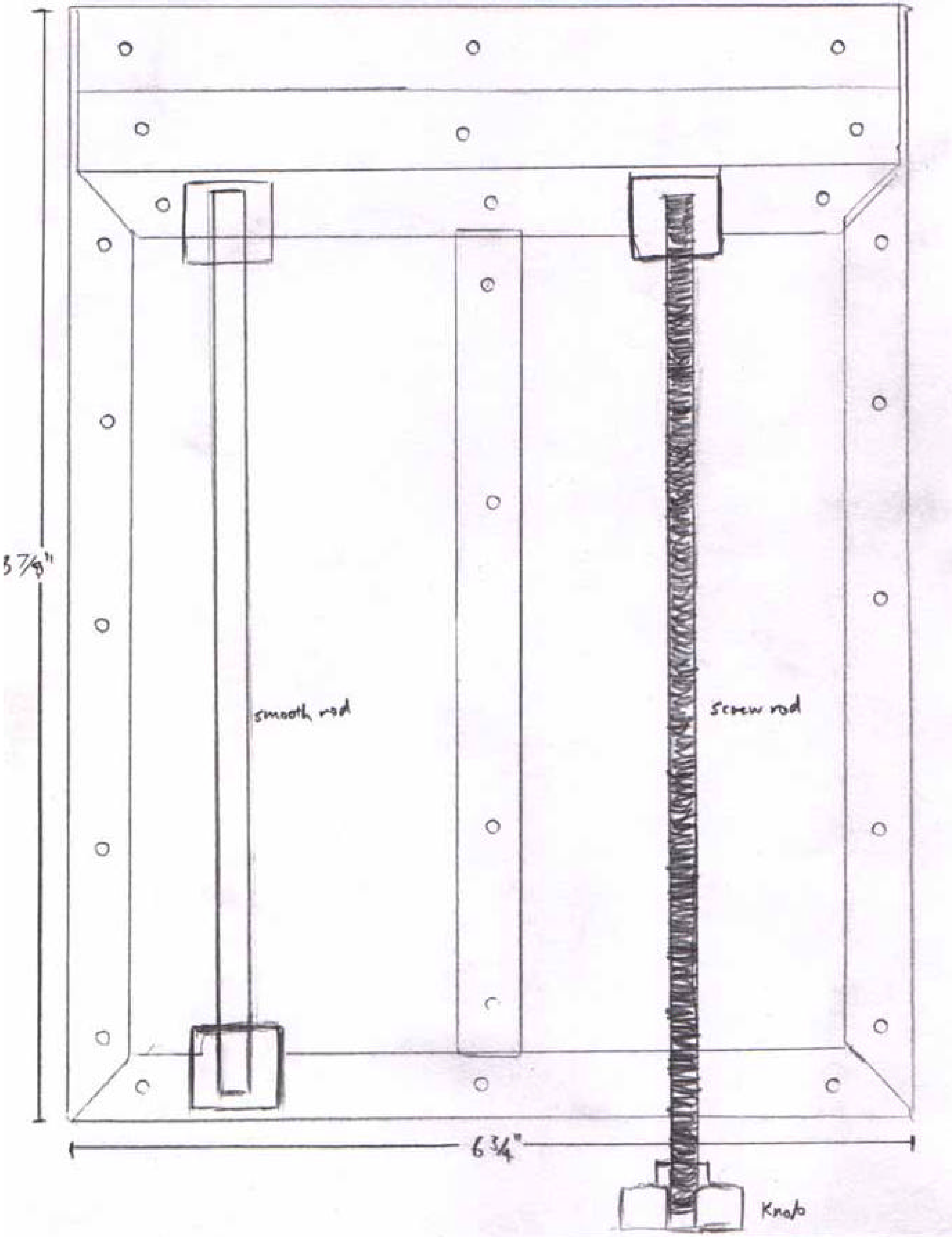


bearing
nut (hexagon)
3/4"
6"



have two of these riveted to stage
mount
bearing
smooth rod
rods inside the base
nut
screw rod
mount

BASE (INTERIOR)



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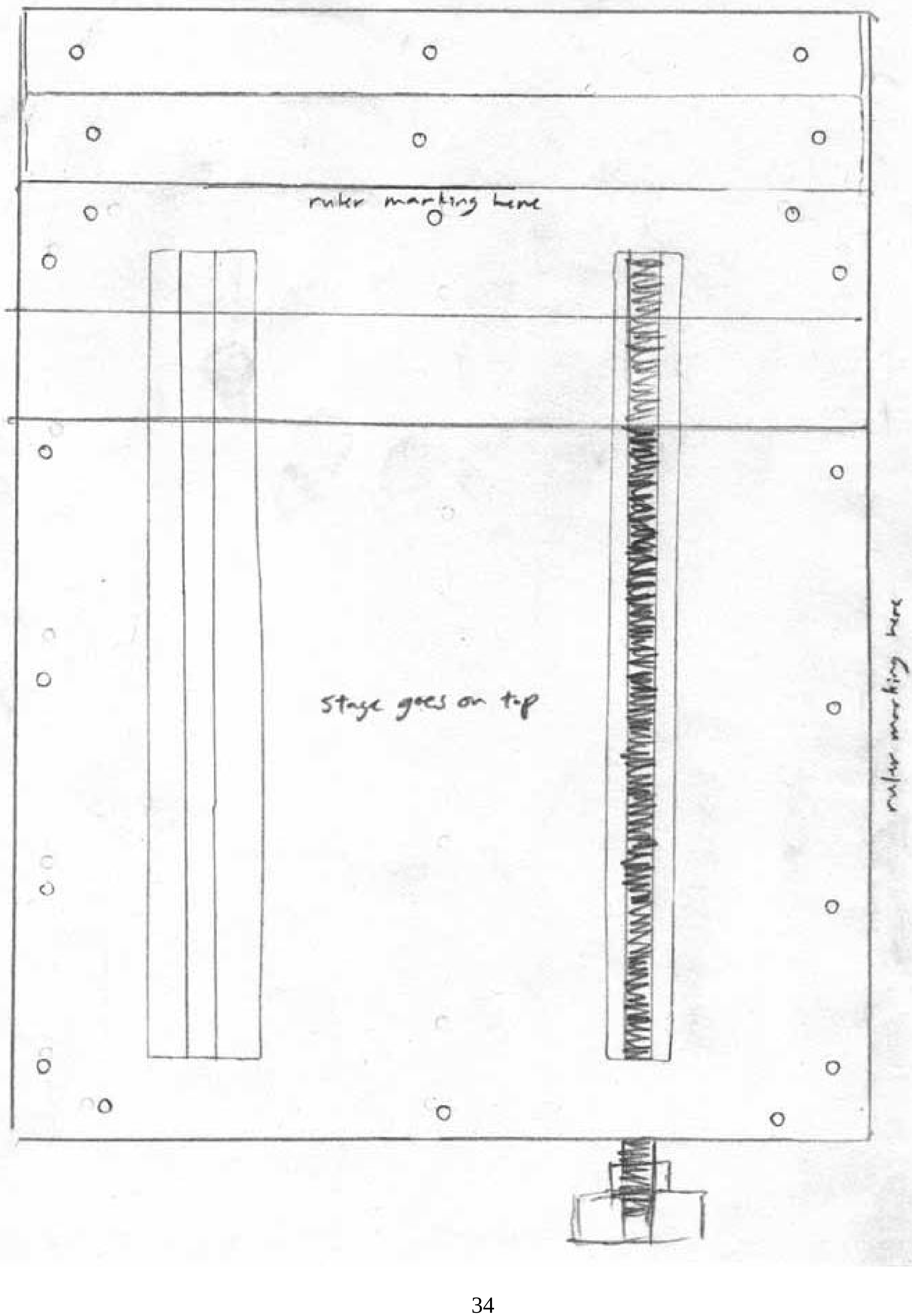

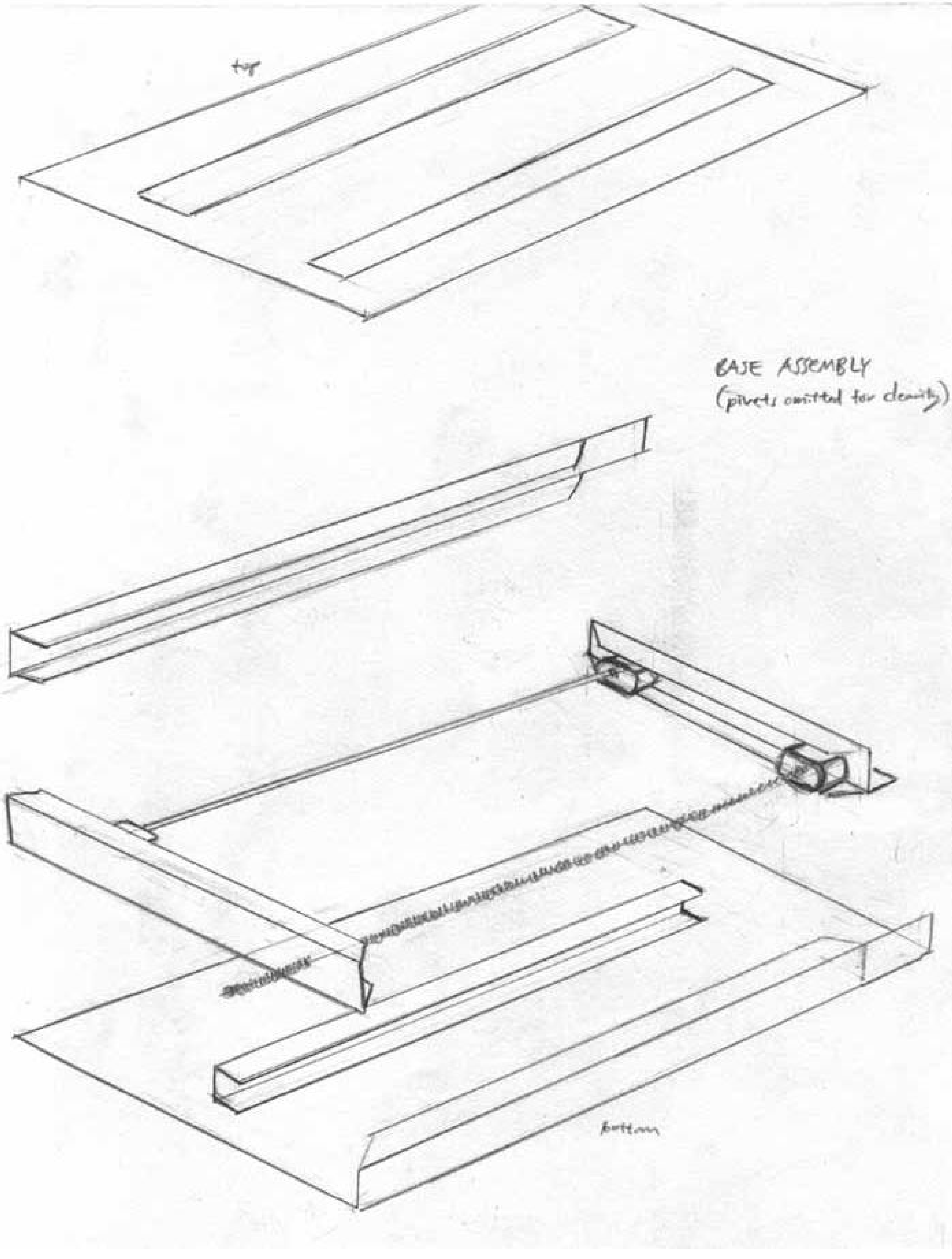
top
BASE ASSEMBLY
(pivots omitted for clarity)
bottom