

# **GVI: Straw Stamp and Slicer - BME 200/300**

Final Report

BME 200/300 Design

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### **Abstract**

Increasing global demand for animal products has encouraged the use of artificial insemination to reduce environmental effects. Genetic Visions-ST conducts a quality control protocol, sequencing bull semen from the straws to ensure the DNA matches the bull listed on the straw, for artificial insemination. The current 1 hour process requires cutting individual straws, pushing the contents into the well plate with a paper clip, and sanitizing after each straw. The team aimed to significantly reduce procedure time by creating a frame, stamper, and slicer. The 3-D printed frame had twelve compartments to hold the straws, and protrusions on the ends to perfectly align the straws above the well plate. The slicer mimicked a paper cutter to effectively cut 12 straws simultaneously. The stamper has a 3D curved printed handle with 12 steel prongs to push out the semen. The team performed compression tests to determine the force to push the semen, cross contamination test with a fluorescein solution, and timed test to confirm protocol time reduction. The results showed that the force to push the contents was less than the bending force of the prongs. The procedure and control groups had no significant difference while having a significant difference between the procedural groups, indicating there was no cross contamination between wells. The procedure was reduced from 1 hour to 26 minutes. Some future improvements include preventing the straws from launching after cutting, easier strategies to align the stamper over the compartments, and finding the best cleaning solutions to stay sterile.

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### Introduction

#### **Motivation and Global Impact**

As the population increases, there will also be an increase in global demand for animal derived products [1]. Genetically improved animals can help this growing issue by providing better meat and dairy products, and also decreasing the environmental harm likely to be caused. To genetically improve animals, artificial insemination is used. At Genetic Visions, they process and control the quality of bull semen to aid the artificial insemination process. However, the current quality control procedure takes around an hour a plate, and eight to ten plates are processed a week, so this procedure can take up to ten hours a week. Decreasing the time to process each plate can help increase the plates processed per week, increasing the efficiency and making the whole artificial insemination process faster.

Furthermore, the semen in each straw is tested to make sure it matches the DNA listed on the straw to make sure no cross contamination occurred. The stamper and slicer must also reduce contamination risk to save time and materials used.

#### **Existing Devices and Current Methods**

Currently at Genetic Visions, the clients are using a pair of scissors to cut each straw individually. Then, each straw is stamped individually by using a paperclip to push the cotton down the straw, pushing the contents out of the straw and into the well plate. Although these methods are accurate, this method is quite time consuming as all 96 straws are processed individually.

One example of a competing product is the MiniCutter for Semen Straws by Nasco Education as depicted in Figure 1 [2]. This is a lightweight product and has an ergonomic handle, increasing the ease of use for the client. To use this product, the straw is placed inside the hole, and a notch is pushed to cut the straw. This straw cutter is able to cut both ¼ and ½ cc straws, which is what the client requested. However, this cutter can only cut one straw at a time, and can not empty the contents of the straw, so a stamper would still be needed. There is not currently a straw stamper on the market that fits the clients requirements.



#### Figure 1: MiniCutter for AI Straws [2]

The clients at Genetic Visions also provided potential design ideas as seen in Figure 2. The first prototype was a stamper, featuring a top plate, with 96 prongs coming out of it. This prototype is ideal, as it can stamp all 96 straws at once, however, the prongs are too large to fit into the straws.

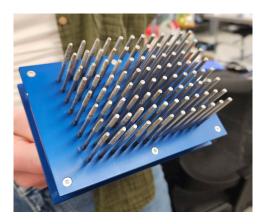


Figure 2: GVI Stamp Prototype

The second prototype was for a cutter. This prototype, as presented in Figure 3, was a paper cutter-like design, featuring a back wall and an arm that can lift up and down. The front wall has 12 individual holes for each straw so contamination can be minimized. Even so, there is still a contamination risk since each straw is cut using the same blade. Also, the blade would have to be quite sharp to be able to cut all 12 straws at once, which poses a safety risk.



Figure 3: GVI Slicer Prototype

#### **Problem Statement**

Currently, quality control procedures investigating quality of bull semen for artificial insemination are time and labor intensive. The process involves cutting and pushing bull semen through a small straw using a straightened paper clip, and transferring the contents to a 96-well plate. This process takes one hour, with 8-10 plates being processed per week. The purpose of the project is to optimize these quality control procedures by designing a straw slicer that should be able to cut 12 straws at a time Additionally, a straw stamper is needed to push bull semen out of the straws in bulk, avoiding cross contamination. All devices should also have removable components for cleaning.

### **Background**

The clients for this project are Sarah Hanson (lab manager), Brett Breidor (lab technician), and Ben Goss (sequencing technician), who are all employees at Genetic Visions-ST. Genetic Visions-ST performs genotyping of production animals as well as sequencing services for a quality control program to ensure the DNA detected from the artificial insemination straws match the bull labeled on the straw.

Genetic Visions-ST uses low-pass sequencing (0.5x coverage), then bioinformatics, to detect if the sperm cells in the straw match the bull that is labeled on the straw with a detection limit of 5%, meaning it can identify contamination if > 5% of the DNA in the sample came from another source [3]. Low pass sequencing is the process of sequencing a genome at a low depth so not every base in the genotype is read, while imputation involves comparison to reference data to form a reconstructed genotype [4]. The low-pass sequencing and imputation combination results in a cost-effective alternative to typical single nucleotide polymorphism arrays, which require more resources and time [4].

To prepare for the low-pass sequencing, each end of the artificial insemination straws are cut off and the contents in each straw are pushed into a 96-well plate. This requires the creation of three devices: a slicer to cut 12 straws, a stamper to push the contents of the straws into the well, and a frame to hold the straws in place during the procedure. Each device must conform to the dimensions of the 96-well plate used by Genetic Visions-ST: 127.8mm x 85.5 mm x 44.1 mm [5].

A main consideration of Genetic Visions-ST while performing the protocol is the prevention of cross-contamination. DNA sample contamination is a common problem in DNA sequencing and can result in systematic genotype misclassification [6]. Genotype misclassification may lead to inaccurate identification of sperm cells in the artificial insemination straws. The emphasis on lack of cross-contamination influences the prototype components and materials. The slicer, stamper, and frame design must prevent the contents of one straw from

seeping into another straw or another straw's designated well on the 96-well plate. Therefore, each device design would benefit from removable components for sanitation and replacement over time.

According to the PDS, the overall goal of the client is to cut down the procedure time from 1 hour to <30 minutes, as 8-10 plates are processed per week. As for design specifications, the slicer must cut 0.20-0.50 inches off the end of each straw, guaranteeing a uniform length for all 12 straws. The slicer must also have a blade guard to cover the blade while not in use. Additionally, the straws must not bend or break during the straw pushing. Each artificial insemination straw has a diameter of 0.002 meters, so 0.32 N of force is required to break the seal and push the contents out of the straw [7]. Furthermore, cross-contamination was heavily emphasized by the clients, so any component of the slicer, stamper, or frame that contacts the inside of the straw must be made of non-porous material and must be removable for sanitation by ethanol or bleach. Finally, each device must have a life-in-service of over one year and there is a \$1000 budget for research and fabrication for all the devices. Additional information regarding the device specifications can be found in Appendix A.

### Preliminary Designs

### Frame Design 1: The Clamp

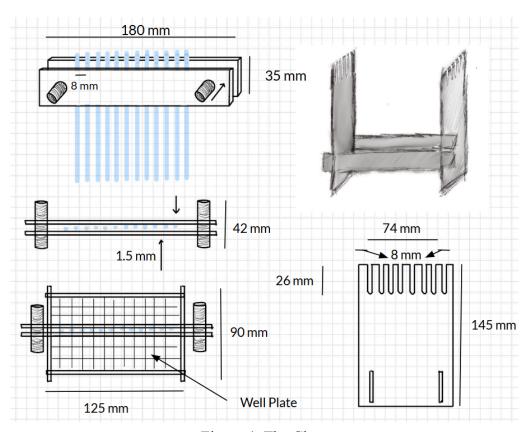


Figure 4: The Clamp

Frame design 1, demonstrated by Figure 4, incorporates a pinching mechanism that holds 12 straws between two plates, with a consistent 8 mm gap between each straw. The dimensions of the plates are 180 mm in length and 35 mm in height. At both ends of the plates, screws with nuts, approximately 42 mm long, will be placed to bring the plates together and tighten. A separate component of this design is the main frame. It consists of two pieces: two tall walls and two side pieces. The two tall walls are 90 mm in length and 145 mm in height, and the two side pieces are 125 mm.

The process of loading this design is that the clamp piece is initially positioned outside of the well plate. The user will load the straws and then tighten. Next, the whole clamp piece will move the straws to the slicer. Once these two parts are complete, the clamp piece will be placed in the notches in the frame. This is self-tightening, meaning a snug grip can be provided. However, the straws are 2 mm in diameter, and each straw is placed 8 mm apart, meaning it will be difficult to place the straws in their designated position and tighten while maintaining precision.

#### Frame Design 2: The Stamp

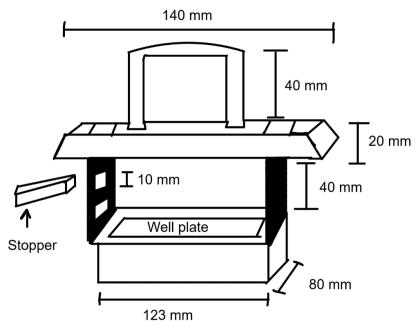


Figure 5: The Stamp

As seen in Figure 5, frame design 2 utilizes a stamping mechanism to efficiently hold and process all 96 straws simultaneously. The piece that will hold the straws is 140 mm in length and 20 mm in height. On the inside that faces the well plate, there will be caps that are 2 mm in diameter and will fit around the tip of the straw. It features an ergonomic handle of 40 mm in height for ease of operation. There will be a track that sits on two sides of the well plate and spans 40 mm in height. On this track, there are two cutouts approximately 10 mm between each other, which allows the user to place a stopper. The stamper piece that holds the straws will fit on this track and be able to move down until where the stopper is placed.

This frame idea will be loaded outside the well plate, and all 96 straws will be cut in the holder. Once cut and snug in the holder, it will be moved to the frame track and loaded on, where the next step will be to push the contents out. The user can control the height of the holder using the stopper. This design allows all 96 straws to be done at once, improving efficiency. However, due to the close spacing of the 96-well plate slots, it will be challenging to align the straws accurately within their designated area.

#### Frame Design 3: The Compartments

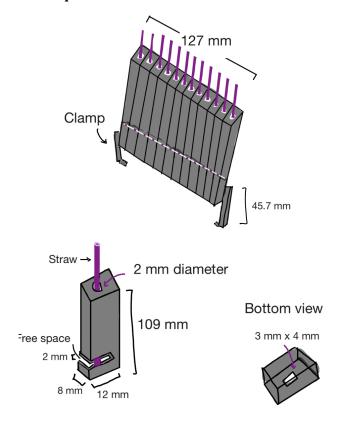


Figure 6: The Compartments

Frame design 3, illustrated in Figure 6, has individual compartments to hold 12 straws at a time. Each compartment is 109 mm in height, 12 mm in width, and 8 mm in length. There will be a 2 mm diameter hole for the straw to snugly fit into. A cutout of 2 mm in height will allow a blade to slice the straws, but note that the back wall will be present to allow for a uniform cut. The 12 compartments are arranged together in a single unit, so they have an overall length of 127 mm. At the bottom of the compartments. There will be a 3 mm by 4 mm cutout at the bottom to catch the clippings of the straws. The compartments will clamp onto the well plate by utilizing a 45.7 mm in height piece placed on the bottom of the design.

The process of this design is similar to the other designs since the straws will be placed into their individual compartments, and then a blade will slice the 12 straws. Once cut, the component will be clipped onto the well plate, lining up with the slots of the row, then stamped and the process will repeat. Due to the individual compartments, there is less open space between each straw and therefore less risk for contamination. However, only 12 straws can be done at a time, and it will be difficult to determine the sizing of the hole. The straws need to be snug when stamping, but need to require minimal effort to remove them once complete.

#### **Stamp Design 1: The Retractable Stamper**

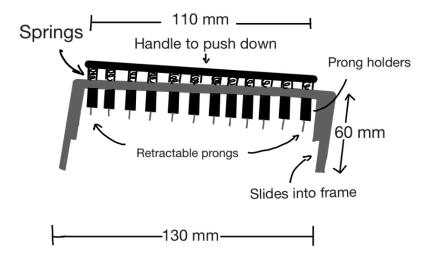


Figure 7: The Retractable Stamper

Figure 7 shows stamp design 1 which incorporates 12 retractable prongs, designed to push the contents of the straws out efficiently. The overall design is 130 mm in length and 60 mm in height, while the handle to push the prongs out is 110 mm in length. This design includes side pieces that slide into the existing frame for alignment. The handle would have springs allowing the prongs to sit in the casting when not used, but be brought down when the handle is pushed.

Before using the stamper, the straws would be in the frame and already cut. The stamper would attach to the frame and push the contents out. The user can line up the casting with the straw before retracting the prongs, allowing for an easier alignment. When not in use, the prongs are retractable, reducing contamination risk. The prongs would additionally be removable due to them being fragile. Since the prongs retract when the handle is not pushed, it will be difficult to sanitize after each row. The user would have to support the component while pushing the handle down.

#### **Stamp Design 2: The Removable Prongs**

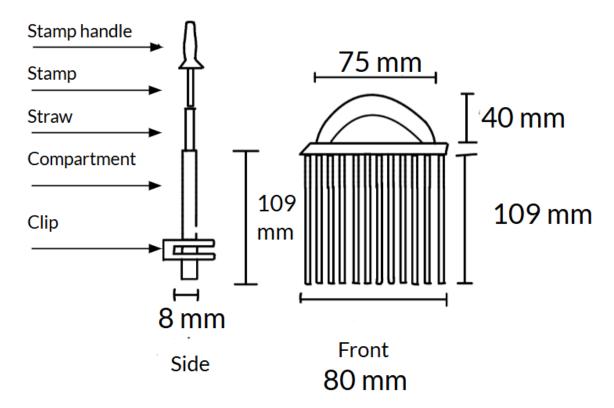


Figure 8: The Removable Prongs

Stamp design 2, as shown in Figure 8, features 12 stationary, removable prongs that remain open to the space. The overall stamp design is 80 mm in length and 149 mm in height. Each prong measures 109 mm in height, while the ergonomic handle is 40 mm in height and 75 mm in length. A clip at the bottom of the design allows it to securely attach to the compartment frame.

Once the cut straws are secure in the compartments and attached to the well plate, the prongs of the stamper will line up with each straw. This process will require the assistance of the user to ensure all prongs are in the designated location. The user will utilize the ergonomic handle to reduce strain and push the contents out. Only 12 straws will be done at a time, and the device will need to be efficiently sanitized. Due to the open nature of the prongs, it will be simple and quick to dip the prongs in a sanitizing liquid. The prongs would be easily removable, allowing them to be replaced as needed. However, the prongs are long and thin, making it difficult to align them in the straws.

### Preliminary Design Evaluation

### Straw Stamp and Slicer Frame Design Matrix

Criteria	The Clamp		The Stamp		The Compartments	
	35 mm		I 10 mm  Violity plate  Viveli plate  80 mm		Clamp  2 mm diameter  2 mm diameter  3 mm x 4 mm  3 mm x 4 mm	
Criteria (Weight)	Raw Score	Weighted Score	Raw Score	Weighted Score	Raw Score	Weighted Score
Contamination risk (25)	3/5	15	2/5	10	5/5	25
Sanitation (25)	4/5	20	4/5	20	2/5	10
Ease of use (20)	3/5	12	3/5	12	3/5	12
Ease of fabrication (15)	3/5	9	4/5	12	4/5	12
Safety (10)	4/5	8	4/5	8	4/5	8
Cost (5)	4/5	4	4/5	4	5/5	5
Total (100)	68		66		72	

Table 1: Straw Stamp and Slicer Frame Design Matrix

The main criteria chosen were contamination risk, sanitation, ease of use, ease of fabrication, safety, and cost as seen in Table 1. Contamination risk was identified as a high concern since it is crucial that individual bull semen samples do not come in contact with each other since this can ruin the DNA sequence result. Sanitation additionally was a high determining factor because it is vital that the frame can easily be clean in between uses to prevent contamination. Ease of use was key to the design as the client requested a device that would create a more efficient process while maintaining precision and a simple design. Thus, the frame should be something simple to hold enough straws without having to manage multiple components. Ease of fabrication was critiqued based on the type of materials required, the mechanism of each component, and the available tools at the university to build the device. Safety was judged based on the frame's ability to prevent the blade from coming in contact with

the client's hand. Lastly, cost was ranked last as most of the devices would utilize simple materials, and the cost for each device would most likely not be able to exceed the budget.

Overall, the Compartments scored the highest out of all the devices, shown in Table 1. One large evaluation was that the compartments would reduce the contamination risk. This design utilizes individual sections for each straw, while the other designs have the straws out in the open. The compartments in the design would be easy to use, as it would have notches on the side to allow the user to align with the straw without worrying about the straw touching the bottom. The other devices would require some adjustment in order for the straw to stay secure. The Compartments also rank higher in ease of fabrication and cost because they would be modeled in AutoCad to 3D print at the Makerspace for around \$7.80, which is easily accessible and replicated to create multiple compartments. Since there would be a small gap only for the blade to cut the straws, it is unlikely that the blade would come in contact with the client's hand, which allows us to rank the Compartments high in safety.

#### **Straw Stamp Design Matrix**

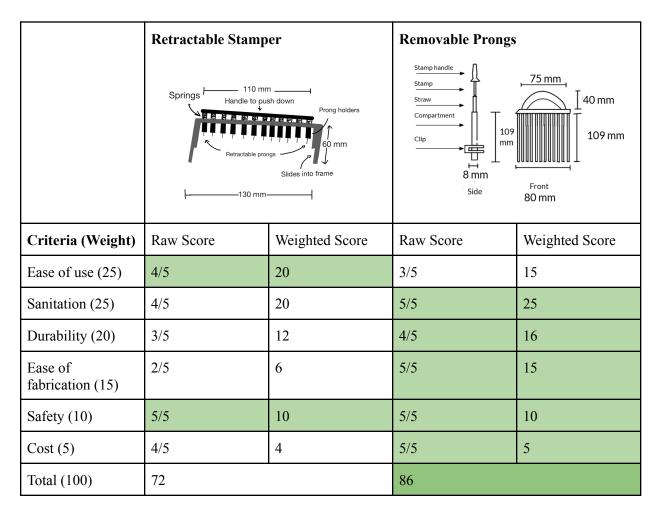


Table 2: Straw Stamp and Slicer Frame Design Matrix

The main criteria chosen were ease of use, sanitation, durability, ease of fabrication, safety, and cost. Table 2 illustrates the design rationale. Ease of use was identified as one of the highest priorities because it was important that the device can easily push out all the bull semen. This process will be repeated among different rows and multiple times a week and should work without experiencing any issues. It was important that the stamp can be effortlessly cleaned between uses to prevent contamination, so sanitation was additionally ranked as a high concern. Durability was another priority because the client expected to use the device 8-10 times per week for at least a year. Thus, the device should be durable enough to last for a long period of time. Ease of fabrication was determined based on the type of materials needed, the mechanism of each component, and the available tools at the university to build the device. Safety was judged based on the physical strain it would have on the client's hand, as they are expected to use the same motion to push out numerous straws. Lastly, cost was ranked last as most of the devices would utilize simple materials, and the cost for each device would most likely not be able to exceed the budget.

The Removable Prongs was chosen out of the two stamps, as seen in Table 2. It ranked higher in sanitation because the prongs could be easily removed to disinfect, clean, and dry as compared to the Retractable Stamper, which has more components to disassemble to clean. The Removable Prongs were more durable because the prongs could be easily replaced once they are near the end of their lifespan and require less maintenance. However, the Retractable Stamper has more components and mechanisms like springs and casing that require more attention to maintain. The Removable Prongs was ranked higher in cost and ease of fabrication because it could be easily 3-D modeled with the materials at the Markerspace for roughly \$4.34, which is significantly less than the set budget.

#### **Final Proposed Design**

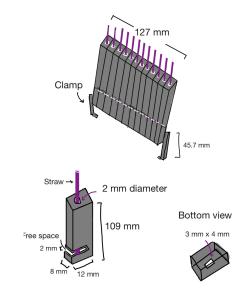


Figure 6: The Compartments

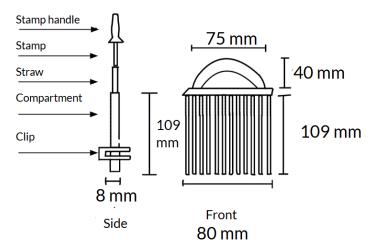


Figure 8: The Removable Prongs

The final proposed design is a combination of the Compartments and the Removable Prongs designs. Both designs sufficiently fulfilled the client's main concern of contamination, sanitation, and ease of use through their ease of separating the straw into their individualized sections and disassembling the devices for cleaning. Each of the straws would be cut at the crimped edge to create an opening for pushing the bull semen. The frame would first clamp to secure itself to the side of the well plate. The clamp allows for easy placement of straws into each section while maintaining proper alignment, as seen in Figure 6. The straw would then be inserted into the compartments to their indicated height. The Removable Prongs Stamp would sit

on top of the compartments and clip to the side to secure them as seen in Figure 7. Once the prongs are properly aligned, the client would be able to push on the handle to push all the bull semen from the 12 straws into the well plate. The client could unclip all the stamps and frames and quickly sanitize them for the next set of straws.

#### **Final Design**

In the final design, four primary components are present in the overall process. As shown in Figure 9 and Figure 11, the elements are the compartments, well plate frame, stamper, and slicer. The compartments consist of a single unit with 12 holes for the straws to be inserted into and pushed until the uncrimped end of the straw is barely visible at the top. As seen in Figure 10, each hole begins as a 3 mm radius circle and funnels into a triangular hole that extends 46 mm downward. The triangle is equilateral with all sides measuring 3.41 mm. This triangular geometry provides a snug but non-deforming fit around the straw, which reduces the risk of the semen leaking out from pressure placed on the straw from rigid walls. Both sides of the compartment unit have one 4.4 mm square hole designed to interface with the pegs of the well plate frame. In addition, there is a rectangular extrusion on the side of the compartments. This feature allows the user to place the compartments in line with the slicer height, which helps distribute the cutting force and improve operational efficiency.



Figure 9: Final printed assembly of well plate frame, compartments, and stamper

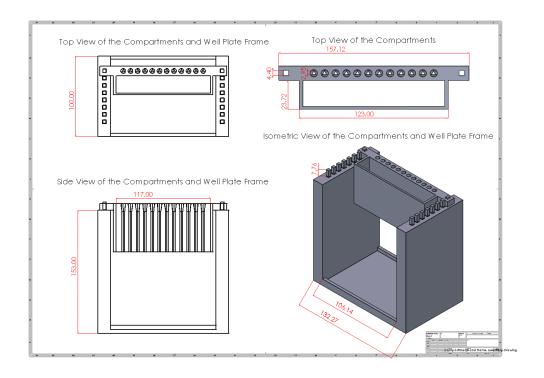


Figure 10: Dimensioned CAD model drawing of well plate frame and compartments

Once the straws are positioned in the compartments, the user cuts off the crimped ends using a 5-inch guillotine-style paper cutter, as shown in Figure 11. The compartment unit is oriented with the extrusion laying flat so the straws align with the blade, and approximately 0.20 inches are removed from the crimped section.



Figure 11: Mini Slicer

After cutting, the compartments are positioned in the well plate frame for stamping. The frame holds the compartments above the wells and maintains proper alignment so the contents of each straw are emptied into the correct well. As seen in Figure 10, the well plate frame is box-shaped and includes two walls measuring 153 mm in length and spaced 106.14 mm apart. Small lips along the bottom edge prevent the well plate from sliding off the supporting surface. Each wall contains eight even spaced pegs corresponding to the rows of the well plate. In addition, when the loaded compartments are placed on the pegs, the height of the walls are designed so the straws discharge the contents without contacting the bottom of the well.

The final step involves expelling the semen out of the straws and into the wells. Illustrated in Figure 12 and Figure 13, the final stamper design consists of a curved handle with an oval hand opening. The handle length is 180 mm with the underside containing 12 circular 1.1 mm holes to seat 1 mm stainless steel rods. As shown in Figure 9, the stamper can be used to simultaneously stamp 12 straws at once, or rotated to apply force to individual straws one at a time.

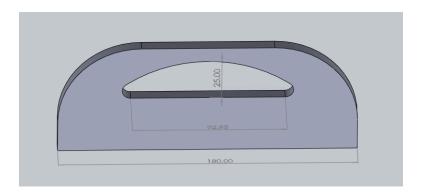


Figure 12: Dimensioned CAD Stamper Front View

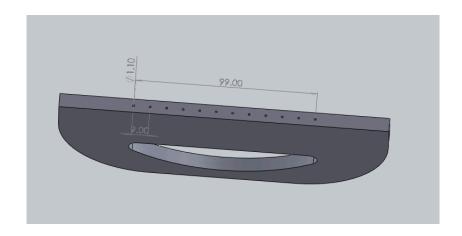


Figure 13: Dimensioned CAD Stamper Bottom View

Throughout the process, the well plate frame and compartments interface directly, while the stamper and slicer function as support components. The compartments and stamper were fabricated out of resin due to its resistance to ethanol, suitability for components requiring frequent cleaning. The well plate frame was printed out of ABS, as it does not directly contact the straws. Collectively, these four pieces function together to create a more efficient process.

#### **Fabrication**

#### **Materials**

To fabricate the design, the well plate frame, compartments, and the stamper handle were 3D printed. Initial prototypes were printed in ABS filament on Bambu Printers in the Makerspace. ABS was selected due to its low cost, durability, and ease of printing, which enabled multiple iterations to be printed. For the final design, the compartments and stamper were printed in Tough 1500 Resin on Formlabs Form Printers in the Makerspace. Resin was chosen as it is resilient and has mechanical properties comparable to polypropylene [8]. Additionally, since it is comparable to polypropylene, it has better chemical resistance than ABS and will be able to withstand being disinfected with ethanol or bleach without being damaged [9]. Non-printed components included the prongs for the stamper, which were fabricated from stainless steel rods cut down to 5.3 inches. Stainless steel was selected for its resistance to ethanol and mechanical similarity to the paper clip currently being used.

For the slicing mechanism, a 5-inch guillotine style paper cutter was purchased. This mechanism because all 12 straws can be cut at once unlike the current scissors they are using. Additionally, the cutter will only need to be disinfected after cutting a row of straws unlike a scissor which needs to be disinfected after every use. Purchasing a pre-manufactured slicer was more cost effective and provided a safer option for users, due to regulations already in place because of the sharp blade. The costs for all of the materials are seen in Appendix B.

#### Methods

The design process began with brainstorming the compartment piece, as this feature dedicated the configurations of the remaining components. The compartment design was transposed into a CAD model while simultaneously designing the well plate frame and stamper. The first printed iteration of the compartments consisted of a single compartment intended to evaluate the fit of the straw within the triangular hole. Using a single compartment prototype for early testing allowed for minimal fabrication time and was easily modified. Once a snug fit was observed, the single compartment design was duplicated in order to facilitate the full 12 straws as a single unit.

While printing the compartments, the well plate frame was modeled and printed to verify dimensional accuracy for the well plate interface. The frame includes eight pegs on each side that allow the compartment frame to slide into these holes and have a uniform fit.

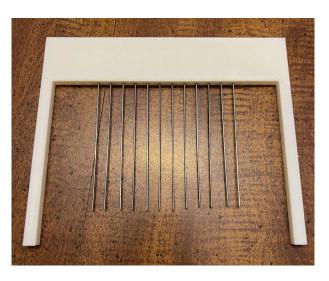


Figure 14: Initial stamper



Figure 15: Initial printed assembly with old stamper

As shown in Figure 14, the initial stamper was modeled as a rectangle with 12 circular holes to fit the stainless-steel rods. The holes were designed to fit flush with the stainless steel rods. Two tabs were incorporated into the compartment design with the goal for the stamper to align with these tabs. Each side of the stamper has a rectangular piece that slides into the compartment design, as seen in Figure 15. This was made with the intent to make it easier to align the prongs with the straws.

The compartment design is fundamental to holding the straws, so it was crucial to design in a timely manner, since the other components work around this. Once the compartment was designed, the well plate frame transitioned to the current high-risk piece. At this stage, all three major components, well plate frame, compartments, and stamper, were modeled but none of the dimensions finalized. An assembly was created with the base piece being the well plate frame. A simple box dimensioned to the well plate size mocked the well plate, and was inserted into the assembly to finalize the well plate frame dimensions. The 12 compartment unit was then added and edited in the assembly environment to incorporate two side holes. One would interface with the pegs on the frame and the other with the stamper. Subsequently, the stamper was inserted and adjusted to ensure proper alignment with the compartments.

After the finalized prototype iteration was printed and assembled, testing revealed that the well plate frame was undersized by 2 mm. This resulted in revising the dimensions and reprinting the well plate frame. Additionally, after inserting the straws and testing the full procedure, problems arose with the stamper. Instead of facilitating alignment, the two side parts of the slicer seemed to impede positioning of the prongs with the straws instead. Therefore, a new iteration was created to omit these side pieces and was replaced with a top oval cutout to act

as a handle instead. When printing this new handle, the bottom holes were not fully extruded due to the printer and the small dimension size. Therefore, a drill with a 1/16" drill bit was used to fully create these holes.



Figure 16: Cutting-board knife combo

Near the end of development, efforts were heavily focused on testing, as well as determining an effective slicing mechanism. Initially, a cutting-board combo knife was used as illustrated in Figure 16. However, cutting all 12 straws simultaneously took a high amount of force and was not ergonomic to the user. Therefore, a 5-inch guillotine paper cutter was purchased and the compartment design was updated to accommodate this specific mechanism.

All CAD modeling was completed in SolidWorks. Early prototypes were printed in ABS to minimize cost and print time. For the final designs given to the clients, the updated stamper and compartments were printed in resin. This resin was more expensive but had better overall physical properties, making up for the increase in price.

### Testing and Results

To evaluate the efficiency and functionality of the straw stamper and slicer, multiple tests were conducted. These tests include an MTS test to measure the force to push the contents of the straw into the well plate, the force to bend the steel rods in the stamper, a timed test to measure process duration reduction, and a contamination test to ensure minimal cross-contamination.

Although included in the preliminary plan for testing, a test to measure the force to slice the straws was not executed due to its lack of feasibility and relevance. The blade on the paper cutter is not removable from the base of the paper cutter, making it difficult to attach to the MTS machine. Additionally, the cutting force for a paper cutter is likely comparable to that of scissors, suggesting that further testing is not critical.

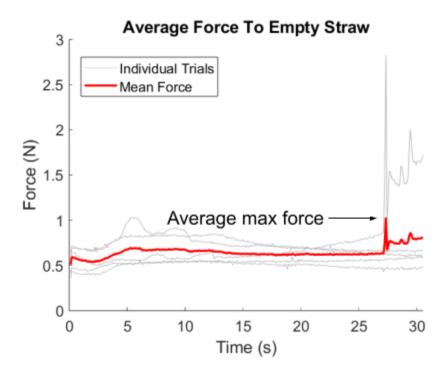


Figure 17: Average Force To Empty Straw

In order to measure the force required to push the contents of the straw into the well plate, a mechanical force test was performed using the MTS machines. The procedure outlined in Appendix G was modified from the original plan for MTS testing to accommodate for the limitations of the MTS machines available. The load cell measures 53 mm in diameter, while the length of one row of straws is 130 mm, meaning an entire row would not fit under the load cell. In order to accommodate, only a single straw was tested. This decision was made under the assumption that the force would linearly increase with the addition of more straws. As seen in Figure 17, after conducting 5 trials, the maximum force required to empty the straw was  $1.120 \pm 0.929$  N. Converting to lbf, the force required is around 0.25 lbf which is well below the original requirement of 1 lbf set by GVI.

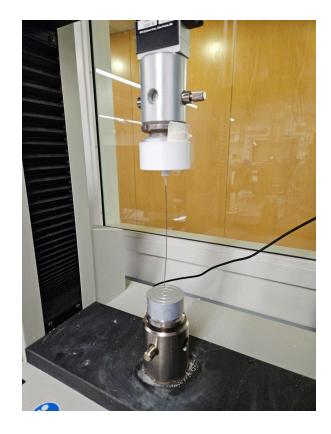


Figure 18: Setup for Rod Bending Force Test

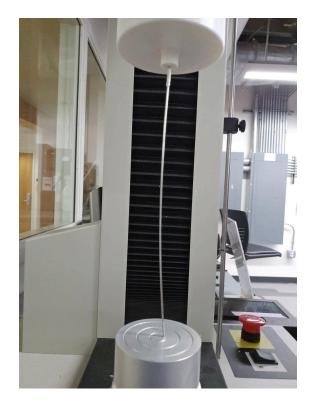


Figure 19: Visual Result of Rod Bending

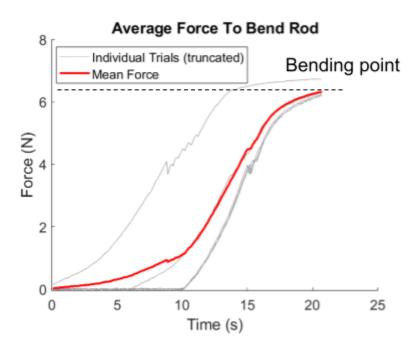


Figure 20: Average Force To Bend Steel Rod

An additional force test was performed to determine the force required to bend the steel rods. This test was included to ensure that the rods would not bend or deform as the stamper pushes into the straws. Verifying the strength of the rods is important for the functionality of the device and ensuring a user-friendly procedure. The rod strength test follows the procedure outlined in Appendix G, except that the straw and compartments are removed, allowing the rod to be placed directly on the MTS base. Once a slight visual bend was observed as seen in Figure 18 and 19, and the force on the monitor plateaued, data collection was stopped. As seen in Figure 20, the force required to bend the rod was  $6.54 \pm 0.100$  N. This value is greater than the force required to empty the straws, meaning the rods will not bend during the procedure, fulfilling the PDS requirements for an easily operable device.

A time test was conducted to determine the duration of the procedure in order to assess if the duration reduction goal of 50% was met. The procedure was performed as GVI would carry out the experiment in their laboratory, but only a single row was tested due to material limitations. The 12 straws were loaded, cut, and stamped using the fabricated devices, over the course of 4 minutes and 30 seconds. Multiplying this by 8 (for the 8 columns in the well plate) would yield a total experiment duration of 36 minutes. Overall, a 40% time decrease was achieved, making the procedure 6 minutes longer than the original goal.

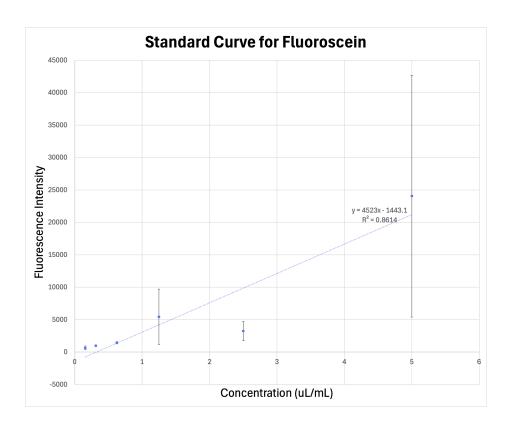


Figure 21: Standard curve of fluorescein for concentrations up to 5 uL/mL

One of the most important design considerations expressed by GVI was ensuring an absence of cross-contamination, as their low-pass sequencing devices can detect contamination levels with a limit of 5% [3]. To examine if the final design complied with the PDS requirement of minimal cross-contamination, a fluorescence-based testing protocol was developed.

As seen in Figure 21, a standard curve for fluorescein was established to determine the desired concentration of fluorescein to use in the contamination test. A desired concentration would be one with minimal variance between trials and a fluorescence intensity value within a readable range for the microplate reader. As seen in Appendix C, six concentrations of fluorescein ranging from 0.15625-5.0 uL/mL were prepared and dispensed in a 96-well black plate and evaluated with a microplate reader at 535 emission and 485 excitation to determine their fluorescence intensity. All tested concentrations had relative fluorescence unit values within the readable range for the microplate reader. The concentrations 0.3125 and 0.625 uL/mL had fluorescence intensity values of  $1000.83 \pm 86.99$  and  $1444.67 \pm 175.51$  (mean $\pm$ standard deviation), respectively. These concentrations yielded the most consistent fluorescence intensities with the smallest variances. 0.625 uL/mL was the concentration chosen to proceed with for the contamination test because it generated consistent microplate readings and was a viable concentration to produce using the 1-10 uL eppendorf pipette provided in the lab and the limited semen provided by GVI for testing.

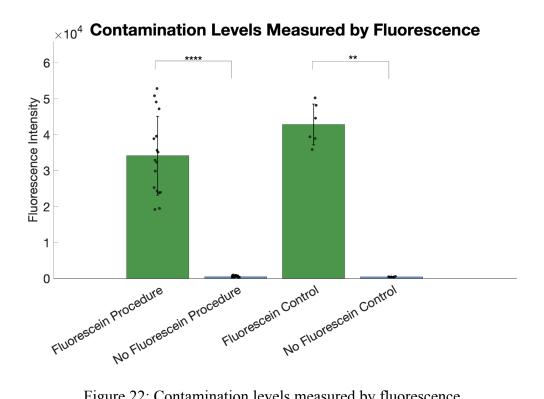


Figure 22: Contamination levels measured by fluorescence

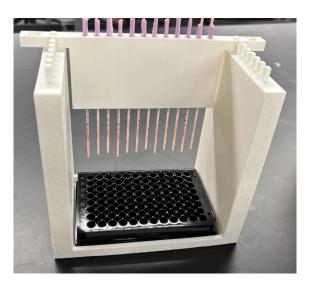


Figure 23: Contamination protocol final design set up



Figure 24: 96-well plate sample containing fluorescein in odd numbered columns and no fluorescein in even numbered columns

Figure 22 displays the fluorescence intensities from the contamination test using the 0.625 uL/mL concentration determined from the standard curve protocol. Before performing a contamination test using the team's final design and the GVI procedure, as seen in Figure 23, positive and negative controls needed to be established to draw comparisons to a condition with no contamination. To begin the protocol, nearly 40 straws were cut and emptied into a centrifuge tube to create a 4.5 mL stock solution of semen. Two fluorescein concentrations were created from the stock solution: 0.0 uL/mL and 0.625 uL/mL. For the control wells, as seen in Figure 24 in row H, each of these solutions were dispensed into one row of the 96-well plate with an eppendorf pipette (alternating 0.0 uL/mL and 0.625 uL/mL), ensuring the entirety of the contents were only going into their respective wells. The experimental condition required a syringe to inject the 0.0 uL/mL and 0.625 uL/mL solutions into their respective straws. These straws were loaded into the compartments (alternating fluorescein and no fluorescein conditions), cut with the slicer design, secured to the well plate holder, and pushed with the stamper. The plate was then processed in a microplate reader (535 emission and 485 excitation) and their fluorescence intensities were evaluated to see if any of the fluorescence from one straw transferred into any wells that are not supposed to have any fluorescein. A more detailed contamination testing protocol can be found in Appendix E.

The 0.0 uL/mL and 0.625 uL/mL solutions had fluorescence intensities of  $501.53 \pm 244.62$  and  $34131.24 \pm 10965.66$ , respectively, for the GVI procedure, and  $426.17 \pm 77.89$  and  $42841.17 \pm 5689.24$ , respectively, for the control trial. The Wilcoxon Rank Sum Test was used for analysis to determine if there were any significant differences between the groups because the data is not normally distributed, there were some outlier data points, and each group is independent. Notably, there were significant differences between the procedural fluorescein and no fluorescein wells (p=7.05e-7), and control fluorescein and no fluorescein wells (p=0.0022). There were also no significant differences between the fluorescein wells for control and procedure groups, and no

fluorescein control and procedure groups. This implies that the control and procedure groups were not significantly different, and that the using final design did not cause any significant cross-contamination during the GVI procedure.

#### Discussion

The results of aforementioned tests indicate areas of improvement for the fabricated devices. Such areas for improvement include design, procedure, and material modifications. The results of the force testing indicate that the procedure can be completed with minimal effort. As the stamper only requires ~1 N of force to operate, little strength is needed to empty the straws, minimizing user fatigue. Additionally, since the rods require ~6.5 N of force to bend, the ease of procedure is ensured by preventing deformating and instability of the rods as they push through the straw. Further, utilizing Euler's Buckling Formula can provide more information on the limits of the buckling force. Assuming a Young's Modulus (E) of 193 GPa [10] and a K value of 0.7 (due to Fixed-Pinned ends of rod), the anticipated force required for buckling (P<sub>cr</sub>) is calculated to be 10.5 N, as seen in Figure 25. This calculation indicates that more force can be applied before the rod experiences complete buckling, reducing the risk of buckling even further. This formula also gives insight into the effects of using a different rod material. Given that the Young's Modulus is directly proportional to the P<sub>cr</sub>, using a material with a higher E value, such as Beryllium [11], would increase the force required to cause buckling.

$$I = \frac{\Pi r^4}{4} \to I = \frac{\Pi (0.0005m)^4}{4} = 4.9 \times 10^{-14} m^4$$

$$Pcr = \frac{\Pi^2 EI}{(KL)^2} \to Pcr = \frac{\Pi^2 (193GPa)^* (4.9 \times 10^{-14} m^4)}{(.7^* 0.13462m)} = 10.5N$$

Figure 25: Euler's Buckling Formula Calculation

The results of the timed test suggest areas for improvement that would decrease experiment duration and increase the ease of the experiment. The primary issue is the excessive time required to align the stamper rods with the straw holes. To address this issue, the funnel height of the compartments could be increased, or additional funnels attached to the straws could be implemented to guide the rods into place during stamping. Alternatively, the straws could be stamped individually, which is expected to be faster than aligning all 12 prior to stamping. Additionally, to improve the ease of procedure, the handle on the slicer could be lengthened to reduce the force required to cut the straws.

Contamination levels as low as 1% can have a significant impact on DNA sequencing results [6], highlighting the importance of performing this test. Specifically, GVI has a detection limit of 5% for cross contamination [3]. The analysis results using the Wilcoxon Rank Sum Test, implies that all devices used in the experiment (slicer, compartments, well plate holder, and stamper) comply with the PDS requirement and do not cause cross-contamination while

performing the GVI procedure. However, the standard deviations were relatively large for all of the groups, and some changes could be made to the procedure. For example, a more successful way to insert equal amounts of solution into the straw, and performing the experiment into the dark to reduce the exposure of light to the fluorescent dye, which could alter results.

It is important to consider the possible sources of error in the tests performed above to ensure accuracy and efficiency of the device. As stated earlier, the mechanical force test for the single straw was performed under the assumption that when stamping 12 straws, the force would linearly increase with the addition of each straw. However, due to user error or slight design inconsistencies, the force may not be evenly distributed, leading to slightly different results for the force applied to each straw. Ultimately, since the force required to stamp one straw is minimal, and the force to bend one rod is 6 times greater than this force, any inconsistencies are unlikely to cause major issues. The distribution of dye in the semen poses another source of error. To create a homogenous solution of fluorescein dye and semen, the solution was vortexed before being drawn up by the syringe to dispense into the straws. However, the dye used did not mix well with the semen due to its oil-content, causing an unevenly distributed mix of semen and dye within the straw. As a result, each straw could have been injected with a slightly different amount of dye, further causing issues with the plate-reader results. Again, this issue is negligible because of the small amount of dye used and had minimal effects on the cross-contamination results. As displayed in Figure 24, bubbles were created in row D, columns 5, 7, 8, 11, and 12 during the stamper pushing portion of the GVI procedure. The bubbles may have led to errors during the microplate reading due to light scattering.

Throughout the course of the project, ethical design choices were made to prioritize user safety, accessibility, and environmental consciousness. As stated in ISO 12100, when designing machinery, risk assessments must be performed to reduce posed harm [12]. In order to comply with this standard, a rectangular extrusion was added to the side of the compartments, allowing the device to lay flat during cutting. This orientation improves stability and reduces risk of injury. In terms of accessibility, the device should be able to be used by all lab members including those with differing hand mobility or size. Given the small force required to empty the straws and the ergonomic handle on the stamper, the stamping process is suited for a diverse range of users. Although the stamper provides benefits to users, the precise motor skills needed to align the rods with the straws leaves room for error and should be improved in the future to further increase accessibility. In addition to user-focused considerations, ethical sourcing and use of test materials were prioritized. Although the semen was inadequate for GVI's quality control (QC) testing, it still exhibited the same physical properties as typical QC samples. Using material that would have otherwise been discarded ensured ethical and sustainable use. Additionally, the device decreases its environmental impact by limiting the need for replacements. Initially, ABS and resin were used to create prototypes, however, ideally nylon would be used. Although resin provides friction and ethanol-resistance, nylon would be an optimal material for the 3D printed components of the device. Nylon is durable, structurally stable, and "resistant to the sterilization

process" [13], so overtime, the pieces will not degrade or deform with repeated use and sterilization

#### **Conclusions**

The team was tasked with developing a device able to hold, cut the ends, and push bull semen out of 96 straws into a 96 well plate with a simpler procedure. The final design consists of a well plate frame, compartments, a stamper, and a slicer. The twelve individualized compartments feature funnels to guide the straw into the hole, while keeping the straws separated during slicing and stamping, and triangular holes to keep the straws secure in place. The compartments also featured a hollow base to ensure the top surface aligns with the slicer, so the user does not have to manually keep them level. The final slicer includes a guillotine style paper cutter that can easily cut 12 straws simultaneously.

The compartments are able to snap into the well plate frame by aligning the side cutout of the compartments into the small protrusions of the edges of the wells for ease of use. The stamper was made out of resin and featured removable prongs, for easy cleaning. The stamper design also included an ergonomic curved handle that would allow the user to freely move to adjust each prong into the individual straws.

Testing demonstrated the ability to stamp and slice the straws effectively, while avoiding cross contamination. MTS testing was done to find the force needed to expel the contents of the straw, while making sure that it does not exceed the force to bend the rods of the stamper. Contamination testing was also done to address the client's largest concern, cross contamination. This testing found that there was no significant difference between the procedure and controls, though a significant difference was observed between the fluorescein and non-fluorescein sample when the GVI procedure was performed. This result confirmed that this design did not cause cross contamination. Possible sources of error were considered in the analysis of testing results. The force testing was only done using one straw, so forces needed to stamp 12 straws simultaneously may differ. Also, with the contamination testing, the dye may have been unevenly distributed in the semen, skewing the results. A timed test was also done to see if the designs reduced the time it takes to complete the procedure. It took around 4 minutes and 30 seconds to complete per row, totaling around 36 minutes for the full well plate, significantly reducing the current time of an hour to complete the procedure. Further timed testing of the full procedure would provide more accurate results on the timing of the procedure with the devices. Nevertheless, the current testing still provided an accurate baseline for the efficiency of the devices

Future improvements include optimizing the design to be able to stamp and slice multiple rows at once, reducing the procedure time further. Furthermore, it is currently difficult to align

the rods of the stamper into all 12 straws at once, so a better way to align these could be designed. More modifications are needed for the slicer device as some straw segments were propelled unpredictably during cutting, which causes some safety and contamination concerns. Potential solutions include creating a barrier that would stop the straws' pieces from propelling or a latch to hold them down before cutting. Since the client stressed the importance of sanitation, the best way to clean the product should be determined while ensuring the material does not deteriorate easily. The end goal is for GVI to perform the full procedure using these designs, and provide feedback to improve them.

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### **Appendix**

### Appendix A- GVI: Straw Stamp and Slicer PDS



# **GVI: Straw Stamp and Slicer - BME 200/300**

Product Design Specifications

BME 200/300 Design

September 18, 2025 Clients: Sarah Hanson, Brett Breidor, and Ben Goss

> Advisor: Professor Justin Williams University of Wisconsin-Madison Department of Biomedical Engineering

#### Team:

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#### **Function**

Currently, Genetic Visions-ST sequences semen from artificial insemination straws, ensuring that the DNA detected matches the bull that is listed on the straw [1]. This quality control program is intensive, as it takes about an hour to cut and push 96 semen straws per 96-well plate. The clients have requested two devices: a slicer and a stamp. The slicer must uniformly cut the ends of 12 straws without cross-contamination. The components of the slicer must be removable, and a blade guard must be incorporated for safety. The stamp must accurately push bull semen out of 96 straws at once without any punctures or deformation to them.

#### **Client requirements**

- A device capable of cutting the ends of 12 insemination straws at a time.
- A mechanism to push the contents of the straws in bulk to a 96-well plate.
- Both devices must have removable components for cleaning.
- Both devices should minimize user error and eliminate any chances of contamination between the straws.
- Reduce the procedure time from 1 hour to a final time of 30 minutes.

#### **Design requirements:**

#### 1. Physical and Operational Characteristics

- a. **Performance requirements**: The device must consist of a cutting component to cut 12 straws at a time, as well as a stamping component that holds 96 straws and pushes the cotton and semen out of each straw into a well plate. The device is intended to be used 8-10 times per week, and the estimated loading for the device is 0.32 N [2] per use. Design should allow for minimization of cross-contamination, and each component of the device must be able to be disassembled for sterilization [3].
- b. **Safety**: Blade guards must be included to cover blades when the device is not in use to prevent any injury to the user. A warning label may be used to bring awareness to the danger of the blade. Disease transmission during straw slicing

and stamping is rare due to lab safety procedures, cattle vaccination, and antibiotic treatment for samples, however a small risk is still posed. If safe practices are not followed, diseases such as Foot-and-Mouth disease or Leptospirosis could infect the user if they were cut by the blade [4]. Utilizing blade guards, wearing gloves, and carefully operating the device are all effective ways to reduce the risk of infection.

- c. Accuracy and Reliability: The slicing component must cut off at least 0.20 inch and at most 0.50 inch of each straw, and the stamping component should push the entire sample into the well plate to maintain consistency in collection. To maintain precision for slicing and stamping, the straws must be held in place to prevent movement or bending [3].
- d. **Life in Service**: This device must function accurately and consistently for a minimum of one year, performing 8-10 procedures per week. The straw cutter will be used 8 times per procedure for about 1 minute per use, while the straw stamp will be used once per procedure for about 10 minutes per use [3].
- e. **Shelf Life**: All of the components of the device must have a shelf-life of at least one year. They will be replaced if they show signs of corrosion or decreased functionality. However, since there will be removable components, replacing specific components could increase the device's overall longevity. This device will be used multiple times during the week. When not in use, it will be stored within the Genetic Visions-ST wet laboratory.
- f. **Operating Environment**: This device will be used in the Genetic Visions-ST wet laboratory and operated by one of the clients. The Food and Drug Administration's regulatory guidelines show that the optimal temperature for wet labs is 68 °F and 77 °F (20°C and 25 °C) with humidity levels between 30% and 50% [5]. The device will come in contact with the filled insemination straws, which are stored in the fridge at ideal temperatures of 4-18 °C to prevent bacteria growth [6].

- g. **Ergonomics**: The device should be easily operable by one of the Genetic Visions-ST's employees. For efficiency and user comfort, the device should also be ergonomically optimized to support operators performing this task repeatedly through the week. The main force needed will be the one to overcome the straw's vacuum seal and push the semen into the well plate. Using the pressure equation P = F/A, the force needed to push the contents of a 0.002 meter diameter straw is 0.32 N [2].
- h. **Size**: The client supplied prototypes to display the functional requirements of the tool. The first prototype, a slicer, measured 11 inches in length and 5 inches in width. It featured a hinge mechanism originating from the base plate and extending upwards approximately 7 inches. The second prototype was a rectangular stamp measuring 5 inches by 4 inches, equipped with spring-loaded pins of 2-inch length. This stamp was designed to interface with straws positioned within a transparent base plate of similar size. When not in operation, both tools are intended to be stored on a personal workbench measuring 3 feet by 2 feet. As the workbench is shared with other tasks, it is essential that the slicing and stamping tools do not obstruct or interfere with daily activities [3].
- i. **Weight**: The client has not specified an optimal weight range for the device. If the equipment is placed in a holder, the holder should not exceed more than 20 kg [7]. This is to ensure safe and efficient transport of the holder between floor level to work bench height. The device itself will be placed on a workbench when not in use. It must be sufficiently lightweight to allow operation using the forearms and shoulders without physical strain. For a repetitive task at this height, the maximum weight of the tool should be between 11kg and 14kg [7].
- j. Materials: The tool must be disinfected after each use, either by immersion in a bleach or alcohol-based solution and through surface wiping. Additionally, the material must be capable of withstanding repeated exposure to these harmful chemical agents. As the client requested, it should be a non-porous material. Various grades of steel exist, including carbon steel, which is susceptible to corrosion when exposed to moisture or oxygen. By applying a chromium oxide

coating to the surface, the chances of corrosion can be greatly reduced. Stainless steels in the 300 and 500 series exhibit enhanced corrosion resistance, while those in the 300 series are noted for weldability [8]. The material must withstand harsh conditions, maintain functionality, and prevent cross contamination.

k. **Aesthetics, Appearance, and Finish**: There are no preferences for the appearance of the device, however, the aesthetics of the device should not impede on the function of the device [3].

#### 2. Production Characteristics

- a. **Quantity**: The client is aiming to have one straw stamper device and one straw slicer device to work with. The client does not have a preference on whether or not both devices are combined into a singular as long as the devices can be easily disassembled for cleaning [3].
- b. **Target Product Cost**: The overall budget is \$1000. The average cost of the jagged tooth blades is around \$20 but will need to be modified based on size [9]. Fine pins to push the cotton cost around \$5 for a pack of 250 [10]. Currently, there does not seem to be other similar products for the straw stamper. The straw slicers have other similar products at an average cost of \$10. ABS Global is selling their straw slicer at a cost of \$6.38 [11]. Valley Vet is selling their straw slicer for \$13.29 [12]. However, the current straw slicer products only cut one straw at a time.

### 3. Miscellaneous

a. **Standards and Specifications**: The straw slicer and stamp must follow international standards that correspond to laboratory devices. Since the bull semen goes through a DNA sequencing process, the components that will contact the bull semen should not cause DNA damage and must exhibit biocompatibility. ISO 10993 defines this as "the ability of a medical device or material to perform with an appropriate host response in a specific application" [13].

In addition to biocompatibility of the device materials, each material's

resistance to corrosion relates to the longevity and accuracy of the device. If corrosion tests are performed on the device materials, they must follow the guidelines set by ASTM F1089, outlining the boil and copper sulfate test, which assess corrosion and copper plating respectively [14].

The safety of the straw slicer and stamp is also a major factor of the design process. ISO 12100, a standard that covers the safety precautions, risk assessment, and risk reductions, must be taken into account when designing the blade and stamp [15]. This will help identify the risks of each design and implement safety components such as a blade guard, better grip material, etc.

- b. **Customer**: The main priorities of the client are to reduce the procedure time from 1 hour to < 30 minutes, while maintaining precision of the devices. For the straw slicer, the clients are partial to their proposed jagged-tooth blade to reduce cross contamination between the 12 straws during each cut. The presence of a blade guard with 12 opening holes for the straws and a "straw stopper" to ensure equal cut length (~1/4 inch) is favored. They strongly prefer the straw slicer to have removable components, allowing for easier and more thorough sterilization. No preferences were given for the straw stamp other than a light-weight design [3].
- c. **Patient-related concerns**: As there is much concern about the risk of cross contamination, the device will need to be sterilized after each use. Because of this, the client would like the device to be easily disassembled for easy cleaning. The product would also need to be able to withstand a cleaning solution, such as bleach after each use. Additionally, the clients value precision the most, over other attributes such as cost and materials [3].
- d. **Competition**: Currently the clients are using straws to cut each individual straw, and a paperclip to stamp each straw. There are other competing products on the market. For example, Agtech Inc has a straw cutter also available. To use this product, the straw is inserted and then a button is pushed which turns a disk inside the mechanism to cut the straw. This product can be taken apart to be cleaned, which the clients specified the product needs However, their straw cutter is only

for ½ cc, while the clients requested the product to work for both ½ cc and ½ cc straws. Also, this straw cutter can only cut one straw at a time, but the client needs to be able to cut 12 straws at the same time [16]. There are many similar products to this plastic semen cutter on the market.

Another product on the market is the MiniCutter for Semen Straws by Nasco Education. This product is lightweight and has an ergonomic handle for easy grip. Similar to the Agtech cutter, this product also has a notch that is pushed for the straw to be cut. However, unlike the Agtech cutter, this product is able to cut both ½ cc and ½ cc straws. The disadvantage to this straw cutter is that it can also only cut one straw at a time, thus it would not work for the clients needs [17].

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**Appendix B- BPAG Table** 

Appendix B- BPAG Table											
Item	Description	Manufac turer	Mft Pt#	Vendor	Vendor Cat#	Date	Q T Y	Cost Each	Total	Link	
Materials											
3D Print	ABS single compartment prototype, Ø 2mm straw hole	_	-	Makerspace	_	10/10 /2025	3. 81	\$0.05	\$0.19	https://docs.google.com/ spreadsheets/d/125EWY r0aojDuu0BGfzzt-YhfG JA1wojkzE-Vt00tw M/e dit?gid=0#gid=0	
3D Print	ABS single compartment prototype with Ø 2.5mm and Ø 3mm straw hole. Stamper rod holder prototype Ø 1.5mm	-	-	Makerspace	_	10/13 /2025	16 .2 3	\$0.05		https://docs.google.com/ spreadsheets/d/125EWY r0aojDuu0BGfzzt-YhfG JA1wojkzE-Vt00tw M/e dit?gid=0#gid=0	
3D Print	ABS 12 compartments together prototype with Ø 3mm straw hole.	-	-	Makerspace	<u>-</u>	10/22 /2025	31 .4	\$0.05	\$1.57	https://docs.google.com/ spreadsheets/d/125EWY r0aojDuu0BGfzzt-YhfG JA1wojkzE-Vt00tw_M/e dit?gid=0#gid=0	
3D Print	MTS test fixture, updated single compartment prototype with smaller diameter hole, and well plate frame	-	-	Makerspace	_	10/27 /2025	45 .8	\$0.05	\$2.29	https://docs.google.com/ spreadsheets/d/125EWY r0aojDuu0BGfzzt-YhfG JA1wojkzE-Vt00tw_M/e dit?gid=0#gid=0	
1mm Steel Rods	12Pcs 1 mm x 300 mm 304 Stainless Steel Round Rod, Metal Shaft, Stainless Steel Smooth Rods for Industry, Metalworking Hobbies and DIY Crafts (12, 1mm x 300mm)	Lyrlidr	-	Amazon	B0F8V G5HJV	10/27 /2025	1	\$6.45	\$6.45	https://www.amazon.co m/Lyrlidr-Stainless-Indu stry-Working-Hobbies/d p/B0F8VG5HJV/ref=sr_ 1_1_sspa?	
UV Detection Dye	Supercool UV Leak Detection Dye, 1 oz. Multi-purpose, Total Dye	TSI Supercool	6722640 16525	Amazon	B008P KV07O	10/27 /2025	1	\$7.46	\$7.46	https://www.amazon.co m/UV-Leak-Detection-D ye-oz/dp/B008PKV07O? th=1	
3D Print	MTS Test Fixture (updated diameter sizes)	-	-	Makerspace	-	11/6/ 2025	31 .1 3	\$0.05	\$1.56	https://docs.google.com/ spreadsheets/d/125EWY r0aojDuu0BGfzzt-YhfG JA1wojkzE-Vt00tw M/e dit?gid=0#gid=0	
3D Print	ABS 12 compartments, base well plate frame, and stamper	-	-	Makerspace	_	11/10 /2025		\$0.05	\$10.6 0	https://docs.google.com/ spreadsheets/d/125EWY r0aojDuu0BGfzzt-YhfG JA1wojkzE-Vt00tw M/e dit?gid=0#gid=0	
3D Print	ABS 12 compartments reprint with smaller holes	-	-	Makerspace	_	11/17 /2025	31	\$0.05	\$1.56	https://docs.google.com/ spreadsheets/d/125EWY r0aojDuu0BGfzzt-YhfG JA1wojkzE-Vt00tw_M/e dit?gid=0#gid=0	
3D Print	ABS frame reprint with wider base	-	-	Makerspace	_	11/17 /2025	20 8. 2	\$0.05	\$10.4 1	https://docs.google.com/ spreadsheets/d/125EWY r0aoiDuu0BGfzzt-YhfG JA1wojkzE-Vt00tw_M/e dit?gid=0#gid=2	

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	Guillotine Paper Cutter Mini 6 Inch Cut									ud_dp_3R02TNW9PH
	Length Small Paper Trimmer Non Slip									42SEXFWNK3&social
	Compact Curved Strip Scrapbooking	D			D0D44					share=cm sw r cp ud
D (01)	Tool Portable for Coupon Craft Paper	DNAMY			B0D44	11/25	١.		\$19.9	dp_3R02TNW9PH42SE
Paper Cutter (Slicer)	Card Photo	JME	-	Amazon	S821T	/2025	1	9	9	XFWNK3
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3D Print	ABS Print	-	-	Makerspace	-	/2025	.8	\$0.05	\$1.04	dit?gid=0#gid=0
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3D Print	ABS Print	-	-	Makerspace	-	/2025	6	\$0.05	8	dit?gid=0#gid=1
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3D Print	ABS Print	-	-	Makerspace	-	/2025	.6	\$0.05	\$0.63	dit?gid=0#gid=2
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3D Print	ABS Print	_	_	Makerspace	_	/2025	6	\$0.05	\$8.78	dit?gid=0#gid=3
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3D Print	Resin Final Print	_	_	Makerspace	_	/2025		\$0.24	3	dit?gid=0#gid=4
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## **Appendix C - Fluorescein Standard Curve Protocol**

- 1. Perform 5 serial dilutions
  - a. 5 uL/mL: 9.995 DI water, 5 uL of dye
  - b. 2.5 uL/mL: 5 mL DI water, 5 mL of stock a
  - c. 1.25 uL/mL: 5 mL DI water, 5 mL of stock b
  - d. 0.625 uL/mL: 5 mL DI water, 5 mL of stock c
  - e. 0.3125 uL/mL: 5 mL DI water, 5 mL of stock d

- f. 0.15625 uL/mL: 5 mL DI water. 5 mL of stock e
- 2. Add 250 uL of each concentration to its designated well
- 3. Tap 96-well plate on hard surface to pop bubbles in solution
- 4. Run in microplate reader (535 emission 485 excitation 30 flashes top read)
- 5. Collect and analyze fluorescence intensity and make standard curve

### **Appendix D - Fluorescein Standard Curve Results**

Conc (uL/mL)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	avg	std
5	55788	22508	12406	16148	10375	27079	24050.66 667	18660.64 206
2.5	2932	3252	2935	5690	1614	3102	3254.166 667	1485.299 902
1.25	2576	6997	12167	1797	3374	5801	5452	4283.759 295
0.625	1237	1652	1345	1314	1558	1562	1444.666 667	175.5069 799
0.3125	953	1095	911	1109	1039	898	1000.833 333	86.98735 54
0.15625	1033	808	553	445	397	735	661.8333 333	267.8884 096

### **Appendix E - Contamination Testing Protocol**

- 1. Cut 40 straws and empty all contents into a 10 mL centrifuge tube
- 2. Vortex the semen and resuspend with eppendorf pipette
- 3. Prepare correct semen and fluorescein solutions (concentrations: 0 uL/mL and 0.625 uL/mL)
  - a. Disperse half of the semen (2.25 mL) into a second centrifuge tube
  - b. 0.625 uL/mL: add 1.41 uL of fluorescein to 2.25 mL of semen in the first centrifuge tube
  - c. Vortex and resuspend both solutions
- 4. Draw up each solution into two separate 1 mL syringes with a 20 gauge needle
- 5. Dispense 50 uL of fluorescein solution into six straws and 50 uL of 100% semen solution into a separate six straws
- 6. Perform GVI procedure (time it)
  - a. Load straws into the compartments, alternating fluorescein and no fluorescein
  - b. Lay compartments down on the paper cutter and cut ~0.20 in off each straw
  - c. Place compartments on the well plate holder hovered above a black 96-well plate
  - d. Align stamper prongs with the straws and push all contents into the well plate

- e. Tap 96-well plate on hard surface to pop bubbles in solution
- f. Run in microplate reader (535 emission 485 excitation 30 flashes top read)
- g. Sanitize the prongs and compartments
- 7. Repeat to get three valid trials
- 8. Run a control trial
  - a. Use eppendorf pipette to dispense 50 uL of each solution into wells (alternating)
  - b. un in microplate reader (535 emission 485 excitation 30 flashes top read)
- 9. Analyze results

# Appendix F - Contamination Testing Results and Code

	Procedure with	Straws		Avg	Std
	Fluoroscein (FP)	No Fluoroscein (NFP)	FP	34131.23529	10965.65613
	24347	834	NFP	501.5294118	244.6198882
	19480	315	FC	42841.16667	5689.244639
	38896	263	NFC	426.1666667	77.88816769
	49088	704			
	19217	325			
	50786	316			
	32882	753			
	39557	305			
	32307	349			
	35123	770			
	52859	310			
	47198	951			
	29893	285			
	23906	405			
	25244	816			
	23800	268			
	35648	557			
Average	34131.23529	501.5294118			
Std	10965.65613	244.6198882			
	Controls with m	nicropipette			

	Fluoroscein (FC)	No Fluoroscein (NFC)		
	50213	566		
	35846	416		
	38914	382		
	48133	340		
	39361	452		
	44580	401		
Average	42841.16667	426.1666667		
Std	5689.244639	77.88816769		

```
data = readtable ("Contamination Curve Plate Map and Concentrations - Organized
Data for MATLAB.csv");
fp = table2array(data(2:18,2));
nfp = table2array(data(2:18,3));
fc = table2array(data(23:28,2));
nfc = table2array(data(23:28,3));
avg std data = data(1:4, 7:8);
avg std array = table2array(avg std data);
% bar graph of straws and controls
figure(1)
hold on
b = bar(avg std array(:,1));
b.FaceColor = 'flat';
b.CData = [0.3 \ 0.6 \ 0.3;
  0.4 0.55 0.75;
   0.3 0.6 0.3;
   0.4 0.55 0.75;1;
errorbar(1:4, avg std array(:,1), avg std array(:,2), 'k.', 'LineWidth', 1.5);
x1 = ones(size(fp)) + 0.1*(rand(size(fp)) - 0.5);
x2 = 2*ones(size(nfp)) + 0.1*(rand(size(nfp)) - 0.5);
x3 = 3*ones(size(fc)) + 0.1*(rand(size(fc)) - 0.5);
x4 = 4*ones(size(nfc)) + 0.1*(rand(size(nfc)) - 0.5);
scatter(x1, fp, 40, 'filled', ...
  'MarkerFaceColor', 'k', 'MarkerEdgeColor', 'k', 'MarkerFaceAlpha', 0.6);
scatter(x2, nfp, 40, 'filled', ...
  'MarkerFaceColor', 'k', 'MarkerEdgeColor', 'k', 'MarkerFaceAlpha', 0.6);
scatter(x3, fc, 40, 'filled', ...
  'MarkerFaceColor', 'k', 'MarkerEdgeColor', 'k', 'MarkerFaceAlpha', 0.6);
scatter(x4, nfc, 40, 'filled', ...
  'MarkerFaceColor', 'k', 'MarkerEdgeColor', 'k', 'MarkerFaceAlpha', 0.6);
set(gca, 'XTick', 1:4, 'XTickLabel', {'Fluorescein Procedure', 'No Fluorescein
Procedure', 'Fluorescein Control', 'No Fluorescein Control'});
```

```
ylabel('Fluorescence Intensity');
title('Contamination Levels Measures by Fluorescence');
% p-tests
[p,h,stats] = ranksum(fp,nfp); % straws compare
% p=7.05e-7, h=1
[p,h,stats] = ranksum(fc,nfc); % controls compare
% p=0.0022, h=1
[p,h,stats] = ranksum(fp,fc); % fluorescein straw vs fluorescein control
% p=0.0742, h=0
[p,h,stats] = ranksum(nfp,nfc); % no fluorescein straw vs no fluorescein control
% p=0.7002, h=0
```

## **Appendix G - MTS Testing Protocol**

- 1. Unlock machine
- 2. Place compartment and straw onto bottom of the MTS machine\*
- 3. Place 3D printed testing fixture onto the load cell
- 4. Place rod into testing fixture
- 5. File > New Custom Test > BME 201
- 6. Set strain rate to 3 mm/sec
- 7. Adjust load cell so the rod is just above and aligned with the straw\*\*
- 8. Zero cross head and load
- 9. Lock machine
- 10. Enter diameter on the monitor tab
- 11. Press play
- 12. Press stop once the rod has caused the cotton in the straw to move
- 13. Export data
- 14. Turn off machine
- \* If completing rod bending test, skip step 2
- \*\* If completing rod bending test, lower the rod so it is just barely touching the base of the MTS machine

### Appendix H - MTS Stamping Force Testing Results and Code

### **Initial forces**

Test run 1: 0.6493 N Test run 2: 0.7136 N Test run 3: 0.4774 N Test run 4: 0.6966 N Test run 5: 0.4363 N

Average initial force: 0.5946 ± 0.1288 N

```
Code:
```

```
close all;
clear all;
[file, path] = uigetfile({'*'});
fullpath = fullfile(path, file);
data = readtable(fullpath);
disp=data(:,1);
force=data(:,2);
time=data(:,3);
force = table2array(force);
time = table2array(time);
figure;
plot(time, force);
xlabel("time (s)")
ylabel("force (N)")
mask = time >= 0 & time <= 1;
maxForce = max(force(mask));
```

### **Max Forces**

Test run 1: 2.8296 N Test run 2: 1.0295 N Test run 3: 0.7077 N Test run 4: 0.8492 N Test run 5: 0.5647 N

Average max force: 1.1962 ± 0.9292 N

```
%% mts push all graphs
close all;
clear all;
% --- Ask user to select 5 trial files ---
[files, path] = uigetfile({'*'}, ...
   'Select 5 trial files', 'MultiSelect', 'on');
% If the user selects only one file, MATLAB returns a char instead of a cell
if ischar(files)
   files = {files};
end
numTrials = length(files);
all forces = cell(1, numTrials);
all times = cell(1, numTrials);
% --- Load all force/time vectors ---
min length = Inf;
for i = 1:numTrials
   fullpath = fullfile(path, files{i});
   data = readtable(fullpath);
   force = table2array(data(:,2));
```

```
time = table2array(data(:,3));
  all forces{i} = force;
  all times{i} = time;
   % Track smallest trial length
  min length = min(min length, length(force));
% --- Truncate all trials to the shortest one ---
forces_trunc = zeros(min_length, numTrials);
times trunc = zeros(min length, numTrials);
for i = 1:numTrials
   forces trunc(:,i) = all forces(i)(1:min length);
   times trunc(:,i) = all times{i}(1:min length);
end
% Shared time vector = time from the first trial
common time = times trunc(:,1);
% --- Compute mean force curve ---
mean force = mean(forces trunc, 2);
% --- Plot ---
figure; hold on;
% Plot all trials in light gray
h trials = plot(common time, forces trunc, 'Color', [0.7 0.7 0.7]);
% Plot mean in red
h mean = plot(common time, mean force, 'r', 'LineWidth', 2);
xlabel("Time (s)");
ylabel("Force (N)");
title ("Average Force To Empty Straw");
% Legend with proper colors (only need one gray handle)
legend([h trials(1), h mean], ...
      {"Individual Trials", "Mean Force"}, ...
      "Location", "best");
print('myFigure','-dpdf')
```