Evaluation of LN_2 Dewar Health Using a Weight-based Monitoring System
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Client:	Dr. Jeffrey Jones - jmjones5@wisc.edu	
Advisor:	Sarah Sandock - sandock@wisc.edu	
Team Members:	Jeffrey Tsai - jtsai8@wisc.edu	(Team Leader)
	Jake Andreae - jandreae@wisc.edu	(BPAG)
	William Guns - wguns@wisc.edu	(BWIG and BSAC)
	Yiqun Ma - yma99@wisc.edu	(Communicator)

***Note: The Journal of Loss Prevention in the Process Industry requires inclusion of the following sections: Highlights, Abstract, Keywords, Introduction, Material and Methods, Theory/Calculation, Results, Discussion, Conclusions, Glossary, and Appendix. The manuscript is presented below.

Evaluation of LN2 Dewar Health Using a Weight-based Monitoring System

Tsai Jeff[a], Ma Yiqun[a], Guns William[a], Andreae Jacob[a], Jones Jeff[b]
[a]Department of Biomedical Engineering and [b]Department of Obstetrics and Gynecology University of Wisconsin-Madison, Madison, Wisconsin

Highlights:

- The operational need problems about cryogenic dewar failure in safeguarding biological samples such as eggs, embryos, and sperm are presented.
- The literature about liquid nitrogen monitoring systems for cryogenic dewars in clinical settings are reviewed.
- A research framework based on a weight-based system to monitor changes in liquid nitrogen evaporation rate is given.

Abstract:

Cryogenic dewars are insulated, vacuum-sealed pressure vessels widely used for storing biological specimens including human reproductive cells and embryos. These samples are invaluable and irreplaceable, so it is important that dewars are kept at low temperatures (< -135°C). Liquid nitrogen is the most popular cryogenic fluid used to keep dewars cold; however, sample-handling, imperfect vacuum seals, and liquid dewar venting are all factors that lead to liquid nitrogen depletion. It is important that cryogenic dewars are refilled periodically to replenish liquid nitrogen levels to prevent dewar failure. Failure occurs when temperature gets too high and is a consequence of poor monitoring and management. There is no industry standard for liquid nitrogen alarms of monitors. Temperature probes are widely used but fail to send alarms until after liquid nitrogen levels are nearly depleted. Liquid level monitoring systems also exist but are primarily used for threshold monitoring. There is an unmet need in the cryogenic storage market for a system that warns of failures in advance rather than simply reporting when they occur or when dewars should be refilled. This article evaluates a weight-based system to monitor changes in liquid nitrogen evaporation rates over time as a method to continuously monitor the overall health of cryogenic dewars. The ability to continuously monitor the health of cryogenic dewars via evaporation rates may provide an early detection system for impending cryogenic dewar failure and therefore potentially prevent a catastrophic event.

Keywords:

Cryogenic, Real-time monitoring, Weight-based system

1. Introduction:

The use of low temperatures in medicine and biological research has been present since antiquity; one of the more common applications in the modern age is the practice of cryopreservation, which utilizes low temperatures to preserve biological samples. Storing biological samples at very low temperatures provides an indefinite longevity to cells. Cryopreservation in liquid nitrogen (LN_2) keeps samples below the glass transition point of water (-135°C), halting all biological activity and preventing degradation, independent of storage time [1]. Failure to monitor and refill LN_2 within cryogenic storage dewars can result in damage to or loss of frozen samples as they gradually thaw due to increasing temperatures. With regard to frozen eggs, embryos, and sperm, losses can be emotionally traumatic for patients and catastrophic financially and legally for the fertility center responsible.

Frozen samples are commonly stored in cryogenic storage dewars filled with LN2 to maintain temperatures around -196 °C [2]. Cryogenic dewars are insulated, vacuum-sealed pressure vessels made from two interspaced layers of metal, typically aluminum or aluminium alloy [3]. Small dewars hold approximately 30 - 60 L of LN_2 and between 500 - 2000 specimens [3]. Vaporized nitrogen that collects in the inner chamber above the LN_2 is vented from the dewar via a pressure release valve, resulting in a gradual loss of LN_2 over time. Evaporation rates depend on several factors including the ambient temperature, sample-handling frequency, and the condition of the dewar's insulated vacuum seal [4]. They can be as low as 0.4% or as high as 3% of the dewar's volume per day [5]. A properly functioning dewar will typically lose all of its LN_2 after 20 – 120 days depending on storage dewar, brand, and condition of the dewar [6]. Consequently, dewars need to be refilled periodically to replace the evaporated LN_2.

In order to mitigate the risk of dewar failure, embryologists track the amount of LN_2 required to refill the dewar weekly. A steady increase in required refill volume indicates that the dewar needs to be replaced. It is common protocol for dewars to be replaced after 10 years of use [7]. Automated monitoring systems are used in combination with manual checks. The most common monitoring metric is temperature. Temperature sensors are typically mounted on the lid of the dewar and communicate wirelessly to alert users with SMS / emails when a safe threshold has been exceeded. One of the main issues with temperature sensors is the gradient of readings that result from the vertical position of the sensor within the dewar. As LN_2 levels drop, the sensor can be in the liquid phase one moment and then in the gaseous phase the next. The effect is gradually warmer temperatures being reported as a function of decreasing liquid level. More

importantly is the fact that storage dewars conduct the cold from the bottom of the dewar to the top of the dewar. This means that a dewar can lose most of its LN_2, yet still maintain the specimens at the appropriate temperature below -135℃ (**Figure 1**). When the LN_2 runs out, a rapid temperature change occurs with very little warning, leaving the user with a very small window to replenishing the dewar before the specimens are rendered unviable. The rapid warming of the dewar also leaves fertility clinics with little time to replenish LN_2 levels before the dewar fails.

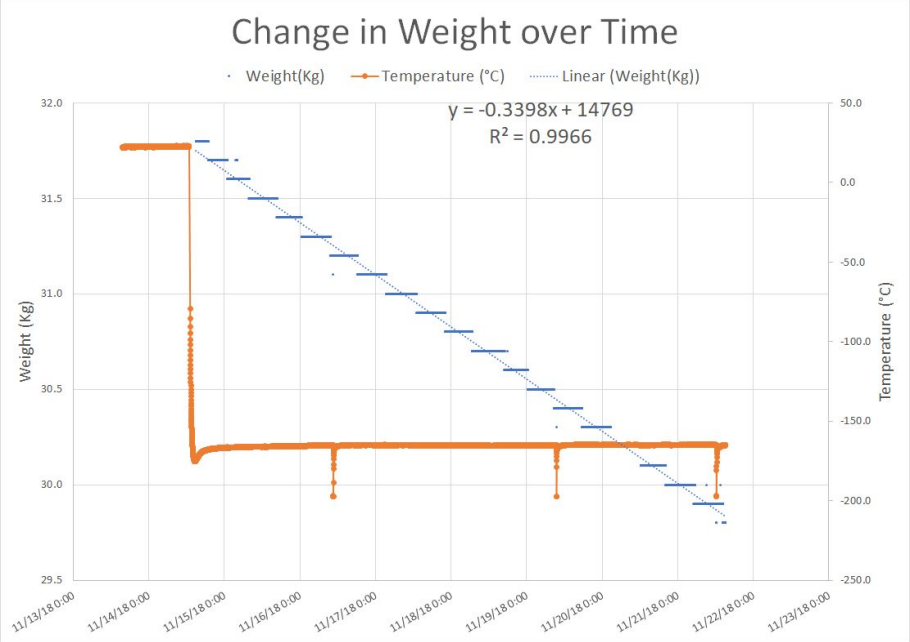


Figure 1: Data collected for the change in temperature and weight of a full LN_2 dewar left undisturbed over a period of two weeks. The orange line represents temperature, and the blue line shows weight. Note that there is a nonlinear change in temperature with respect to the amount of LN_2. Moreover, it is shown that there is a relatively linear trend in the decrease of weight over time, with an R^2 value of 0.9966.

There is a need for an external method of monitoring that does not require internal probes that can be used in conjunction with temperature sensors as a redundant system for safeguarding specimens. We propose a weight-based monitoring system capable of continuous weight monitoring over extended periods of time. Weight could be a low-cost solution for real-time, automated monitoring. Unlike temperature or threshold monitoring systems, weight could be used as a proxy for LN_2 evaporation rate to observe and analyze failure risk for dewars. Evaporation rate is an ideal quality control metric that can be used to quantify dewar health, predict when a dewar will empty, and stratify risk across employees and customer segments.

2. Materials and Methods:

Two cryogenic dewars were used in this study - a new (< 1 yr.) Worthington 35VHC (Dewar A) and an old (> 20 yrs.) MVE – XC 47/11 (Dewar B). Dewars were placed atop CPWplus 75M (Adam Equipment®) scales and weight was continuously recorded for a 24-hour period under both Control and Experimental conditions using an interface in conjunction with a Network Telemetry Monitoring System (Networked Robotics). The Adam Equipment® CPWplus 75M manufactured scales had a 165lb capacity and data output capabilities, and the Networked Robotics® Tempurity™ System measured weight in centigrams (to 0.02 kg). Dewar canisters were empty and no samples were added.

Under Control conditions (baseline), dewars were filled with LN2 and, except for measuring the LN2 level 3x/week, the dewars were otherwise left undisturbed during the monitoring period. Experimental conditions were identical to the Control, except the dewar's foam core access plug was removed and left off during the monitoring period. Three iterations of testing were performed for each dewar under each condition.
The data collection was performed at Generations Fertility Care. This fertility clinic (IVF unit) is a joint venture of UW Health and UnityPoint Health-Meriter and has provided Reproductive Endocrinology and Infertility care since 1974.

3. Theory/Calculation:

We hypothesized that the age and condition of a storage dewar is directly related to the rate of LN_2 evaporation. Here, we collected weight and temperature data from two clinically used cryogenic dewars to determine if there exists a relationship between evaporation rate and dewar age. The ability to detect differences in LN_2 evaporation rate under these experimental conditions may provide a means for early detection of impending cryogenic dewar failure.

At the end of the 24-hr period, evaporation rates were calculated by taking the difference between two adjacent weight data points and dividing the difference by the time elapsed between the two data points (**Eq. 1**).

$$r = \frac{W_0 - W_1}{\Delta t} \qquad \textbf{(Eq. 1)}$$

Where r = evaporation rate
W_0 = most recent weight data point
W_1 = previous weight data point

Δt = time elapsed between the two data points

Evaporation rates were calculated every minute, 10 minutes, and 24 hours. The Tempurity™ System collected one data point every minute. The minute-by-minute evaporation rate represents the real-time evaporation rate. A sudden increase in the minute-by-minute rate indicates either human operation (sample-handling) or a worsening of the dewar's condition rendering it more prone to failure. Under baseline conditions, the minute-by-minute rate is expected to be approximately zero. This is due to the fact that evaporation is slow, and the precision of the Tempurity system (0.02 kg) cannot reflect the weight loss during the 1-minute interval.

The 10-minute evaporation rate represents the average evaporation rate calculated over the last 10 minutes. A sudden change in the 10-minute rates indicates two possibilities: 1) a human operator has added LN_2 or removed/added samples to the dewar, or 2) a sudden failure persisted and alarm notification should be sent to the dewar user.

The rate calculated daily (every 24-hr) is used for evaluating and comparing dewar evaporation rates. Because the 24-hour period produces a significant amount of data points, uncontrollable noise interference can be treated as negligible. The 24-hour evaporation rates were converted to L/day from kg/day (**Eq. 2**) to match the manufacturer-specified rate.

$$r_L = \frac{r_w}{0.808\ kg/L} \qquad \textbf{(Eq. 2)}$$

Where r_L = evaporation rate in L/day
r_w = evaporation rate in kg/day
0.808 kg/L = density of LN2 at -195.79 °C

4. Results:

Under both Control and Experimental conditions, the change in weight of both dewars exhibited a linear relationship with time ($R2 \geq 0.99$), while the temperature within the dewars remained relatively constant throughout the monitoring period. Linear regression analysis of the Control condition data revealed an LN_2 evaporation rate of 0.340 liters LN_2/day and 0.414 liters LN_2/day for Dewar A and Dewar B, respectively. Both dewars exhibited slightly higher evaporation rates than the manufacturer specified rates of 0.270 LN_2/day and 0.390 LN_2/day, respectively. Similarly, linear regression analysis of the Experimental condition data revealed an LN_2 evaporation rate of 0.954 liters LN_2/day and 1.38 liters LN_2/day for Dewar A and Dewar B, respectively. Differences between the Control and Experiment LN_2 evaporation rates for both dewars could be detected within a few hours (**Figure 2**).

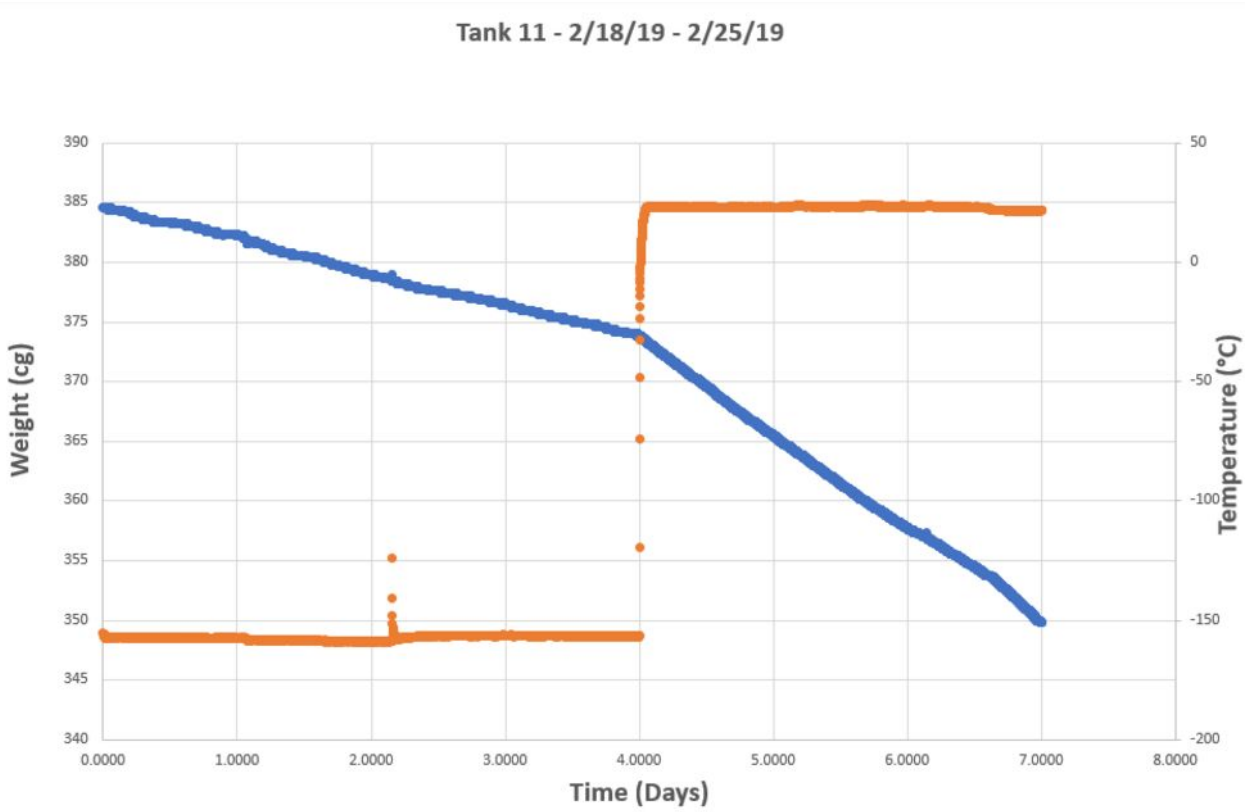


Figure 2: Evaporation Rates for Tank 11 under both Control and Experimental conditions. There is a significant increase in LN_2 evaporation rate for dewars with a compromised seal lacking where the foam coure access plug was removed (right) compared to baseline (left).

5. Discussion:

Cryogenic storage dewars are not perfect nor are the human operators. While methods for monitoring LN_2 dewars currently exist, recent cases related to dewar failure have been reported. Two recent dewar failures involving the University Hospital Cleveland Medical Center in Ohio and Pacific Coast Fertility in San Francisco resulted in a loss of more than 6,000 human eggs and embryos belonging to over 1,000 families because low LN_2 levels went unnoticed [8,9]. Lawsuits citing gross negligence, recklessness, and breach of contract have been rewarded up to $350,000 per individual affected. There is still a need for additional safeguards beyond just temperature or threshold monitoring.

This article has demonstrated that significantly different evaporation rates can be detected in dewars of varying conditions, including those with obvious impairments. It has also been shown that varying rates can be detected between dewars of different age. However, it should be noted that conclusions about the relationship between dewar age and dewar performance cannot be made due to the fact that the two dewars used for this study are of a different make and model.

By comparing the evaporation rates of dewars to those that are considered to be irreparably damaged, it is possible to determine which dewars exhibit excessive evaporation rates that make them no longer viable. The evaporation rates determined using the Experimental conditions can be used as value indicators for dewar failure. For example, these rates can be hardcoded into a program for weight-based monitoring and used as upper limit threshold end points that when exceeded will send an alert to the user. This ability to quantify the health of LN_2 dewars in real-time by analyzing the evaporation rate can help facilitate more effective maintenance of these units, which can reduce the time, energy, and financial resources involved.

The application of this technology is not intended to be a stand-alone monitoring system that replaces existing safety systems. Rather, it is intended for use as a redundant alarm system to further safeguard against failure. This method of monitoring is external to the dewar and does not require internal probes, meaning that it can easily be used in conjunction with temperature probes and adopted as a complementary system by dewar users. For establishments that place dewars on roller-bases, the scale can be designed to fit directly onto the roller base itself (Appendix A). A weight-based system also has the potential to be serve as a cost-effective alternative to temperature monitoring systems for cases involving less critical samples (i.e. commercial cell lines) where financial and ethical liability is significantly reduced. Moreover, it has the potential to replace threshold monitoring systems all together owing to the significant advantage of offering real-time and continuous monitoring compared to discrete monitoring of current threshold sensors.

6. Conclusions:

A weight-based monitoring system offers a simple, safe, and cost-effective alternative for monitoring of cryogenic storage dewars that is real-time and continuous. It has the ability to detect relatively small changes in LN_2 evaporation rates across multiple brands and makes of dewars. The ability to continuously monitor the health of cryogenic dewars via evaporation rate may provide an early detection system for impending cryogenic dewar failure and therefore potentially prevent a catastrophic event. The ability to report evaporation rate can also be used for quality control testing of dewars to ensure that they are maintaining LN2 according to the manufacturer specified guidelines. Ultimately, the system helps safeguard against failure and

provides facilitates more effective monitoring and maintenance by reducing the time, energy, and financial resources involved when manually measuring LN_2 levels.

6. Glossary:

Cryogenics refers to the branch of science that addresses the production and effects of extremely low temperatures.

Dewars are insulated, vacuum-sealed pressure vessels widely used for storing biological specimens including human reproductive cells and embryos.

Dewar health refers to the overall performance of the dewar and its ability to maintain LN_2 at levels specified by the manufacturer.

Real-time relates to a property of a system where input data is processed within milliseconds so that it is available virtually immediately as feedback.

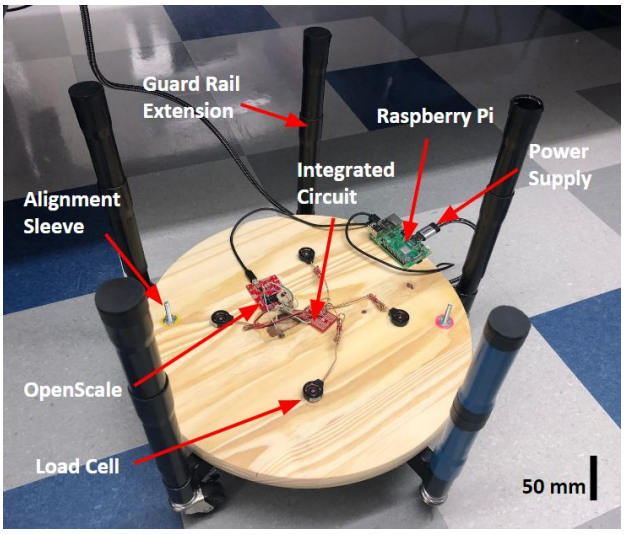
Evaporation rate is defined as the amount of LN_2 vented from a dewar. For the purposes of this article, it is expressed in L/day.

7. Appendix:

7.1 Custom Scale Fitted to an Existing Roller Base:

For facilities that use wheeled roller bases to support their dewars, we propose implementing a custom scale fit to the existing base. The scale shall neither compromise the integrity nor the stability of the LN_2 dewar. In addition, the scale shall neither impede general functionality nor maintenance of the dewar. Included below is a SolidWorks rendering of a sample design of a Worthington VHC 35 cryogenic dewar sitting atop a custom scale (**APP Figure 1**). A physical prototype that has been constructed is also presented below, however clinical testing is still needed to evaluate its efficacy (**APP Figure 2**).

APP Figure 1: SolidWorks rendering of the fully assembled roller base and integrated scale with the LN2 tank placed directly on the assembly.



APP Figure 2: The custom scale features a Raspberry Pi connected to an OpenScale used to read the output from four load cells.

7.2 Materials

7.1.1 Hardware

The materials used in the design include a VHC 35 roller base, two beveled wooden disks, four 200 lb capacity compression load cells, a SparkFun OpenScale board, a Raspberry Pi microcontroller, and ten stackable PVC guide posts. The roller base is made from aluminum and has plastic wheels which allow for any 2-D motion (i.e. translation and rotation). The FX1901 load cells are optimized for laboratory or hospital use and designed to provide superior resolution. The OpenScale is designed to integrate input from multiple load sensors and contains an ATmega328P microcontroller to process, transfer, and format data.

7.1.2 Software

The current prototype uses a Python script to read from the Serial out of the SparkFun OpenScale and plots a graph of the measured data in real time. This script is used purely for demonstration and proof of concept, to show that the design is capable of monitoring the system in real time.

7.2 Methods

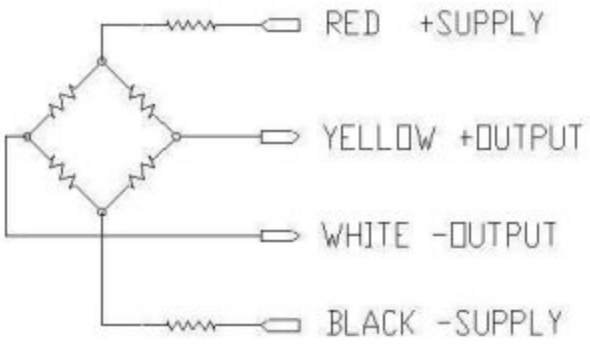
7.2.1 Roller Base Platform Scale

The platform scale was fabricated by evenly spacing four load cells radially between two wooden disks. One of the wooden disks (t = 1'' , D = 17.5'') was centered and secured to the VHC 35 roller base using five 2'' deck screws. A drill press was first utilized to ensure accurate hole positioning followed by a hand drill to secure the five deck screws to the base. The positions for the sensors were then marked on the second plate and four pilot holes were drilled. The second wooden disk was then clamped to the secured disk. Using the pilot holes as a guide, our team center punched the location of the load cells on the secured plate. At this time, two holes were also drilled for sleeves on which our team secured two ¼'' bolts and t-nuts on the first plate in order to align the sensors. The top disk was then removed and the load cells were glued in place. Due to the thickness of the wooden disks, PVC pipes were secured to the posts of the roller base in order to lengthen the original guide posts so as to prevent tipping and/or translation of the VHC 35 cryogenic tank. It should be noted the four sets of double stacked PVC pipes spray painted black are firmly secured to the base's original guide posts while the last double stacked PVC pipe spray painted blue is removable to facilitate loading and unloading of the tank.

7.2.2 Circuit Design

The overall circuit design connects input from the four load cells in parallel to the OpenScale board, which then sends the information to the Raspberry Pi through its Serial out for further data manipulation. Each load cell has 4 outputs that were soldered to wires so they could be connected in parallel (**APP Figure 3**). The wires were shrink wrapped to help secure connections and improve wire management. Upon initial testing the team found that the voltage output of the load cells exceeded the maximum capacity input of the OpenScale, so a voltage divider was added using values of R_1 = 1k, R_2 = 10k to scale down readings to more appropriate values. The

parallel output was then connected to the load cell screw terminals on the OpenScale board. The OpenScale is connected by micro USB to the Raspberry Pi, which uses a script to read and graph incoming data in real time.



APP Figure 3: Wiring information for each of the load cells. Each of the outputs was soldered with additional wires (not shown) for connection to a Breadboard. Image taken from the FX1901 datasheet, originally created by the manufacturer, TE Connectivity.

7.3 Final Design Summary

The final prototype utilized sturdy and reliable materials to demonstrate a proof of concept. It features a roller base specific to the Worthington - VHC 35 and four FX1901 compression load cells. The capabilities of this prototype feature continuous weight versus time plotting. Each load cell has a maximum compressive capacity of 200 lbs, and can therefore bear a 800 lb limit (F.S. $\geq$ 8). Additionally, the added thickness of the custom scale is compensated by way of extended guard rails.

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