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# 1.0 PAYLOAD DESCRIPTION

## 1.1 Mission Goal

The goal of the HADES 2013 team is to design and construct a payload that samples microbial aerosols from the float altitude of 36-38 km. These samples shall be returned uncontaminated to the clean room facilities at LSU for analysis. Analysis of the samples will determine the total number of cells collected, and efforts will be made to culture, isolate, and characterize microorganisms from the samples.

## 1.2 Science Questions

I. Are there microbial aerosols present in the stratosphere?

Hypothesis: The number of cells per volume of air at 36-38 km will be less than those commonly found in the troposphere ($<10^4$ cells $m^{-3}$) due to decreases in water and nutrient availability, temperature, and pressure, and increased ultraviolet radiation (1). Microorganisms can be injected into the stratosphere by dust storms, volcanic activity, severe weather, and anthropogenic sources (6, 14, 15). Residency times for cells smaller than 10 µm can range from months to years due to the laminar flow of stratospheric winds.

II. Are the microorganisms collected at 36-38 km viable and can they be cultured in standard microbiological media?

Hypothesis: Given conditions of high UV fluence, low water availability, low temperature, and the potential presence of oxidative species (e.g, $H_2O_2$ and $O_3$) in the stratosphere, only spore-forming microbes or species with *Deinnococus*-like resistance would be capable of surviving.

## 1.3 Science Objectives

1. Obtain an aerosol sample from a target altitude in the stratosphere.
2. Evaluate the amount of microbial contamination associated with assembly, flight, recovery, and analysis.
3. Determine the concentration of microbial cells collected at 36-38 km.
4. Determine the viability of microorganisms collected at 36-38 km.
5. Determine the environmental conditions (i.e., temperature, pressure, humidity, radiation) from 1.5 to 36-38 km.
6. Verify the results of previous microbiological analysis detailed in the SMITH 2011-12 science reports.

The payload shall sample for microorganisms in the stratosphere and those samples shall be returned safely to the laboratory for analysis. Three identical systems shall be analyzed after flight. The sample chamber shall be exposed to stratospheric air, while a second on board chamber shall remain sealed. A third chamber shall remain in the LSU clean room to establish the background level of microbial contamination. Three chambers shall allow us to determine if cells are being introduced during payload transport and flight operations. Direct enumeration of cells visualized using nucleic acid stains shall allow us to estimate the total number of cells collected in a given volume. The 2013 payload shall monitor temperature, pressure, and relative humidity during flight.

## 1.4 Science Requirements

1. Minimize the amount of background contamination before flight with a rigorous decontamination protocol.
2. Sample aerosols at target altitude for the duration of float (minimum of 12 hours).
3. Simultaneous assessment of laboratory and flight-associated contamination.
4. Direct enumeration of cells with multiple analyses.
5. Isolate microorganisms from aerosol samples collected at 36-38 km.

Background contamination must be minimized according to the protocol described in Section 4.1. By quantifying our background contamination we can determine the lowest number of cells required for a 3σ signal (Section 4.4.2). The payload must sample continuously during float to collect the largest volume possible. Our past flights have sampled 12 (2011) and 8 hours (2012). In addition to the chamber that is opened during float, we will also assess the biomass in two identical chambers. We shall use a variety of quantification techniques to determine the concentration of cells in a given cubic meter of air at float. We shall attempt to isolate viable microorganisms in the lab for future characterization.

## 1.5 Technical Requirements

1. Measure the temperature from -70 to +40° C.
2. Measure the pressure from 0-1000 mbar.
3. Measure the relative humidity from 0-100%.
4. Collect aerosols from target altitude for a minimum of 12 hours while rotating the chambers at 1 rotation second$^{-1}$.
5. Return the samples uncompromised to the laboratory for microbiological analysis.

To characterize the environment in the stratosphere, several parameters must be measured: temperature, pressure, and humidity (Figure 1). During the 2012 flight, the payload encountered temperatures ranging from -7 to +34° C and the pressure ranged from 0 to 1000 mbar. Relative humidity decreased to <1% in the stratosphere, indicating a desiccating environment.

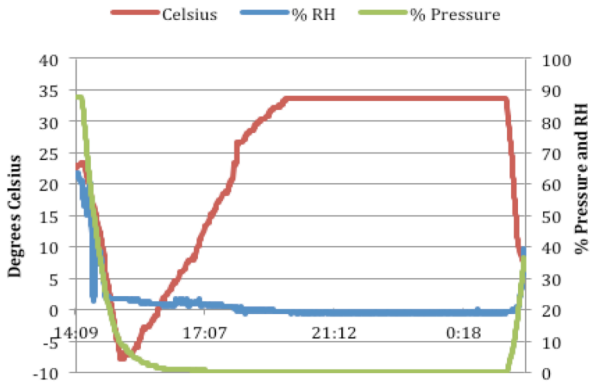



**Figure 1**: Environmental parameters measured during the HASP 2012 campaign.

During HASP 2011 and 2012, the SMITH payload successfully sampled 25 mL min-1 at 36-38 km for the duration of float. The 2013 payload will sample a greater volume of air than the previous payloads in anticipation of recovering a detectable sample. The volumetric flow rate of the 2013 payload can be estimated using the calculation below. The rod length (L) = 21.65 mm and the width of the rod (W) = 1.60mm are multiplied to give the area (A) = 34.64 mm$^2$. The area (A) is the forward facing area of the rod. The volume (V) swept by the rod in one revolution is equal to the area of the rod times the arc length that it sweeps through in a full rotation. The distance from the axis of rotation to the center of a rod holder (R) will be 120 mm. The arc length that a rod sweeps through in one revolution is then equal to 2π times the normal distance ($R \pm \frac{L}{2}$) from the axis of rotation to the center of a rod.

One rod on the near side of the rod holder to the center of rotation will have volume swept:

$$V_{inner} = A \times 2\pi \times \left(R - \frac{L}{2}\right) = 23800 \frac{mm^3}{rev}$$

One rod on the far side of the rod holder to the center of rotation will have a volume swept:

$$V_{outer} = A \times 2\pi \times \left(R + \frac{L}{2}\right) = 28500 \frac{mm^3}{rev}$$

To find the total volume swept in one revolution we can sum the volumes swept in one revolution for all the inner rods and volumes swept in one revolution for all the outer rods. Where the number of rods on the inner side is ($N_{inner}$) = 40 rods and the number of rods on the outer side is ($N_{outer}$) = 40 rods.

$$V_{total} = (N_{inner} \times V_{inner} + N_{outer} \times V_{outer}) = 2{,}090{,}000 \frac{mm^3}{rev} = 2.09 \frac{Liters}{rev}$$

To find the total volumetric flow rate (VFR) you can multiply the total volume swept in one revolution by the angular velocity of rotation (ω). Where ω is estimated to be 60 revolutions per minute.

$$VFR = V_{total} \times \omega = 125 \frac{Liters}{min}$$

A 12-hour flight shall result in a total sampling volume of 90 $m^3$, a significant increase over the past two sampling missions.

## 1.6 Principle of Operation

During the HASP 2011 and 2012 campaigns, the SMITH team flew an aerosol sampler to recover microorganisms present in the stratosphere. Full details of the results of this flight are available in the SMITH 2011-12 Science Reports. The 2011 and 2012 payloads collected an estimated volume of 5.0 x $10^{-3}$ to 1.3 x $10^{-2}$ $m^3$ at 36 km and 7.2 x $10^{-3}$ to 1.8 x $10^{-2}$ $m^3$ at 38 km, respectively. However, there was no significant microbial signal detected during either mission. Instead of pulling air across a 0.2-µm filter, the 2013 design shall collect aerosol by impaction. A chamber will hold 40 commercially available Rotorods (3.50E-05 $m^2$) coated with silicon grease (Figure 2). As the chamber is rotated, each rod will pass through a volume of air. Particles present in the atmosphere will become entrapped in the grease and returned to the lab for analysis.

**Figure 2**: Sampling chamber with 40 silicon covered rods

The advantages of holding 40 rods include: multiple analysis techniques can be performed in triplicates and several rods can be combined to increase the signal. The team has developed stringent protocols to decontaminate the payload prior to flight. The chambers shall remained sealed prior to, and immediately after, sampling to minimize contamination. Preliminary data suggests we may have sampled at the upper limit of the biosphere, and a greater sample volume will allow us the opportunity to verify these results. A prototype of the 2013 HASP payload was flown on a sounding balloon flight in September 2012. Data from this flight can be seen in Section 4.4.2.

## 1.7 Science Background

There is a knowledge gap regarding the quantification of microorganisms above altitudes of ~10 km. As seen in Table 1, sampling missions have isolated organisms in the stratosphere as early as 1936, but little information has been provided on the inherent levels of background contamination. Although we have

not recovered any viable organisms from >30 km, we have provided the limits of detection and quantification for stratospheric samples.

Table 1: History of microbiological sampling of the stratosphere

| Date | Altitude (km) | Sample Method | Biology Measured | Volume (L) |
|---|---|---|---|---|
| 1936[13] | 11-21 | Balloon | 5 *Bacillus* sp.,1 *Penicillium* sp., 1 *Macrosporium* sp., 2 *Aspergillus* sp. | Unknown |
| 1978[9] | 48-77 | Meteorological Rocket | *Mycobacterium* sp. & *Micrococcus* sp. | Unknown |
| 2003[15] | 30-41 | Balloon, liquid neon cryopump | Isolated *S. pastuerii, B. simplex*, the fungus *Engyodontium album* | 57 |
| 2004[6] | 20 | Airplane, impactor surfaces | *Bacillus* luciferins, *Bacillus sphaericus* | Unknown |
| 2006[8] | 19-41 | Balloon, liquid neon cryopump | 7 cells $L^{-1}$ (based on counting clumps), *Bacillus* sp., *Staphylococcus* sp., *Engyodontium* sp. | 19-81 |
| 2007[7] | 20 | Airplane, impactor surfaces | *Micrococci, Microbacteria, Staphylococcus* sp., *Brevibacterium* sp. | Unknown |
| 2008[16] | 10-12 | Airplane (Gulfstream-2), Vaccuum pump and filter | Isolated 1 *Deinococcus* | 48-78 |
| 2010[14] | 20 | Airplane, impactor surfaces | Isolated *Bacillus* sp. | Unknown |

We will attempt to recover any viable microorganisms collected and to determine the concentration of cells at float altitudes. The HASP 2013 payload design will allow us to utilize multiple analysis techniques including: direction enumeration of cells via microscopy, measuring the amount of adenosine triphosphate (ATP) collected as an estimate for biomass, and standard culturing techniques. This year we will add analysis methods that allow us to quantify the total number of bacterial endospores that may not be accounted for with the previously mentioned techniques.

## 2.0 PAYLOAD DESIGN

### 2.1 Principle of Design

Figure 3 shows a high-level system diagram of the payload designed to operate at the HASP float altitude. The main purpose of this payload collect aerosol samples at a range of altitudes in the mid stratosphere. Since HASP will float at approximately 36-38 km, this will be the targeted altitude for sampling. The payload will carry a sample chamber and a flight control chamber. Both chambers will be physically identical, but the flight control chamber will remained sealed for the duration of the flight. The flight control chamber measures the background contamination throughout the flight without actively sampling. The chambers shall be rotated centrifugally such that when the sampling chamber is opened, air with flow across the rods at the target altitude. In the event the sample chamber fails to open correctly during flight, it will be possible to open the flight control. This procedure may cause the loss of the flight control, and will only occur as a last resort.

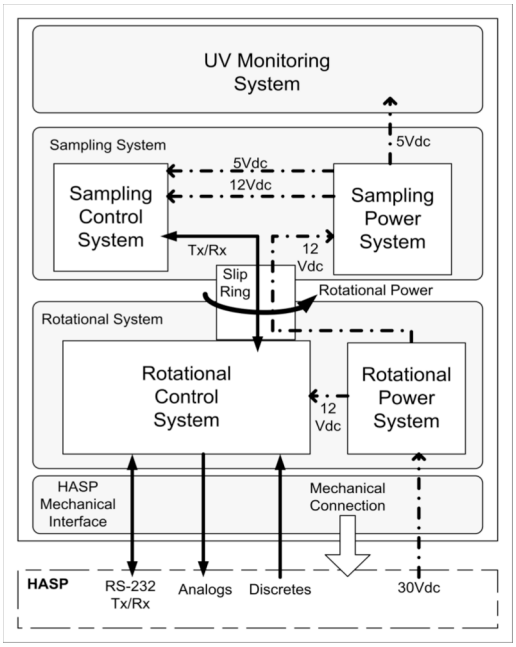



**Figure 3:** High-level system diagram

The electronics includes all of the monitoring and controlling of the payload and collecting the environmental data. The motor and actuator systems will be monitored through temperature, electrical current, and rotation per second (RPS) sensors. The motor system and heaters will be controlled through the power distribution system commanded through HASP.

Before flight, both chambers will be sterilized and sealed as detailed in Section 4.1. They will remain in this state until HASP reaches float. Once at float, a radio command will be uplinked to the payload to open the doors sealing the sampling chamber followed by a command to initiate the motor to begin the chambers' centrifugal rotation. Confirmation of successful door opening will be downlinked to the ground station. Then the chambers' rotational speed will be monitored. Data from all sensors (temperatures and speeds of systems and environmental data) will be relayed to the ground station throughout the flight. The rotational system will have radio controlled power throttling allowing for change in power consumption and heat production. Both the motor and actuator systems' temperatures will be closely monitored. If either system's temperature approaches the lower limit of its operational range, ground control can command heaters to turn on. This will heat the system until it returns to safe operational temperature

range. Before cut down, a command to stop sampling will be uplinked to the payload. This will turn off the rotational motor system and close the doors, completely sealing the chamber. Once confirmation of a successful chamber closing is received, the payload will then remain in this state until it is returned to the lab for processing.

## 3.0 Subsystem Integration

### 3.1 Mechanical Interface

The overall concept for the payload is shown in Figure 4. The overall payload body shall be 27 cm x 29 cm x 30 cm. The rotational system box and slip ring (A: 27 cm x 29 cm x 9.2 cm) will house and protect the motor, electrical control, gearing, and power system that will create the rotation used to collect microorganisms. The power and control electronics for the sampling system are housed in sampling system box (B: 15 cm x 22 cm x 5.1 cm). The sampling system electronics box will house the power and control circuitry required to open and close the chamber doors using linear actuators and supply power to the UV monitoring system (C: 6.4 cm x 15 cm x 4.3 cm). The UV monitoring system box will houses the necessary electronics to power and record data from the UV sensors. The sample and control chambers (yellow arrows, 8.3 cm tall) are mounted on top of the sampling system box. These chambers contain the rods used to collect microorganisms in the stratosphere (Section 1.6). The control chamber will be built as a fully functional system, identical to the sampling chamber, but will only be used should the sampling chamber malfunction. The main purpose of the three payload boxes is to protect the payload's internal components from debris and shield components from sunlight during the flight. The rotational system box will be mounted directly onto the large PVC HASP interface plate using four ¼" screws.

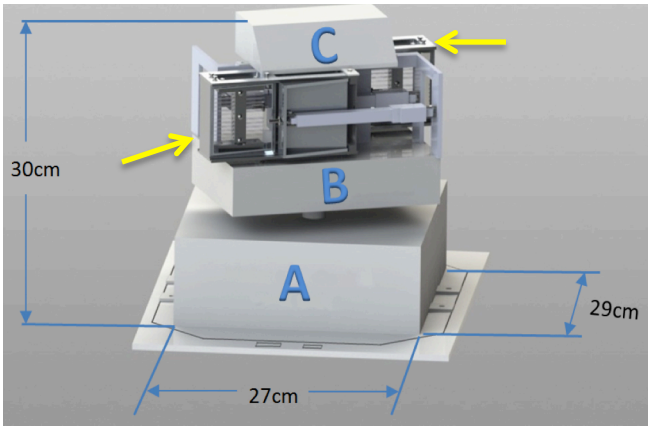



**Figure 4:** Overall payload concept and dimensions

The estimated weights for the major payload components are listed in Table 2. We shall be under the 20 kg weight limit.

| **Table 2**: Estimated weight budget | |
|---|---|
| **Part** | **Weight (g)** |
| DC Motor | 2110 |
| Gear Train and Control/Power Electronics | 470 |
| Aluminum Structures | 4760 |
| Actuators and Control/Power Electronics | 675 |
| Sampling Chamber | 560 |
| Control Chamber | 560 |
| UV Sensor Foam Structure and System | 355 |
| Insulating Foam | 100 |
| Heaters | 50 |
| **Total** | **9640** |

The payload boxes shall be insulated with Styrofoam and painted white to reflect sunlight. The electronics shall produce enough heat maintain an operational temperature. The motor and slip ring will have heaters and temperature sensors attached to allow us to regulate temperatures during flight.

The payload mounting footprint can be seen in Figure 5. The 27 cm x 29 cm x 9.2 cm frame will be attached HASP interface plate by four ¼" chromium bolts.

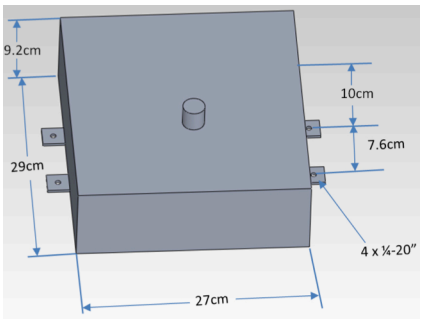



**Figure 5:** Payload mounting footprint

## 3.3 Power Interface

Figure 6 displays the power system diagram for the payload. HASP provides a limited power of 75 W to each large payload. The voltage at which the power is supplied is 29 to 33 VDC. The power system must power the rotational DC motor, two linear actuators, and all circuit boards. Also in the case that the motor gets below operational temperature (to be experimentally determined), the power system will turn on the heaters. To successfully power the components, a 30 V to 12 V DC-to-DC converter and a 12V to 5V DC to DC convertor will be used to reduce the current draw. The boards will always be powered on. The motor, linear actuators, and heaters are regulated through the control systems and the discrete lines from HASP (Table 3).

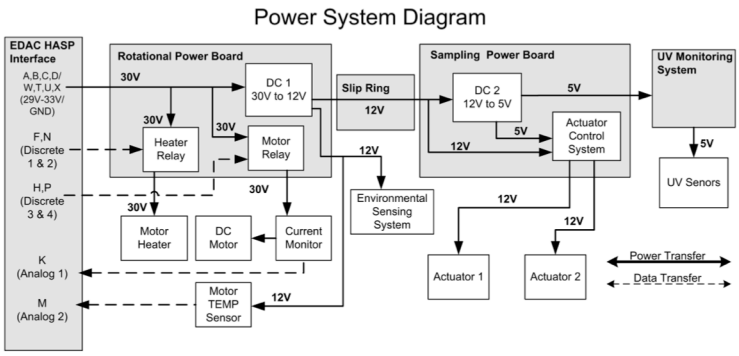



**Figure 6**: Power system diagram

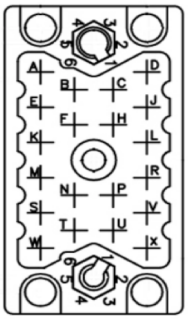


**Figure 7**: EDAC Connector

A twenty-pin EDAC 516 connecter will be used to interface with HASP power supply (Figure 7). Pins A, B, C, and D on the EDAC will be used for +30 VDC and W, T, U, and X will used to ground the power supply, as seen in Table 3. Pins F and N will be used to control the heater relay which will turn on the heaters. Pins H and P will be connected to an additional relay that will turn on the motor to begin rotating the collection chambers. Pins A-D, will be used in parallel to provide the appropriate power supply of 2.5 Amps at 30 VDC to the payload. The power system requires two DC/DC converters to step down voltages from 30 VDC to 12 VDC and from 12 VDC to 5 VDC. Besides the motor, heaters, and actuators, all the other components will be powered throughout the duration of the flight.

**Table 3:** EDAC pin assignments

| Function | EDAC Pins | Wire Color | Purpose |
|---|---|---|---|
| +30 VDC | A, B, C, D | White with red stripe | Power payload |
| Power Ground | W, T, U, X | White with black stripe | Ground payload |
| Discrete 1 | F | Brown | Heater is turned on |
| Discrete 2 | N | Green | Heater is turned off |
| Discrete 3 | H | Red with white stripe | Motor is turned on |
| Discrete 4 | P | Black with white stripe | Motor is turned off |

Table 4 shows the estimated voltage, current, duty cycle, power, and power consumed for each component. The power usage of each component is determined multiplying the voltage by the current: $V * I$. The total power calculated is the maximum power the payload draws if all components were active at the same time. The duty cycles were calculated using some of the results from the previous flight: $total\ run\ time\ /\ total\ flight\ time$. The power consumed is determined as follows: $I * duty\ cycle * total\ flight\ time$. The total flight time is 16 hours (with a 12-hour float). This was based on HASP 2011 data. The DC-to-DC converters are not 100% efficient; during voltage reduction process they dissipate heat, causing power to be lost. Therefore, the total power consumed becomes $P_{in} * efficiency$. The efficiency of each converter is estimated to be 85%. These efficiencies are already taken into account in Table 4.

**Table 4:** Power budget

| Component | Voltage (V) | Current (mA) | Duty Cycle Over Entire Flight (%) | Power (W) | Power Consumed (Amp hours) |
|---|---|---|---|---|---|
| Rotational DC motor | 30 | 1000 | 75 | 30 | 12 |
| (2) Linear actuators | 12 | 420 | 0.1 | 5.0 | 0.31 |
| Heaters | 30 | 600 | 5.0 | 18 | 0.48 |
| Environmental Board | 12 | 48 | 100 | 0.58 | 0.53 |
| Arduino | 9 | 55 | 100 | 0.5 | 0.37 |
| GPS Shield | 3.3 | 70 | 100 | 0.23 | 0.17 |
| Trimble GPS | 3.3 | 27 | 100 | 0.089 | 0.07 |
| **Total** | | | | **54.4** | **13.9** |

**Table 5:** Converter efficiency

| Type of DC/DC Converter | Purpose | Efficiency (%) |
|---|---|---|
| Converter 1: 30V to 12V | Actuator Power | 85 |
| Converter 2: 12V to 5V | Actuator Control Board and UV Sensors | 85 |

## 3.4 Data Interface

### 3.4.1 Data Handling

A controller is required to handle the communication between HASP and our payload. The controller must have a serial RS-232 connector. We will require seven ADC channels to accommodate the three environmental sensors and four onboard monitoring temperature sensors. Eight bit ADC channels will suffice for our environmental sensors. I/O pins will be required to interface with the sample and control actuators. Table 6 shows the format of the record that will be stored and downlinked during flight. The real time clock needs a total of 4 bytes. The temperature sensors also need a total of 4 bytes at 1 byte per sensor. The rotation sensors for our payload will need 2 bytes each initially. The numbers in these two bytes can later be converted into rotations per second, which can be stored in one byte giving a total of two bytes for the rotation sensors. There will be eight state bits, which will store the status of the sample and control chambers and the rotation of the device. The environmental sensors will need a total of 3 bytes. The last command sent will require two bytes and the telemetry period, the command response, the pump control error response, and the environmental error response will all require one byte each.

**Table 6:** Data record format. RTC: real time clock

| **Record Format** | **Byte Size** |
|---|---|
| RTC date | 1 |
| RTC hour | 1 |
| RTC minute | 1 |
| RTC second | 1 |
| Rotational motor temperature sensor | 1 |
| Rotational motor RPS | 1 |
| State bits | 1 |
| Environmental pressure | 1 |
| Environmental temperature | 1 |
| Environmental humidity | 1 |
| Telemetry period | 1 |
| Last command sent high byte | 1 |
| Last command sent low byte | 1 |
| Command response | 1 |
| Error Response | 1 |
| **Total** | **15** |

### 3.4.2 Downlink Data Format

The data will transfer from HASP via a RS-232 serial connection that will use 8 data bits, no parity bit, 1 stop bit, and no flow control. The serial connection will be a DB9 DTE (Data Terminal Equipment) connector (Figure 8). Only the transmitted data, received data, and signal ground lines will be used. The payload will downlink a data record when the time since the last data record sent has surpassed the telemetry period. The telemetry period will initially be set to ten seconds. All data records will be comma delimited and sent to ground control in ASCII. Each data record will end with a carriage return and a line feed. This will provide ground control with a near real time status of the payload. The team can then mitigate any problem if they occur. A current estimate is 15 bits per second (bps) for the payload.

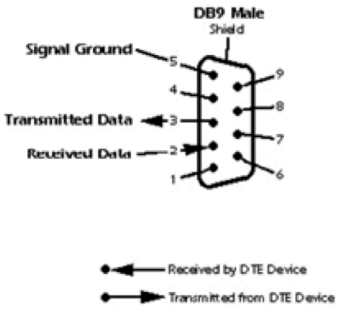



**Figure 8:** DB9 Pins used for the HASP serial interface

### 3.4.3 Uplink Command and Data Format

The serial connection to HASP provides the ability to uplink 2 byte commands. The first byte is used to specify which command is to be complete and the second byte is used as the argument for this command. This offers 256 possible unique commands for the payload with each command having an argument. Table 7 contains the current list of commands we can uplink. The sampling process starts once the commands to begin rotating and open the chambers are issued. To stop the sampling process, a command must be sent to close the chambers and stop rotating. Additional commands can be used to manually control key functions. These functions include commands like changing the telemetry period.

**Table 7:** Total commands

| |
|---|
| ▪ Begin rotating |
| ▪ Open sample chamber |
| ▪ Close sample chamber |
| ▪ Open control chamber |
| ▪ Close control chamber |
| ▪ Turn on heaters |
| ▪ Turn off heaters |
| ▪ Stop rotating |
| ▪ Change telemetry rate |
| **Total Commands: 9** |

## 4.0 Microbiological Analysis

### 4.1 Pre Flight Procedures

The payload decontaminating procedure employs a series of techniques to kill microbes and reduce cellular contamination. The payload will be assembled in a class 100 clean hood that is housed within a class 10,000 clean room (4). Instruments involved in preparation will be heat sterilized at 120° C for 20 minutes. In addition, all surfaces will be exposed to germicidal UV-C (254 nm) light for 20 minutes and rinsed in 3% hydrogen peroxide (v/v) to oxidize cellar macromolecules, such as nucleic acids. The materials will be then rinsed with a 70%

ethanol (v/v) solution to remove residual salts. After drying, the sampling device will be assembled and placed in a gas-pourous sterilization pouch and exposed to ethylene oxide (EO) at a concentration of 0.45-.65 Mg meters$^{-3}$ at 55° C and 30-50% RH for 4 hours (11). EO is effective for its bactericidal properties as well as its ability to inactivate spores (2). The SMITH payload for HASP 2011 and 2012 were processed in the identical manner. Three identical chambers will be constructed to monitor levels of contamination incurred during payload handling; one chamber will sample the stratosphere for the duration of float, one chamber will remain sealed during flight, and one will in the lab. This approach will allow us to assess the level of background contamination present in the sampling chambers.

### 4.2 Post Flight Analysis

Based on the HASP 2011 post flight timeline, we anticipate a minimum of two days for the recovery and transportation of the payload back to the laboratory at LSU in Baton Rouge. During this time, the payload will be stored at ~4° C to prevent extensive thermal shock to the samples. Once in the clean room, the filters will be recovered and classical microbiological and microscopic techniques will be employed to quantify the cells present and to verify the results and conduct a variety of assays to assess if viable cells are present in the samples. The payload will be disassembled and the chambers will only be opened under class 100 conditions.

### 4.3 Limits of Detection for Multiple Assays for Rods

Each rod has a sampling area of 3.50E-05 m$^2$ and each payload contains a minimum of 40 rods. The minimum number of cells required to achieve a signal is based on previous flight results from ~10 km (Table 8).

**Table 8:** Minimum requirements for September Campaign 2012 to achieve the level of detection 1 σ above the background. †

Assuming 0.1% culturability using standard enrichment media techniques. *Priority Level 1: must begin within 24 hours of return to LSU; Priority Level 2: must begin within 48 hours of return to LSU; Priority Level 3: must begin within 7 days of return to LSU; Priority Level 4: begins when microscopic analysis is completed.

| Technique | Cells rod$^{-1}$ | Priority Level* |
|---|---|---|
| ATP | 10 | 1 |
| SYBR gold | 300 | 3 |
| Live/Dead | 700 | 2 |
| Culturing† | 1000 | 2 |
| XTT† | 1000 | 2 |
| Endospores | TBD | 4 |

## 4.4 Cell Concentration Measurements

### 4.4.1 Microscopic Analysis

Three microscopic methods will be used to directly enumerate the number of DNA-containing cells in the samples. One of the drawbacks to these methods is inorganic particles may autofluoresce and must be distinguished from bona fide cells.

#### *4.4.1.1 SYBR Gold*

Cells will be stained with SYBR Gold (Molecular Probes, Inc., cat. no. S-11494) and visualized at 1000X using an Olympus bx51epiflourescence microscope. Blanks will be counted in parallel to determine the background contamination in the sample. For each sample, 60 fields of view will be counted. The area of each field of view is ~$28 \times 10^5$ $\mu m^2$. The number of cells per sample will be estimated by dividing the total rod area of the sample by the area counted and scaling accordingly. The data will be compared to the biomass estimates made from the ATP extractions to more accurately determine the amount of cells present in a given volume of air in the stratosphere.

#### *4.4.1.2 LIVE/DEAD*

The LIVE/DEAD BacLight (Molecular Probes, Inc., cat. no, L7012) stain allows the distinction of potentially viable cells from those which are inferred to be dead. The STYO 9 dye stains all DNA containing cells green, whereas propidium iodide is only able to enter cells with compromised membranes, staining such cells red (3). This assay allows for the estimation of the number of potentially viable cells (those with intact membranes) to be compared to the number of cells not likely to be viable. In addition to distinguishing between live and dead cells, the total number of cells counted will serve as an alternate measurement of total cell concentration.

#### *4.4.1.3 Thioflavin T*

Thioflavin T binds to amyloid folds in proteins and can be used to visualize vegetative cells and bacterial endospores simultaneously. This will be our first opportunity to quantify endospores, as they are impermeable to the dyes used in previous years. This will provide a comprehensive biomass concentration measurement.

### 4.4.2 Quantification of ATP as an Estimate of Biomass

The measure of ATP in an environmental sample is our most sensitive assay to estimate biomass recovered from atmospheric samples. Purified ATP (100 nmol/L) supplied by the manufacturer, was diluted into sterile filtered, autoclaved water to create a standard curve (Figure 9). The amount of light produced, measured in relative light units (RLUs), in the reaction is directly proportional to the amount of ATP in the sample.

During September 2012, we tested our payload design using a sounding balloon to sample from 1.5-27 km. Within 4 hours of recovery, five subsamples were assayed for the amount of adenosine triphosphate (ATP) collected. Each of the three chambers that were exposed to the atmosphere measured a significant signal above the background, and the sample chambers were significantly different form each other ($p<0.05$, $df=2$, $F=3.88$). The sample from HASP 2013 shall be assayed for the total amount of ATP collected from the target float altitude. This will allow us to compare the amount of ATP to appropriate controls and estimate the biomass collected in a given volume of atmosphere.

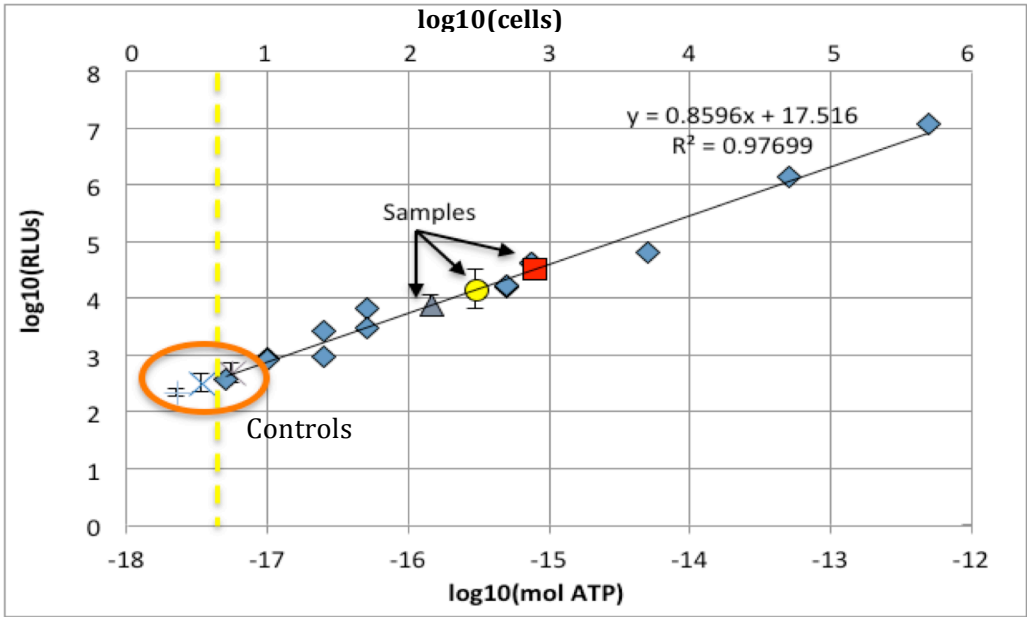



**Figure 9:** ATP as an estimate of biomass collected from 1.5-27 km. ATP measurements were made from each chamber (n=5) and the data has been log transformed. The standard curve is shown in blue. The yellow line represents a limit of detection 3σ above the blanks. Statistical analysis revealed that there was no difference in the controls (orange circle, $p=0.12$, $df=2$, $F=1.0E+09$).

### 4.5 Culture Based Techniques

Previous stratospheric sampling missions have reported isolating microorganisms in the lab, but the existing data does little to account for background contamination incurred before or after flight (3, 4, 5, 8, 9, 10). The majority of the isolates are endospore-forming *Bacillus,* but fungal isolates, as well as a single Gram-negative *Staphylococcus,* have also been reported (3, 4, 5, 8, 9, 10). In an attempt to verify these results, we will conduct culturing experiments. Rods shall be placed into1% R2A liquid media and incubated aerobically at 4° C. After 1 month of incubation, the samples shall be moved to 25° C. Subsamples will be routinely plated on solid media and monitored for growth.

## 5.0 TESTING AND INTEGRATION PROCEDURES

### 5.1 Testing at LSU

- Calibrate payload temperature sensors
- Calibrate environmental sensors
- Thermal vacuum test of motor and pump system at various voltages
- Verify the functionality of each subsystem
- Verify correct voltages across different boards (sensor, communication and power)
- Run the flight software and do a complete system test
- Use terminal to test serial communication

### 5.2 HASP Testing and Integration

- Monitor current drawn by different pump systems at different voltages
- Verify communication to and from HASP
- Mount payload to HASP platform
- Perform thermal test
- Perform vacuum test
- Perform 10 G vertical and 5 G horizontal impact analysis
- Connect HASP power and serial connectors
- Run flight software
- Troubleshoot for any faults

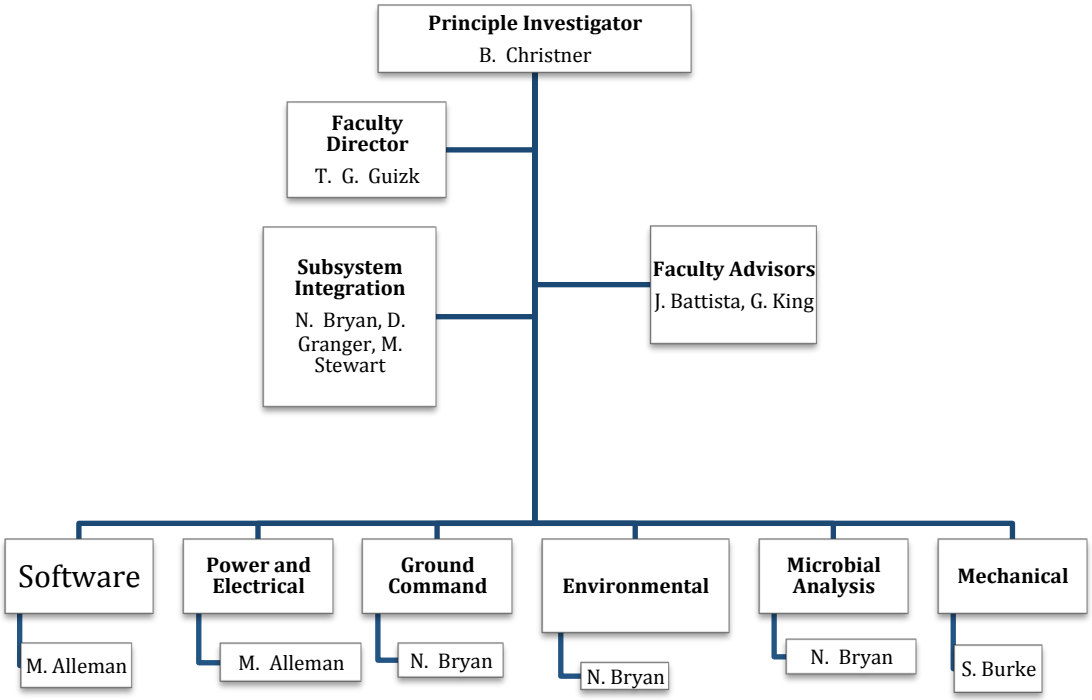
## 6.0 TEAM ORGANIZATION

### 6.1 Management

The payload for HASP 2013 will be developed and operated under the support of the NASA EPSCoR MARSLIFE project at Louisiana State University (LSU). Project manager Noelle Bryan leads the team and will be present at Integration and Flight. The faculty advisors are B. Christner (Science Advisor) and T. G. Guzik (Payload Advisor). B. Ellison, D. Granger, and M. Stewart will serve as technical staff mentors. Biological Sciences faculty members will also be available for consultation.

S. Burke and M. Alleman (undergraduates) will return for a third year. After completing the SMITH 2013 project, students will gain first-hand experience with project management, experiment construction, data collection, analysis, and interpretation. The data obtained on the cell concentrations in the stratosphere will be a major component of N. Bryan's graduate thesis.

## 6.2 Project Organization



## 6.3 Preliminary Schedule

| Task Name | Duration | Start | Finish |
|---|---|---|---|
| **SMITH Payload** | **315 days** | **Mon 10/1/12** | **Fri 12/13/13** |
| **Preliminary Design** | **76 days** | **Mon 10/1/12** | **Mon 1/14/13** |
| Mission Definiton Review | 18 days | Mon 10/1/12 | Wed 10/24/12 |
| System Requirement Review | 25 days | Thu 10/25/12 | Wed 11/28/12 |
| Definition of Preliminary Design | 5 days | Wed 10/31/12 | Tue 11/6/12 |
| **Final Design** | **53 days** | **Thu 11/1/12** | **Mon 1/14/13** |
| HASP Proposal | 1 day | Fri 12/14/12 | Fri 12/14/12 |
| Selection Announcement | 1 day | Mon 1/14/13 | Mon 1/14/13 |
| **Electronic Design** | **39 days** | **Thu 11/1/12** | **Tue 12/25/12** |
| Environmental Sensors | 5 days | Thu 11/1/12 | Wed 11/7/12 |
| Sample sensors | 39 days | Thu 11/1/12 | Tue 12/25/12 |
| **Software Design** | **47 days** | **Mon 11/5/12** | **Tue 1/8/13** |
| Data Storage | 5 days | Mon 11/5/12 | Fri 11/9/12 |
| Downlink | 5 days | Mon 11/12/12 | Fri 11/16/12 |
| Uplink | 5 days | Mon 11/19/12 | Fri 11/23/12 |
| Write Preliminary Code | 32 days | Mon 11/26/12 | Tue 1/8/13 |
| **Mechanical Design** | **5 days** | **Mon 12/3/12** | **Fri 12/7/12** |
| Sample system | 5 days | Mon 12/3/12 | Fri 12/7/12 |
| Mounting | 2 days | Wed 12/5/12 | Thu 12/6/12 |
| Electronics Box | 1 day | Fri 12/7/12 | Fri 12/7/12 |
| **Subsystem Intergration Phase** | **34 days** | **Tue 1/15/13** | **Fri 3/1/13** |
| Order Materials | 4 days | Tue 1/15/13 | Fri 1/18/13 |
| Integrate Temperature Sensors | 5 days | Mon 1/21/13 | Fri 1/25/13 |
| Calibrate Temperature Sensors | 5 days | Mon 1/28/13 | Fri 2/1/13 |
| Build Mechanical Housing | 20 days | Mon 2/4/13 | Fri 3/1/13 |
| **Preliminary PSIP Document** | **35 days** | **Mon 3/4/13** | **Fri 4/19/13** |
| Exact Power and Weight Budget | 3 days | Mon 3/4/13 | Wed 3/6/13 |
| Determine Exact Downlink Data Format | 3 days | Thu 3/7/13 | Mon 3/11/13 |
| List Uplink Commands | 2 days | Tue 3/12/13 | Wed 3/13/13 |
| Discreet Command and Analog Output Usag | 2 days | Thu 3/14/13 | Fri 3/15/13 |
| Write Rough Draft | 7 days | Mon 3/18/13 | Tue 3/26/13 |
| Edit Rough Draft | 9 days | Wed 3/27/13 | Mon 4/8/13 |
| Edit Preliminary Version | 9 days | Mon 4/8/13 | Thu 4/18/13 |
| Final Document Due | 1 day | Fri 4/19/13 | Fri 4/19/13 |
| Preliminary HASP Thermal / Vacuum Testing | 5 days | Mon 5/20/13 | Fri 5/24/13 |
| Final Testing and Payload Adjustments | 20 days | Mon 5/27/13 | Fri 6/21/13 |
| **Final PSIP Document** | **15 days** | **Mon 6/3/13** | **Fri 6/21/13** |
| Edit Preliminary PSIP Document | 14 days | Mon 6/3/13 | Thu 6/20/13 |
| Final Document due | 1 day | Fri 6/21/13 | Fri 6/21/13 |
| **FLOP** | **25 days** | **Mon 6/24/13** | **Fri 7/26/13** |
| Write Detailed Timeline for Launch Day | 3 days | Mon 6/24/13 | Wed 6/26/13 |
| Write Detailed Procedures for Flight Line Set | 5 days | Thu 6/27/13 | Wed 7/3/13 |
| Write Rough Draft | 12 days | Thu 7/4/13 | Fri 7/19/13 |
| Edit Rough Draft | 4 days | Mon 7/22/13 | Thu 7/25/13 |
| Final Document Due | 1 day | Fri 7/26/13 | Fri 7/26/13 |
| **Student Payload Integration at CSBF** | **6 days** | **Mon 7/29/13** | **Mon 8/5/13** |
| Mount Payload to HASP Platform | 2 days | Mon 7/29/13 | Tue 7/30/13 |
| Test and Debug Flight Software | 2 days | Wed 7/31/13 | Thu 8/1/13 |
| Perform Thermal Vacuum Test | 2 days | Wed 7/31/13 | Thu 8/1/13 |
| Perform Shock Test | 2 days | Fri 8/2/13 | Mon 8/5/13 |
| **HASP Flight Preparation** | **19 days** | **Mon 8/5/13** | **Thu 8/29/13** |
| Fixing Problems Encountered at Integration | 19 days | Mon 8/5/13 | Thu 8/29/13 |
| Target Flight Ready | 1 day | Fri 8/30/13 | Fri 8/30/13 |
| Target Launch Data and Flight Operations | 1 day | Mon 9/2/13 | Mon 9/2/13 |
| **Flight/Science Report** | **70 days** | **Mon 9/9/13** | **Fri 12/13/13** |
| Analysis of Data | 22 days | Mon 9/9/13 | Tue 10/8/13 |
| Write Rough Draft | 23 days | Wed 10/9/13 | Fri 11/8/13 |
| Edit Rough Draft | 24 days | Mon 11/11/13 | Thu 12/12/13 |
| Final Draft | 1 day | Fri 12/13/13 | Fri 12/13/13 |

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