HASP Student Payload Application for 2012

Payload Title: Sampling for Microorganisms In The High (SMITH) Atmosphere		
Payload Class: (check one) ☐ Small X Large	Institution: Louisiana State University	Submit Date: 12/16/11
Project Abstract: The Sampling for Microorganisms In The High atmosphere (SMITH) payload will sample for the presence of cells at 36 km. The payload will also characterize the environment microorganisms may encounter in the stratosphere including temperature, pressure, and humidity. The SMITH 2012 concept will be an extension of the SMITH 2011 payload's data. The SMITH team is led by Noelle Bryan, under the guidance of Dr. B. Christner, P.I. The team members include M. Alleman, K. Blackburn, B. Broekhoeven, S. Burke, and A. Spring.		
Team Name: SMITH		Team or Project Website:
Student Team Leader Contact Information:		Faculty Advisor Contact Information:
Name:	N. C. Bryan	B. C. Christner
Department:	Dept. of Biological Sciences	Dept. of Biological Sciences
Mailing Address:	282 Life Sciences Bldg.	282 Life Sciences Bldg.
City, State, Zip code:	Baton Rouge, LA	Baton Rouge, LA
e-mail:	Nbryan5@lsu.edu	xner@lsu.edu
Office telephone:	(225) 578-1734	(225) 578-1734
Cell:	(225) 326-4628	(225) 247-3150
FAX:	(225) 578-2597	(225) 578-2597

Contents

List of Figures

List of Tables

1.0 PAYLOAD DESCRIPTION

1.1 Mission Goal

The goal of the SMITH 2012 payload is to sample for cells that may be present at an altitude of 36 km. During HASP 2011, the SMITH payload sampled at 36 km for 13 hours and collected approximately 20 m^3. Microbiological analysis of the sample gave conflicting reports of the number of cells present at this altitude. To validate the results of this flight, we propose to fly a second payload and increase the volume of air sampled. A second SMITH payload will provide data on unprecedented research on the high altitude limits of life.

1.2 Science Questions and Hypotheses

I. Are cells present in the atmosphere at an altitude of 36 km?

Hypothesis:

Based on the sampling of 20 m^3 during the HASP 2011 flight, we predict the concentration of cells at an altitude of 36 km will be less than 1000 cells m^{-3}.

II. What is the high altitude limit of the biosphere?

Hypothesis:

Due to decreased relative humidity (RH), temperature, and pressure, and increased ultraviolet (UV) radiation with altitude, the upper boundary of the biosphere is an altitude below 36 km.

1.3 Science Objectives

- Sample a minimum volume of 40 m^3 during the course of the flight
- Recover the particles > 0.2 µm from large volumes of air and return the samples uncompromised to the lab for analysis
- Quantify the number of cells collected during flight using a variety of standard microbiological techniques
- Monitor the environmental parameters of the stratosphere (i.e., temperature, pressure, humidity, radiation)
- Quantify and characterize contamination associated with laboratory- and payload- based handling procedures
- Verify the results of previous microbiological analysis detailed in the SMITH 2011 science report

1.4 Principle of Operation

During the HASP 2011 campaign, the SMITH team flew a high volume air sampler to monitor cells in the atmosphere. Full details of the results of this flight are available in the SMITH 2011 Science Report. The payload successfully collected an estimated volume of 20 m^{-3} at 36 km for a 13 hour float. The samples were returned to the lab for microbiological analysis. The team has developed stringent protocols to decontaminate the payload prior to flight. The filter housing remained sealed prior and immediately after sampling to minimize contamination. Although the payload was functionally a success, there are improvements required to more accurately determine the number of cells present at 36 km.

We propose:

- To collect a larger volume of air to increase the number of potential cells collected
- To develop an alternative method of monitoring the solenoid valves (further detailed in mechanical section)
- To develop a more efficient program for data storage and communication
- To monitor the hermetic seal of the sample chambers post flight with a pressure gauge
- To accurately measure the flow rate of the entire system at 5 mbar prior to flight
- To develop more sensitive protocols for quantifying the number of cells collected during flight (i.e., improve limits of detection for adenosine triphosphate (ATP) extractions)

The previous modifications will allow us to accurately determine the number of cells present at 36 km. Preliminary data suggests we may have sampled at the upper limit of the biosphere, and a second flight will allow us the opportunity to verify these results.

1.5 Science Requirements

As a continuation of SMITH 2011, the SMITH 2012 payload will sample for the presence of microorganisms at an altitude of 36 km. We have successfully sampled over 20 m^3 of air at 1 kPa and we propose to improve upon our existing method. A minimum two-fold increase in the volume sampled is required to expand our current list of microbiological techniques employed post flight.

1.6 Technical Requirements

To characterize the environment in the stratosphere, several parameters must be measured including temperature, pressure, and humidity. During the previous flight, the payload encountered temperatures ranging from -48.9 to 33.5° C and the pressure ranged from 1 to 100 kPa. Relative humidity decreased to 2% in the stratosphere.

- Measure temperature in the range of -70 to 30° C, to a resolution of 1°C and at a rate of once per minute
- Measure pressure in the range of 0 to 100 kPa, to a resolution of 0.2 kPa and at a rate of once per minute
- Measure relative humidity in the range of 0.1-100%, to a resolution of 1% and at a rate of once per minute

A continued collaboration with Southern University and A&M College (SU) will allow increased student involvement on the SMITH payload. SU will be responsible for fabrication and testing of the environmental subsystem. All measurements must follow the technical requirements set forth by the SMITH proposal. Integration of the environmental subsystem will occur under the guidance of both the SU and SMITH teams. The SU team will by Dr. L. Henry, Department of Physics. As both teams are located in Baton Rouge, LA, collaboration will occur on a frequent basis.

1.7 Science Background

SMITH 2011 Stratospheric Sampling

During HASP 2011 SMITH monitored environmental parameters during flight. Sunrise was at 06:45 CDT on September 8th 2011 and launch was initiated at 09:08. Float was reached at 11:14 CDT on September 8th 2011 and sunset was 19:19. The average altitude at float was 117,000 feet (35.700 meters). The average pressure at float was one kilopascal and the average relative humidity at float was two percent. Cut down was initiated at 00:44 CDT on September 9th 2011.

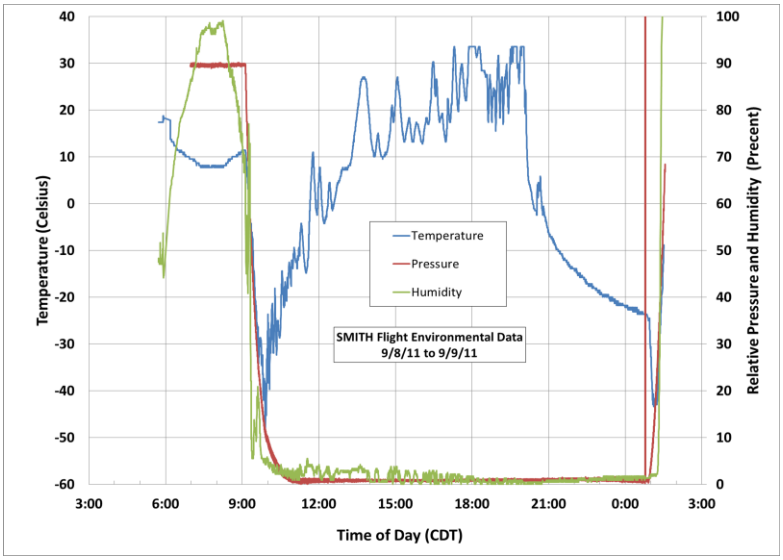


Figure 1: Environmental parameters recorded by SMITH during HASP 2011 flight.

Although research focused on the detection of a target organism in the atmosphere is increasing, there is a large knowledge gap regarding the vertical distribution of microorganisms. As seen in Table 1, sampling missions have isolated organisms in the stratosphere as early as 1936 (5). More recent studies have isolated organisms, but fail to provide data demonstrating the effectiveness of their decontamination protocol (5, 6, 7). Fulton's aircraft sampling in 1966 was the last focused effort to determine the number of colomy forming units (CFUs) at increasing altitudes. The numbers of CFUs ranged from ~500 CFU m^{-3} at 700 meters to <10 CFU m^{-3} at 3 km. By measuring only CFUs, Fulton's work may have resulted in an underestimation of the total concentration of cells by a factor of two to five. Since then there have been several missions that have cultured organisms after sampling the stratosphere, but little has been done to detail the sterilization protocols and controls.

Year	Mission	Altitude (km)	Microorganisms	Source
1936	USA, Balloon	11-21	5 *Bacillus* sp.	Rogers and Meirer, 1936
1975	Russia, Meteorological Rocket	48-77	*Mycobacterium* sp. & *Micrococcus* sp.	Imshenetsky, 1976
2001	India, Balloon	41	5 *Bacillus* sp., *Staphylococcus* sp.	Wainwright, et al., 2003, & Suresh et al., 2004
2003	USA, Aircraft	20	2 *Bacillus* sp.	Griffin, 2005
2004	USA, Aircraft	20	*Micrococci*, *Microbacteria*, *Staphylococcus* sp., *Brevibacterium* sp.	Griffin, 2008
2008	USA, Aircraft	20	*Bacillus* sp.	Smith et al., 2010

Table 1:Stratospheric Sampling Missions.

2.0 PAYLOAD DESIGN

2.1 Principle of Design

The main purpose of this payload is to take biological samples at a range of altitudes in the mid stratosphere. Figure 2 shows a high level system diagram of the payload designed to operate at the HASP float altitude. Since HASP will float at approximately 36 km, this will be the targeted altitude for this flight. The payload will consist of a sample chamber and a flight control chamber. The flight control chamber measures the background contamination throughout the flight without actively sampling. The sample chamber actively pulls air across a filter at the target altitude. Both chambers will be physically identical, but the flight control chamber will remained sealed. In the event the sample chamber fails to operate during flight, it will be possible to use the flight control chamber as the sample chamber. Since we would lose our flight control, this will be done only as a last resort.

The electronics includes all of the monitoring and controlling of the payload and collecting the environmental data. This is detailed in the electronics high level system diagram in Figure 3. The pump systems will be monitored through temperature and rotation per second (RPS) sensors. The pump systems and heaters will be controlled through the power distribution system commanded through HASP.

Before flight, both chambers will be sterilized and sealed. 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 solenoid valves sealing the filter chamber followed by a command to initiate the pump for air sampling. Confirmation of successful solenoid valve opening will be downlinked to the ground station, at which point pump speed will be monitored. Data from all sensors, including temperatures and speeds of systems and environmental data sensors will be relayed to the ground station throughout the flight. The pumping system will have radio controlled power throttling allowing for change in power consumption and heat production. The pumping system temperature will be closely monitored and if it falls below operational range, the engine will be turned off. Should this occur, ground control can command heaters to turn on, resulting in heating of the pumping system until it is again in operational temperature range.

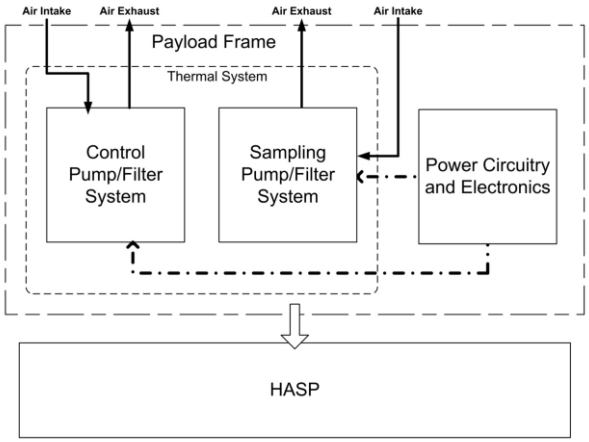


Figure 2: High level system diagram

Before cut down, a command to stop sampling will be uplinked to the payload. This will turn off the pumping system and close the solenoid valves, completely sealing the filter chamber. Once confirmation of a successful filter chamber closing is received, the payload will then remain in this state until it is returned to the lab for processing. If for some reason the sampling system fails, commands can be relayed to the control system that follow the same procedure as stated above. The sampling device requires a minimum of 40 m^3 of atmosphere to pass through the filter. The pumping system will be specified to produce the flow rate required to obtain this sample size in a minimum of 12 hours. The flow rate of this system is determined by the pump's displacement volume per crankshaft revolution multiplied by the crankshaft's revolutions per minute (RPM).

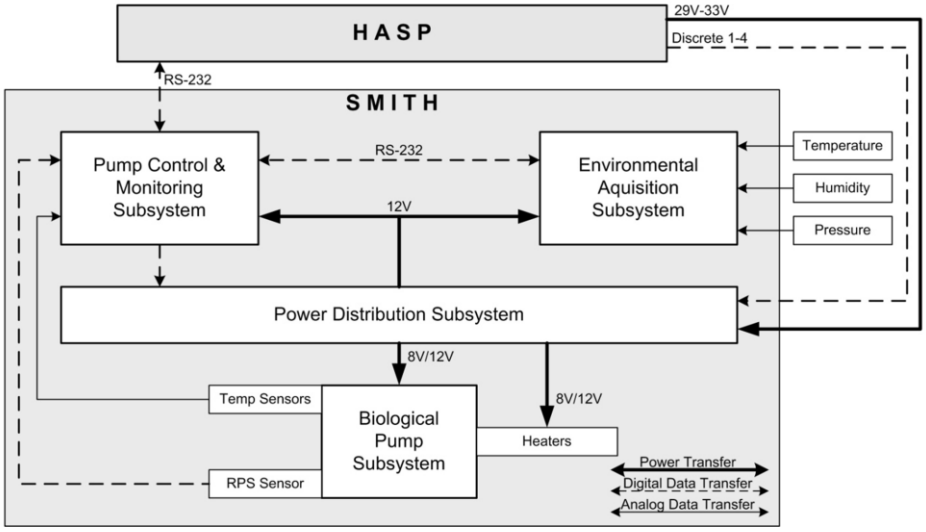


Figure 3: Electronics High Level System Diagram

3.0 SUBSYSTEM ITEGRATION

3.1 Mechanical Interface

Figure 4 shows the overall concept for the payload. The main structural frame (A) will house and protect the filter chambers used to collect microorganisms and also protect the pumping system used to pull air through the filters during sampling. These components make up the sampling and control systems. The sampling system is the system designated to be used to collect the sample of microorganisms in the stratosphere. The control system will be built as a fully functional system identical to the sampling system but designated to only be used should the sampling system malfunction.

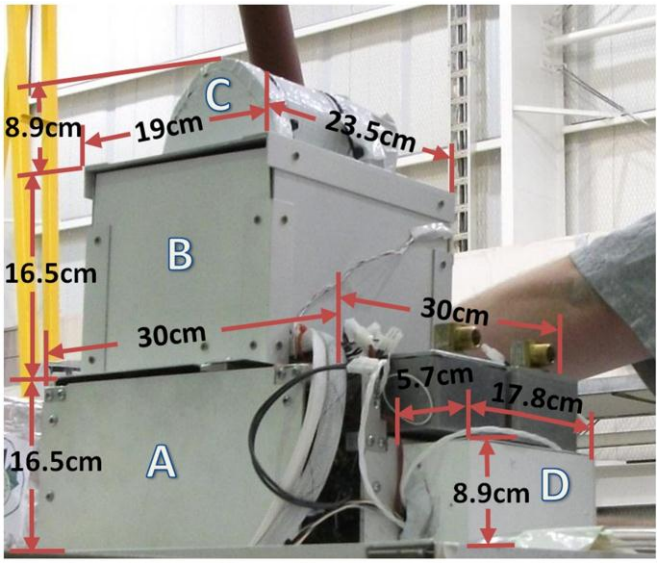


Figure 4: Payload Concept

The overall payload body will most likely include a 16.5cm x 30cm x 30cm main structural frames (A) at the bottom, 16.5cm x 19cm x 23.5cm electronics box (B) in the middle, and a 8.9cm in diameter starting capacitor (C) on the top as seen in Figure 4. Also on the bottom right may again be a 8.9cm x 5.7cm x 17.8cm relay box (D). The main purpose of the payload body is to protect the payload from debris and shield components from sunlight during the flight. The electronics box will house two electronic stacks, the payload power circuitry, and absorbed radiation dosimeters. The two stacks will be mounted onto the electronics box floor via eight stand offs and eight nuts. The 0.5 farad starting capacitor (used for initial pump startup) will mount using plastic semicircle holsters that came with the capacitor and four 8/32 screws. The capacitor may be covered in white duct-tape to protect the capacitor and plastic holsters from being damaged by the sun's rays. The relay box will mount directly to the PVC HASP interface plate by 8/32 screws and was constructed using rivets. The main structural frame (Figure 5) will be mounted directly onto the large PVC HASP interface plate using four ¼" screws.

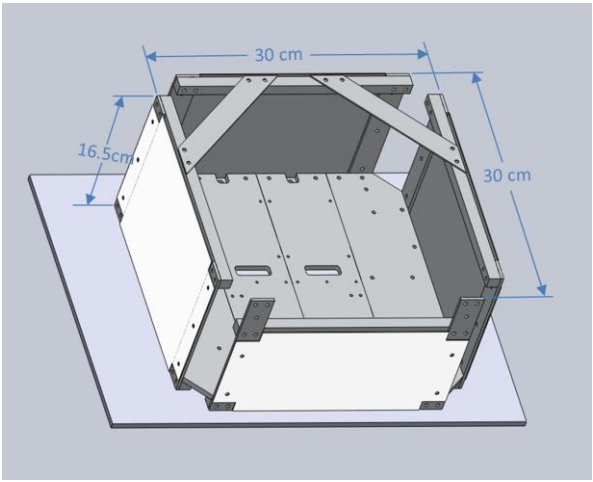


Figure 5: Main Structural Frame

The purpose of the pump/filter system is to collect a sample of microorganisms by pulling a volume of air though the filter. In Figure 6, the D/C motor will receive electrical power supplied by the SMITH power circuitry. This will drive the D/C motor crankshaft causing the pump to function and pull air through its air intake. The pumps air intake will be connected to the end of the filter assembly, causing air to be pulled through the filter assembly. The filtered air is then exhausted to the atmosphere via the pump exhaust. For this experiment the pump selected for this experiment will need a twofold minimum increase in displacement volume compared to the pump selected last year and needs to create more air flow per amp-hour consumed. Also the new pump should be able to operate in a larger range of temperatures allowing for more time of sampling. A positive displacement pump will most likely be selected again for this experiment because it does not require the large head pressure to be effective.

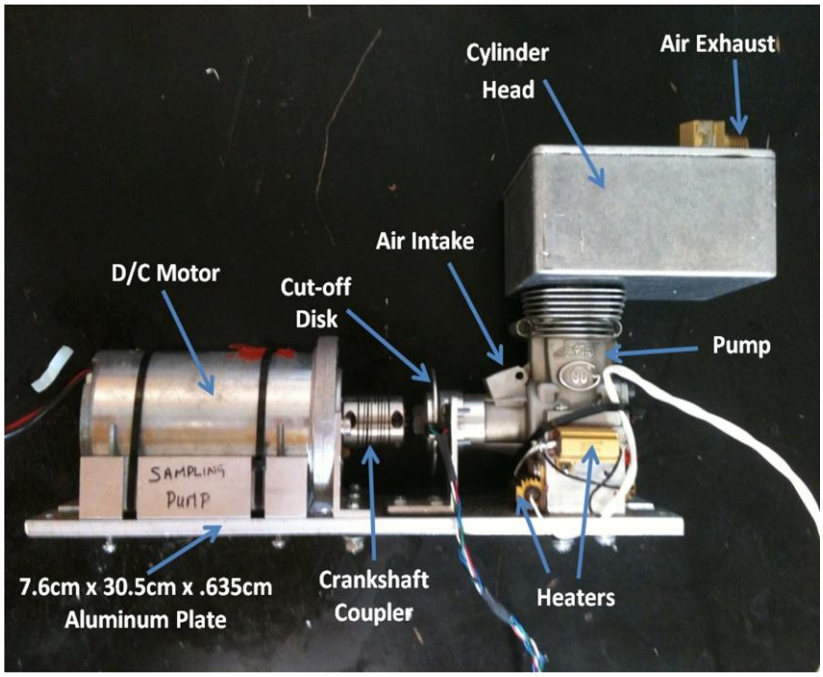


Figure 6: Pump System

Table 3 lists several key components and their estimated weights. We predict the pump system will be the heaviest component of our payload. All the components estimate to be 41lbs. which is below the allocated 44 lbs.

Table 2: Estimated Weight Budget

Item	Weight (lbs.)
Pump System	30
Main Structural Frame	2
Sampling Chamber (x2)	5
Electronics (sensors & circuitry)	2
Electronics Housing	2
Total	41

3.3 Power Interface

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. SMITH must power two motors (one at a time), four solenoid valves (two at a time), and all circuit boards. To successfully power these components,

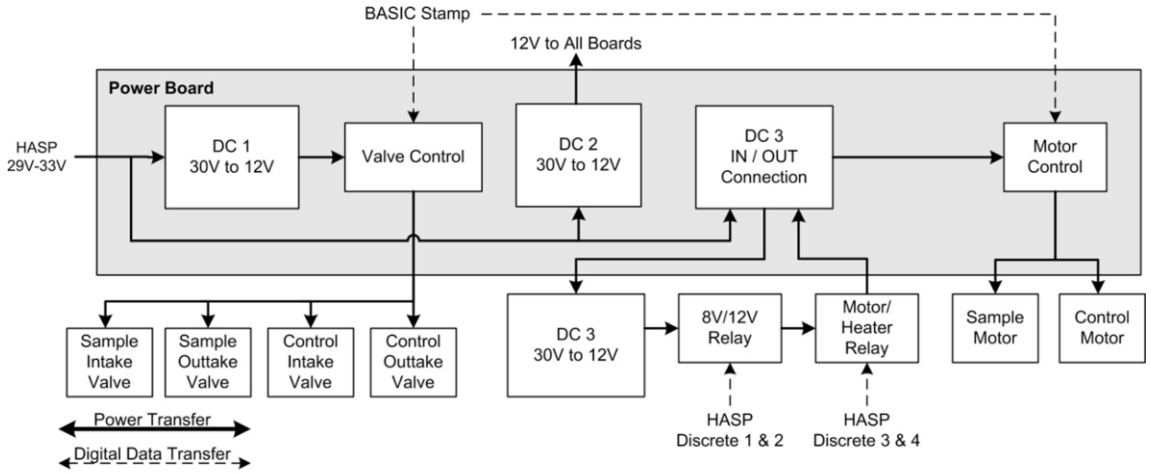


Figure 7: Power System

three 30 V to 12 V DC/DC converters will be used to reduce the current draw. As shown in Figure 7, the boards will always be powered and the motors and valves are controlled through the BASICStamp and the discrete lines from HASP.

A twenty pin EDAC 516 connecter, shown in Figure 8, will be used to interface with HASP power supply. 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 X. Pins F and N will be used to control the relay to alternate between 8 V and 12 V to the motors and heaters. Pins H and P will be connected to an additional relay that will alternate between allowing either the motors or the heaters to be powered on.

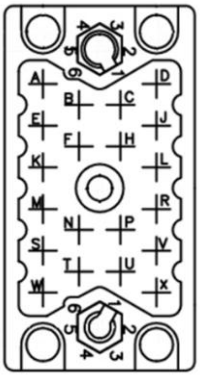


Figure 8: EDAC Connector

Function	EDAC Pins	Wire Color	Purpose
+30 VDC	A, B, C, D	White with red stripe	Power SMITH
Power Ground	W, T, U, X	White with black stripe	GND SMITH
Discrete 1	F	Brown	DC Converter 3 Outputs 8V
Discrete 2	N	Green	DC Converter 3 Outputs 12V
Discrete 3	H	Red with white stripe	Allows the Motors to be turned on
Discrete 4	P	Black with white stripe	Allows Heaters to be turned on

Table 3: EDAC Pin Layout

Pins A-D, will be used in parallel to provide the appropriate power supply of 2.5 Amps at 30 VDC to the payload. SMITH requires three DC/DC converters to step down voltages from 30 VDC to 12 VDC. Besides the motors, heaters, and valves, all the other components will be powered throughout the duration of the flight. Table 4 shows the 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 draw if all components were active at the same time (with the exception of the control pump system). The duty cycles were calculated using the results from the previous flight: $total\ run\ time\ /\ total\ flight\ time$. The power consumed is determined as follows: $I * duty\ cycle * total\ flight\ time$. For Table 4 the total flight time used in the calculations was 16 hours, based on HASP 2011 data.

Component	Voltage (V)	Current (mA)	Duty Cycle Over Entire Flight (%)	Power (W)	Power Consumed (Amp hours)
Sample/Control Pump	8	2200	54	17.6	19
Sample/Control Pump	12	2400	26	28.8	10.0
Heaters	12	1500	5	18	1.2
Intake Valve	12	190	80	2.28	2.4
Outtake Valve	12	190	80	2.28	2.4
BalloonSat 1	12	55	100	0.66	0.9
Communication Board 1	12	20	100	0.24	0.3
Temperature Board	12	48	100	0.576	0.8
BalloonSat 2	12	55	100	0.66	0.9
Communication Board 2	12	20	100	0.24	0.3
Sensor Board	12	48	100	0.576	0.8
			Total	**71.912**	**39.0**

Table 4: Power Budget

The DC/DC converters we will be using 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 listed in Table 5. These efficiencies are already taken into account in Table 4.

Type of DC/DC Converter	Purpose	Efficiency (%)
Converter 1: 30V to 12V	Solenoid Valves	95
Converter 2: 30V to 12V	Boards	95
Converter 3: 30V to 12V	Motors & Heaters	86

Table 5: Converter Efficiency

4.0 DATA INTERFACE

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 aboard SMITH. Eight bit ADC channels will suffice for our environmental sensors. Six I/O pins will be required to interface with the sample and control pumps and the sample and control intake and exhaust valves. Table 6 shows the format of the record that we will store and downlink 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 pumps and the sample and control intake and exhaust valves. 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.

Record Format	Byte size
Real Time Clock – Date	1
Real Time Clock – Hour	1
Real Time Clock – Minute	1
Real Time Clock – Second	1
Sample pump temperature sensor	1
Sample motor temperature sensor	1
Sample rotation sensor	1
Control pump temperature sensor	1
Control motor temperature sensor	1
Control rotation sensor	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
Pump Control Error Response	1
Environmental Error Response	1
Total:	**20**

Table 6: Record Format

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 and can be seen in Figure 9. Only the transmitted data, received data, and signal ground lines will be used. SMITH will downlink a data record when the time since the last data record sent has surpassed the telemetry period. The telemetry period will

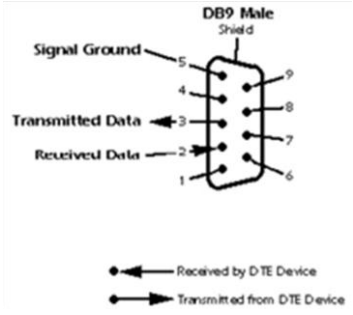


Figure 9: DB9 Pins used for the HASP serial interface

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 SMITH team can then mitigate any problem if they occur. A current estimate is 15 bits per second (bps) for SMITH.

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 is the current list of commands we can uplink.

The sampling process starts once the commands to turn on the pump and open the intake and exhaust valves are issued. To stop the sampling process, a command must be sent to turn off the pump and close the intake and exhaust valves. Additional commands can be used to manually control key SMITH functions. These functions include commands like changing the telemetry period. The flight control chamber has the ability to receive the same sets of commands as the sampling chamber. This is in case of a failure in the primary sampling chamber. As the design progresses, additional commands will be added if necessary.

Commands	
Sample Pump:	
• Turn on pump	
• Turn off pump	
• Open intake valve	
• Close intake valve	
• Open exhaust valve	
• Close exhaust valve	
• Turn on pump and open intake and exhaust valves	
• Turn off pump and close intake and exhaust valves	
Control Pump:	
• Turn on pump	
• Turn off pump	
• Open intake valve	
• Close intake valve	
• Open exhaust valve	
• Close exhaust valve	
• Turn on pump and open intake and exhaust valves	
• Turn off pump and close intake and exhaust valves	
• Change Telemetry Period	
Total Commands:	**9**

Table 7: Total Commands

5.0 Microbiological Analysis

5.1 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 (2). 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 then soaked overnight in sodium hypochlorite (500 PPM) 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 (3). EO is effective for its bactericidal properties as well as its ability to inactivate spores (1). The SMITH payload for HASP 2011 was 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 sample for 5 min, and one will remain sealed after sterilization to serve as a procedural control. This approach allowed us to assess the level of background contamination present in the units during HASP 2011, and is critical for critically evaluating results from filters that sampled stratospheric air during flight.

Figure 10: Filter chamber assembly includes the sample intake solenoid valve (SI), the valve handle, the filter cartridge, and the sample outtake valve (SO).

Figure 10 shows a portion of the filtration sampler from HASP 2011, which was comprised of 2 solenoid valves, a polypropylene filter housing (Sterlitech Corp., SKU 501200) with a 47 mm 0.2 µm polycarbonate track etch (PCTE) filter (Sterlitech Corp., SKU PCT0247100), and all the plumbing was hermetically sealed. The entire assembly was sterilized with the solenoid valves closed, and EO penetrated the system via an open ball valve, which was opened before EO treatment and subsequently closed without removing the system from the sterilization pouch. While the overall design will remain the same, a few minor improvements based on experience with the payload on HASP 2011. We intend to replace the plastic filter housing with a more durable stainless steel or aluminum housing and add a pressure gauge monitor the seal of the chambers post flight.

5.2 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 result's and conduct a variety of assays to assess if viable cells are present in the samples. The payload will be disassembled under class 100 conditions, the chambers will be opened, and the filters will be transferred to 10 mL of 0.22 µm-filtered autoclaved phosphate buffered saline solution (PBS).

5.3 Cell Concentration Measurements

A primary goal of this mission will be to determine if there is a concentration of airborne cells in air at 36 km > 5 cells m^{-3} , the level of detection for our most sensitive measure of microbial concentration. Based on a pump rate of 60 L min^{-1} , and the background contamination observed during HASP 2011, it should be possible to detect microbes in air containing 10 cells m^{-3} after filtering for at least 6 hours.

To do this, two microscopic methods will be used to directly enumerate the number of DNA-containing cells in the samples after their collection from the aqueous suspension via filtration on a 5 mm filter spot. One of the draw backs to these methods is the inability to stain endospores that may be present in the samples. Malachite green is a stain that will distinguish endospores from vegetative cells and will be added to our list of procedures. In addition, inorganic particles may autofluoresce and must be distinguished from cells.

Cells will be stained with SYBR Gold (Molecular Probes, Inc., cat. no. S-11494) and visualized at 1000X using an Olympus bx51epiflourescence microscope. For each sample, 1 ml of the resuspended particles will be filtered onto a 5 mm spot of a 0.22 µm black polycarbonate membrane (GE Osmonics, cat. no. K02BP03500) and stained for twenty minutes in the dark with 25X SYBR gold. Blanks of the water and PBS solutions will be filtered in parallel to determine the background contamination in the solutions that may contact the sample. For each sample, 60 fields of view will be counted. The area of each field of view is ~28 x 10^5 μm^2. The number of cells per sample will be estimated by dividing the total filter area of the filter spot by the area counted and scaling accordingly. The number of cells sampled will be used to estimate the total number of cells per volume of air.

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 (2). This assay allows for the estimation of the number of potentially viable cells (with intact membranes) to be compared to the number of cells not likely to be viable. Blanks of water and the PBS solutions will be filtered in parallel and counted. 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.

Adenosine triphosphate (ATP) persists only in viable cells and can be used for as a proxy for microbial biomass. Biomass calculations can then be directly compared to the concentration of cells estimated from microscopic analysis. The commercially available ATP Biomass Kit HS (BioThema, cat. no. 266-311) reports limits of detection in the range of 50 cells ml^{-1}. Laboratory experiments will determine the lowest limit of detection of biomass from airborne samples. The current limit of detection for SMITH 2011 analysis was 10^3 cells ml^{-1}. An increased sample size would also aid in the future attempts of ATP analysis.

For HASP 2011, culturing attempts utilized two different growth media: 1% R2A, (0.01 g L^{-1} carbon content) and 1% nutrient broth (0.05 g L^{-1}). Although both media have been widely documented in the recovery of airborne microorganisms, neither was successful. This may be due to the low number of cells recovered. However, it also indicates our payload and sample processing are introducing negligible contamination. In order to improve upon our culturing methods, laboratory experiments will be conducted to determine the growth conditions most favorable to desiccated and UV irradiated cells.

6.0 TESTING AND INTEGRATION PROCEDURES

6.1 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

6.2 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 10g vertical and 5g horizontal impact analysis
- Connect HASP power and serial connectors
- Run flight software
- Troubleshoot for any faults

7.0 TEAM ORGANIZATION

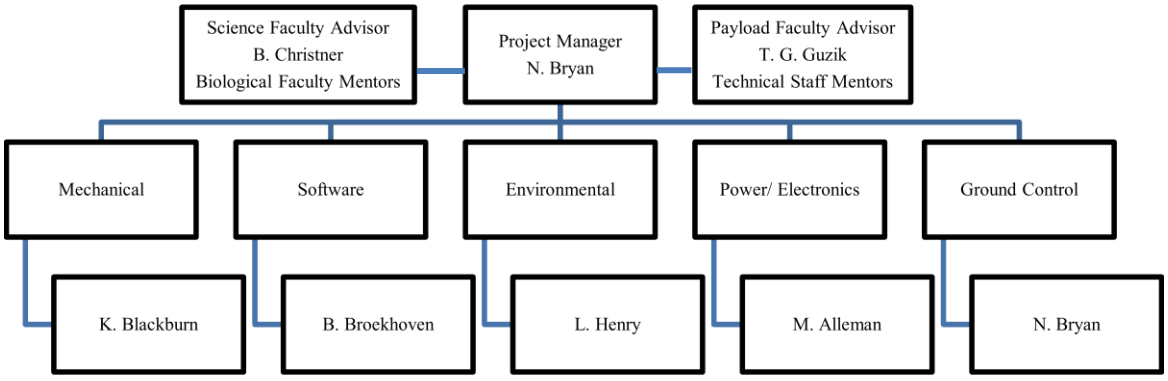
7.1 Management

The SMITH project for HASP 2012 will be developed and operated under the support of the NASA EPSCoR MARSLIFE project at Louisiana State University (LSU). The SMITH project is led by project manager Noelle Bryan. The faculty advisors for the payload are B. Christner (Science Advisor) and T. G. Guzik (Payload Advisor). B. Ellison, D. Granger, D. Smith, and M. Stewart will serve as technical staff mentors. Biological Sciences faculty members will also be available for consultation.

Several of the students involved in the SMITH 2011 project will be returning for a second year including S. Burke, M. Alleman, and B. Broekhoven. After completing the SMITH 2011 project, students gained first-hand experience with project management, life-cycle, experiment construction, data collection, analysis, and interpretation. This skill set will be further developed on the SMITH 2012 project. The data obtained on the cell concentrations in the stratosphere will be a major component of N. Bryan's graduate thesis. We look forward to further collaboration with SU and the opportunity to incorporate additional students, such as K. Blackburn and A. Spring (Figure 11).

7.2 Project Organization

Figure 11: Organizational Chart



7.3 Preliminary Schedule

ID	WBS	Task Name	Duration	Start	Finish
1	**1**	**SMITH Payload**	**337 days**	**Thu 9/1/11**	**Fri 12/14/12**
2	**1.1**	**Preliminary Design**	**327 days**	**Thu 9/1/11**	**Fri 11/30/12**
3	1.1.1	Mission Definiton Review	18 days	Thu 9/1/11	Mon 9/26/11
4	1.1.2	System Requirement Review	25 days	Tue 9/27/11	Mon 10/31/11
5	**1.1.3**	**Definition of Preliminary Design**	**5 days**	**Thu 12/15/11**	**Wed 12/21/11**
6	1.1.3.1	Trade Study of Pumps	5 days	Thu 12/15/11	Wed 12/21/11
7	**1.1.4**	**Final Design**	**66 days**	**Tue 11/1/11**	**Tue 1/31/12**
8	1.1.4.1	HASP Proposal	1 day	Mon 12/19/11	Mon 12/19/11
9	1.1.4.2	Selection Announcement	1 day	Mon 1/16/12	Mon 1/16/12
10	**1.1.4.3**	**Electronic Design**	**44 days**	**Tue 11/1/11**	**Fri 12/30/11**
11	1.1.4.3.1	Environmental Sensors	5 days	Mon 12/26/11	Fri 12/30/11
12	1.1.4.3.2	Pump sensors	39 days	Tue 11/1/11	Fri 12/23/11
13	**1.1.4.4**	**Software Design**	**37 days**	**Mon 12/12/11**	**Tue 1/31/12**
14	1.1.4.4.1	Data Storage	5 days	Mon 12/12/11	Fri 12/16/11
15	1.1.4.4.2	Downlink	5 days	Mon 12/12/11	Fri 12/16/11
16	1.1.4.4.3	Uplink	5 days	Mon 12/12/11	Fri 12/16/11
17	1.1.4.4.4	Write Preliminary Code	32 days	Mon 12/19/11	Tue 1/31/12
18	**1.1.4.5**	**Mechanical Design**	**21 days**	**Mon 1/2/12**	**Mon 1/30/12**
19	1.1.4.5.1	Pump system	21 days	Mon 1/2/12	Mon 1/30/12
20	1.1.4.5.2	Filter System	5 days	Mon 1/16/12	Fri 1/20/12
21	1.1.4.5.3	Mounting	5 days	Mon 1/16/12	Fri 1/20/12
22	1.1.4.5.4	Electronics Box	5 days	Mon 1/23/12	Fri 1/27/12
23	1.1.5	Status Report Due End of Months	219 days	Tue 1/31/12	Fri 11/30/12
24	**1.2**	**Subsystem Intergration Phase**	**55 days**	**Mon 1/2/12**	**Fri 3/16/12**
25	1.2.1	Order Materials	21 days	Mon 1/2/12	Mon 1/30/12
26	1.2.2	Integrate Temperature Sensors	5 days	Tue 1/31/12	Mon 2/6/12
27	1.2.3	Calibrate Temperature Sensors	5 days	Tue 2/7/12	Mon 2/13/12
28	1.2.4	Build Mechanical Housing	20 days	Tue 2/14/12	Mon 3/12/12
29	1.2.5	Status Report Due	2 days	Thu 3/15/12	Fri 3/16/12
30	**1.3**	**Preliminary PSIP Document**	**70 days**	**Mon 3/19/12**	**Fri 6/22/12**
31	1.3.1	Exact Power and Weight Budget	3 days	Mon 3/19/12	Wed 3/21/12
32	1.3.2	Determine Exact Downlink Data Format	3 days	Wed 3/21/12	Fri 3/23/12
33	1.3.3	List Uplink Commands	2 days	Mon 3/26/12	Tue 3/27/12
34	1.3.4	Discreet Command and Analog Output Usage	2 days	Tue 3/27/12	Wed 3/28/12
35	1.3.5	Write Rough Draft	7 days	Thu 3/29/12	Fri 4/6/12
36	1.3.6	Edit Rough Draft	9 days	Mon 4/9/12	Thu 4/19/12
37	1.3.7	Preliminary Version Due Date	1 day	Fri 4/20/12	Fri 4/20/12
38	1.3.8	Edit Preliminary Version	19 days	Mon 5/28/12	Thu 6/21/12
39	1.3.9	Final Document Due	1 day	Fri 6/22/12	Fri 6/22/12
40	1.4	Preliminary HASP Thermal / Vacuum Testing	5 days	Mon 5/21/12	Fri 5/25/12
41	1.5	Final Testing and Payload Adjustments	20 days	Mon 7/2/12	Fri 7/27/12
42	**1.6**	**FLOP**	**42 days**	**Thu 5/31/12**	**Fri 7/27/12**
43	1.6.1	Write Detailed Timeline for Launch Day	3 days	Thu 5/31/12	Mon 6/4/12
44	1.6.2	Write Detailed Procedures for Flight Line Setup	5 days	Tue 6/5/12	Mon 6/11/12
45	1.6.3	Write Rough Draft	14 days	Tue 6/12/12	Fri 6/29/12
46	1.6.4	Edit Rough Draft	19 days	Mon 7/2/12	Thu 7/26/12
47	1.6.5	Final Document Due	1 day	Fri 7/27/12	Fri 7/27/12
48	**1.7**	**Student Payload Integration at CSBF**	**5 days**	**Mon 7/30/12**	**Fri 8/3/12**
49	1.7.1	Mount Payload to HASP Platform	1 day	Tue 7/31/12	Tue 7/31/12
50	1.7.2	Test and Debug Flight Software	1 day	Mon 7/30/12	Mon 7/30/12
51	1.7.3	Perform Thermal Test	1 day	Wed 8/1/12	Wed 8/1/12
52	1.7.4	Perform Vacuum Test	1 day	Thu 8/2/12	Thu 8/2/12
53	1.7.5	Perform Shock Test	1 day	Fri 8/3/12	Fri 8/3/12
54	**1.8**	**HASP Flight Preparation**	**19 days**	**Mon 8/6/12**	**Thu 8/30/12**
55	1.8.1	Fixing Problems Encountered at Integration	19 days	Mon 8/6/12	Thu 8/30/12
56	1.9	Target Flight Ready	1 day	Fri 8/31/12	Fri 8/31/12
57	1.10	Target Launch Data and Flight Operations	1 day	Mon 9/3/12	Mon 9/3/12
58	**1.11**	**Flight/Science Report**	**73 days**	**Wed 9/5/12**	**Fri 12/14/12**
59	1.11.1	Analysis of Data	22 days	Wed 9/5/12	Thu 10/4/12
60	1.11.2	Write Rough Draft	23 days	Mon 10/8/12	Wed 11/7/12
61	1.11.3	Edit Rough Draft	26 days	Thu 11/8/12	Thu 12/13/12
62	1.11.4	Final Draft	1 day	Fri 12/14/12	Fri 12/14/12

Figure 12: Anticipated Project Schedule

8.0 REFERNCES

1. Bragt, J. et al. (1971) Effects of sterilization on components in nutrient media. Wageningen, Veeman & Zonen Publishing.
2. Bulous, L. et al. (1999) BacLight: application of a new rapid staining for direct enumeration of total and viable bacteria in drinking water. Journal of Microbial Methods, 37:77-86.
3. Cooper, D. W. (1988) Rationale for proposed revisions to the federal standard 209B cleanrooms. Journal of Environmental Sciences, 31.
4. Mendes, G. C. C. et al. (2007) Ethlyene oxide sterilization of medical devices: a review. American Journal of Infection Control, 35: 574-81.
5. Rogers, L.A. and F. C. Meir. (1936) The collection of microorganisms above 36,000 feet. National Geographic Society Stratosphere Series, 2: 146.
6. Wainwright, M. et al. (2003) Microorganisms cultured from stratospheric air samples obtained at 41 km. Federation of European Microbiological Societies Microbiology Letters, 218: 161-165.
7. Yang, Y. et al. (2008) UV- resistant bacteria isolated from the upper troposphere and lower stratosphere. Biological Sciences in Space, 1: 18-25.