HADES Science Report

HASP Flight 2013

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1.0 Mission Description

1.1 Mission Goal

The HADES team achieved the goal of designing and constructing a payload to sample for microbial aerosols from the HASP float altitude of ~36 km. The samples were returned uncontaminated to the lab facilities at Fort Sumner, NM. Analysis was performed to: detect viable cells (via the molecule adenosine triphosphate, ATP), determine the total number of cells collected, and isolate culturable microorganisms in the lab.

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, 12, 13). Residency times for cells smaller than 10 μm can range from months to years due to the laminar flow of stratospheric winds.

II. Are microorganisms collected at 36-38 km viable and can they be cultured using 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

Objective	Success	Failure
Obtain an aerosol sample from a target altitude in the stratosphere.	X	
Evaluate the amount of microbial contamination associated with assembly, flight, recovery, and analysis.	X	
Determine the concentration of microbial cells collected at 36-38 km.	X	
Determine the viability of microorganisms collected at 36-38 km.	X	
Determine the environmental conditions (i.e., temperature, humidity) from 1.5 to 36-38 km.	X	

The payload successfully sampled for microorganisms in the stratosphere and those samples were returned safely to the laboratory for analysis. The addition of the control chamber that was not exposed to the atmosphere allowed us to determine the amount of background contamination of the system. With a clearly

defined level of noise, we were able to successfully determine if there was a bona fide microbial sample from the stratosphere. The payload monitored internal temperatures and relative humidity (RH) during flight.

1.4 Science Requirements

Objective	Success	Failure
Minimize the amount of background contamination before flight with a rigorous decontamination protocol.	X	
Sample aerosols at target altitude for the duration of float.	X	
Simultaneous assessment of background contamination.	X	
Direct enumeration of cells with multiple analyses.	X	
Attempt to isolate microorganisms from aerosol samples collected at 36-38 km.	X	

Background contamination was minimized according to the protocol described in Section 4.1. By quantifying the background contamination we determined the lowest number of cells required for a 1σ signal (Section 4.2.1). The payload must sample continuously during float to collect the largest volume possible.

1.5 Technical Requirements

Objective	Success	Failure
Measure internal and payload temperatures (-70 to 40° C).	X	
Measure the RH from 0-100%.	X	
Collect aerosols from target altitude while rotating the chambers at ~1.0 rotation second^{-1}.	X	
Return the samples uncompromised to the laboratory for microbiological analysis.	X	
Know the status of the payload rotation during flight.	X	
Know the position of the chamber doors during flight.	X	

2.0 Payload Operation

2.1 Principle of Operation

Figure 1 shows a high-level system diagram of the payload. The main purpose of the payload was to collect aerosol samples at a range of altitudes in the mid-stratosphere. The payload carried two sampling chambers, each containing forty commercially available Rotorods® (3.50×10^{-5} m^2) coated with silicon grease. As the sample chambers rotated, each rod passed through a volume of air. Particles present in the atmosphere became embedded in the grease and were returned to the lab for analysis. A third chamber was flown but the doors of the chamber were never opened. The impactor rods that were not exposed to the atmosphere served as a measurement of the background contamination incurred during flight and recovery.

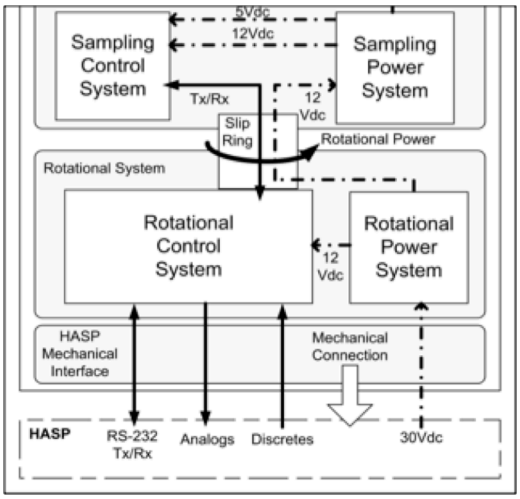


Figure 1: High-level system diagram of the HADES payload.

The electronics included all of the monitoring and controlling of the payload and collection of the environmental data. The motor and actuator systems were monitored through temperature, electrical current, and rotation per minute (RPM) sensors. The motor system and heaters were controlled through the power distribution system commanded through HASP. Before flight, all chambers were sterilized and sealed as detailed in Section 4.1.

2.2 Rotation During Flight

Prior to launch, the payload began rotating to avoid possible freezing due to low temperatures in the tropopause. At float, the rotation of the motor was stopped to allow for a visual confirmation of the open doors. Payload rotation resumed in approximately 40 seconds. Before cut down, a command to stop rotating was uplinked to the payload. The total number of rotations completed was recorded during flight (Figure 2). The payload rotated for a total of 10.8 hours, completed 5.6×10^4 rotations, and sampled 1.5-m^3 rod^{-1}.

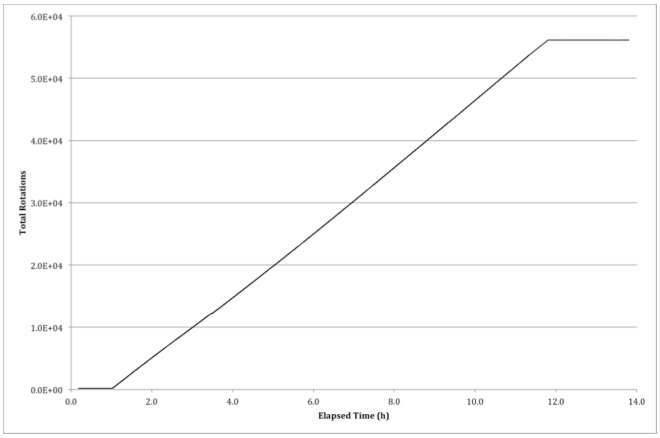


Figure 2: The total number of rotations completed during flight was monitored to ensure sample collection.

2.3 Door Position During Flight

The position of the linear actuators was downlinked in the form of ADC counts (Figure 3). This provided the ground team with near real time data on the location of each door, on each chamber, for the duration of flight. The starting positions of the doors were measured as ADC values >750 ADC. The doors were opened once the payload reached float altitude. The average altitude at float was 37 km (+/- 1.0 km). Confirmation of successful door opening (ADC values <100) was downlinked to the ground station and verified from the real time video on board HASP. Just before HASP lost video feed, the doors were closed to seal the chambers. The closed-door position was confirmed through downlinked data and live video feed. The payload functioned as planned for the duration of flight.

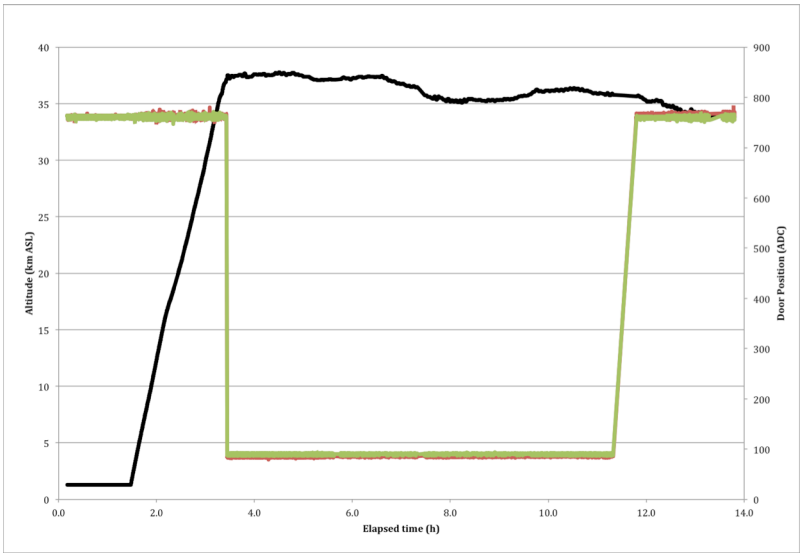


Figure 3: HADES recorded altitude during flight (black line). The position of the doors of each chamber was monitored during flight (colored lines).

2.4 Environmental Monitoring

2.4.1 Relative Humidity

The HADES payload monitored RH during flight (Figure 4). The lack of moisture in the stratosphere is a major environmental stressor for microorganisms. The average RH during float was 4.0% (+/- 7%). A desiccating environment for a microorganism is considered to be less than 30%.

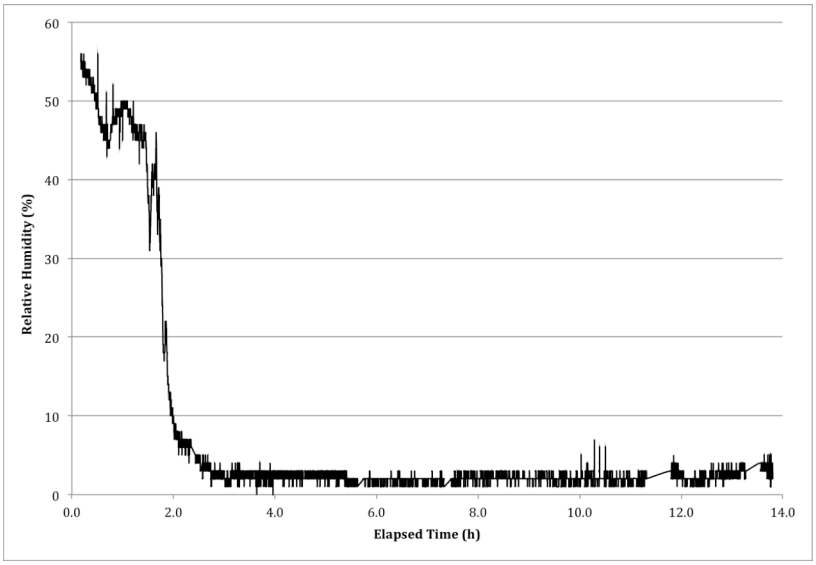


Figure 4: HADES recorded RH during flight.

2.4.2 Payload Component Temperatures

During the HASP Integration week, the HADES payload failed to operate the doors during the cold temperature challenge. The payload monitored the temperatures of the sampling chambers (Figure 5). The team added manually operated heaters to two sampling chambers in the event the chambers became cold during flight. Although chamber temperature plummeted to -51° C at the tropopause, the chambers maintained a safe temperature during float and the heaters were not employed.

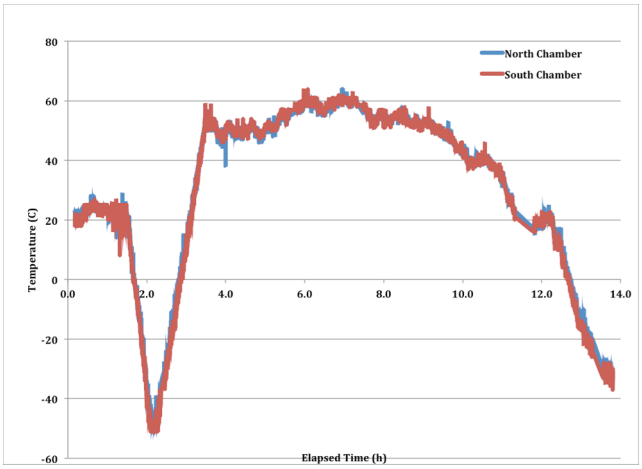


Figure 5: HADES monitored the temperatures of the sampling chambers during flight.

3.0 Summary of Subsystems

3.1 Mechanical Subsystem

The overall concept for the payload is shown in Figure 6. The overall payload body was 52.6 cm x 25.4 cm x 29.2 cm. The rotational system box housed the slip ring and protected the motor, electrical control, gearing, and power systems (Figure 6, A). The power and control electronics for the sampling system were housed in the sampling system box (Figure 6, B). The sampling system electronics box housed the power and control circuitry required to open and close the chamber doors using the linear actuators (Figure 6, C). The sample and control chambers were mounted on the sampling system box (Figure 6, D). The main purpose of the payload boxes was to protect the payload's internal components from debris and shield components from sunlight during the flight. The rotational system box was mounted directly onto the large PVC HASP interface plate using four ¼" screws.

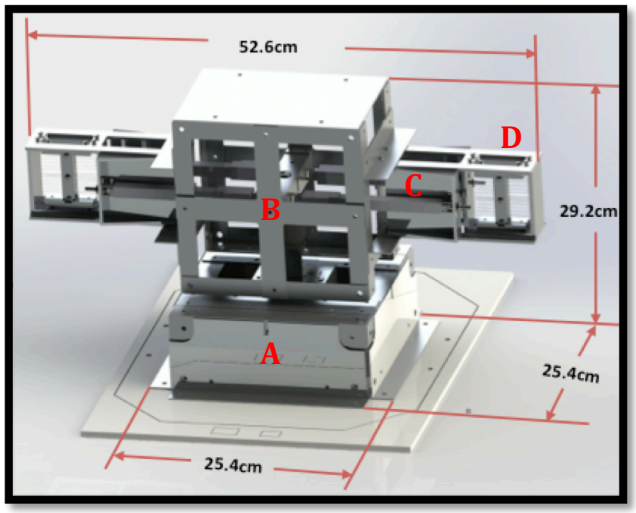


Figure 6: Dimensioned drawing of the HADES payload.

3.1.1 Weight Budget

Table 1: Hades Weight budget. W: west chamber, E: east chamber

Part Name	(oz)	(lbs)	(g)
W Top Door with clevis	2.8	0.175	79
W Bottom Door with clevis	2.8	0.175	79
W Chamber with O-rings and filter	3.8	0.238	108
W Rail Right	1.3	0.081	37
W Rail Left	1.3	0.081	37
W Holder Sleeve A.	0.6	0.038	17
W Holder Sleeve A.	0.6	0.038	17
W Holder Half 1 5Up	0.1	0.006	3
W Holder Half 2 5Up	0.2	0.013	6
W Holder Half 1 (Bottom)	0.1	0.006	3
W Holder Half 2 (Bottom)	0.2	0.013	6
W Spacer with 2 corner attachments and 6 screws	0.9	0.056	26
W Right Connecting Bracket (short)	0.6	0.038	17
W Left Connecting Bracket (short)	0.6	0.038	17
W Ground Plate with rubber	0.8	0.050	23
E Top Door with clevis	2.8	0.175	79
E Bottom Door with clevis	2.8	0.175	79
E Chamber with O-rings and filter	3.8	0.238	108
E Rail Right	1.3	0.081	37
E Rail Left	1.3	0.081	37
E Holder Sleeve B.	0.6	0.038	17
E Holder Sleeve B.	0.6	0.038	17
E Holder Half 1 7Up	0.1	0.006	3
E Holder Half 2 7Up	0.2	0.013	6
E Holder Half 1 (Bottom)	0.1	0.006	3
E Holder Half 2 (Bottom)	0.2	0.013	6
E Spacer with 2 corner attachments and 6 screws	1.0	0.063	28
E Right Connecting Bracket (short)	0.6	0.038	17
E Left Connecting Bracket (short)	0.6	0.038	17
Top X Brace	1.4	0.088	40
Bottom X Brace	1.4	0.088	40
Top Square with rivets	4.9	0.306	139
Bottom Square with Rivets	5.2	0.325	147
Bottom Motor Box	20	1.250	567
Lazy Susan	6	0.375	170
Slip Ring	2	0.125	57
HASP Mounting Angles	4	0.250	113
Cer Post with clevises and top and bottom Screws	3.6	0.225	102

Foam	2.6	0.163	74
(26) 8-32 Wing Nuts	2.9	0.181	82
(24) 1/4" 8-32 Screws	1.3	0.081	37
Estimate For (16) 8-32 3/5" screws and 16 8-32 locking nuts 16/24 * weight (24) 1/4" 8-32 Screws + (26) 8-32 wing nuts	2.8	0.175	79
160 Greased Glass Rods	1.49	0.093	42.4
8 Carabineers	1.1	0.069	31
8 AA Battery Pack	4.8	0.300	136
LiSO2 Battery Pack	2.7	0.169	77
8 Linear Actuators	15.4	0.963	437
H-Bridge with power connector	1.8	0.113	51
Antenna	0.7	0.044	20
Arduino and Shield	3.1	0.194	88
Electric D/C Motor	16	1.000	454
Total Weight:	138	8.6	3900

3.2 Power Subsystem

Figure 7 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. In the event that the motor gets below operational temperature (to be experimentally determined), the power system was capable of turning 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 were used to reduce the current draw. The motor, linear actuators, and heaters are regulated through the control systems and the discrete lines from HASP (Table 2). Due to complications calibrating the ultraviolet (UV) sensors, the UV monitoring system was not employed during the HADES 2013 flight.

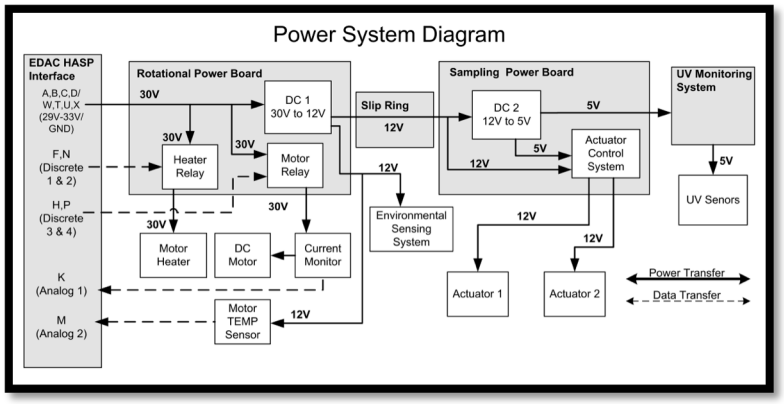


Figure 7: Power system diagram

A twenty-pin EDAC 516 connecter was used to interface with HASP power supply (TABLE 2). Pins A, B, C, and D were used for +30 VDC and W, T, U, and X were used to ground the power supply. Pins F and N were used to control the heater relay to turn on the heaters. Pins H and P were connected to an additional relay that turned on the motor to begin rotating the payload. Pins A-D, were used in parallel to provide the appropriate power supply of 2.5 Amps at 30 VDC to the payload.

Table 2: EDAC Pin Assignments

Function	EDAC Pins	Purpose
+30 VDC	A, B, C, D	Power payload
Power Ground	W, T, U, X	Ground payload
Discrete 1	F	Motor On
Discrete 2	N	Motor Off
Discrete 3	H	Motor Heater On
Discrete 4	P	Motor Heater Off
Analog 1	K	Motor Current Monitor
Analog 2	M	Motor Temperature Sensor

Table 3 reports the measured current draw of multiple HADES components.

Table 3: Measured current draw at 30 VDC

Component	Voltage (V)	Current (mA)	Duty Cycle Over Entire Flight (%)	Power (W)	Power Consumed (Amp hours)
Rotational DC Motor	24	1000	75	24	12
Linear Actuators (4)	12	840	0.1	10.8	0.45
Heaters (3)	12	1800	25	21.6	7.2
LAMB Shield	12	48	100	0.58	0.53
Arduino	12	55	100	0.70	0.37
GPS Shield	3.3	70	100	0.23	0.17
Total				57.91	20.72

The power system required two DC/DC converters to step down voltages from 30 VDC to 12 VDC and from 12 VDC to 5 VDC (Table 4). Besides the motor, heaters, and actuators, all the other components were powered throughout the duration of the flight.

Table 4: DC/DC Converters

Type of DC/DC Converter	Purpose	Part Number (Digikey)	Efficiency (%)
30V to 24V	Rotational DC Motor Power	811-1889-5-ND	89
30V to 15V	Microcontroller Power	102-2552-ND	87

3.3 Data Subsystem

3.3.1 Data Handling

Table 5 shows the format of the record that will be stored and downlinked during flight. During flight the GPS position and functionality of the payload were monitored. The team was able to monitor in real time: the rotations of the payload, the position of the doors, temperatures of key components, and RH of the atmosphere.

Table 5: Data Record Format	
<1>, <2>, <3>, <4>, <5>, <6>, <7>, <8>, <9>, <10>, <11>, <12>, <13>, <14>, <15>, <16>, <17>, <18>, <19>, <20>, <21>, <22>, <23>, <24>, <25>, <26>, <27>	
1	Type
2	Timer, milliseconds since boot
3	GPS time, hour minute second
4	GPS auto door control on/off
5	North/South doors open/closed
6	Thermostat on/off
7	North/South Heater on/off
8	RPM
9	Rotation count
10	GPS checksum
11	GPS fix
12	GPS satellites tracked
13	Latitude
14	Longitude
15	Altitude
16	North Top Actuator Position
17	North Bottom Actuator Position
18	South Top Actuator Position
19	South Bottom Actuator Position
20	North Top Actuator Temperature
21	North Bottom Actuator Temperature
22	South Top Actuator Temperature
23	South Bottom Actuator Temperature
24	Interior/Electronics Temperature
25	North Chamber Exterior Temperature
26	South Chamber Exterior Temperature
27	RH
1 = On 0 = Off	

3.3.2 Downlink Data Format

The data was transferred from HASP via a RS-232 serial connection with 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 downlinked a data record when the time since the last data record sent has surpassed the telemetry period. The telemetry period was set to ten seconds. All data records were comma delimited and sent to ground control in ASCII. Each data record ended with a carriage return and a line feed. This provided ground control with a near real time status of the payload.

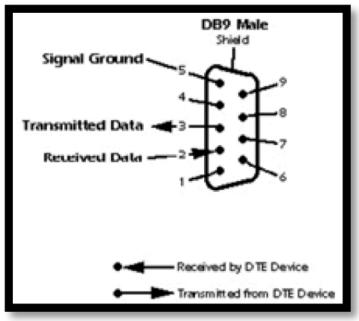


Figure 8: DB9 Pins for the HASP serial interface.

3.3.3 Uplink Command and Data Format

The serial connection to HASP provided the ability to uplink 2 byte commands. The first byte was used to specify which command is to be completed and the second byte was used as the argument for this command. Table 6 contains the list of commands used for uplink. To stop the sampling process, a command was sent to close the chambers and then to stop rotation. Additional commands were available to manually control key functions.

Table 6: Commands used for uplink

1st Byte Command	2nd Byte Argument	Description	Action
01	01	Manual North chamber door control	Door open
	00		Door close
02	01	Manual South door control	Door open
	00		Door close
03	01	Manual chamber door control	Door open
	00		Door close
04	01	Manual North actuator heater control	Heaters on
	00		Heaters off
05	01	Manual South actuator heater control	Heaters on
	00		Heaters off
06	00	Manual telemetry downlink	Send data
07	XX*5 (sec)	Change frequency of data record	Change record period
08	XX	Manual audible verification frequency	Force beep
09	01	Automatic heater control	Heaters on
	00		Heaters off
10	01	Automatic actuator control	Actuators on
	00		Actuators off

4.0 Microbiological Analysis

4.1 Pre-Flight Procedures

The payload decontaminating procedure employed a series of techniques to kill microbes and reduce cellular contamination. Instruments involved in preparation were heat sterilized at 120° C for 20 minutes. In addition, all surfaces were 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 were then rinsed with a 70% ethanol (v/v) solution to remove residual salts. The sampling rods were dipped into silicone grease and loaded into the sampling chambers (Figure 9). The chambers were placed in a gas-porous sterilization pouch and exposed to ethylene oxide (EO) at a concentration of 0.45-0.65 Mg meters^{-3} at 55° C and 30-50% RH for four hours resulting in a 12-log kill of *B. niger* spores (11). EO is effective for its bactericidal properties as well as its ability to inactivate spores (2).

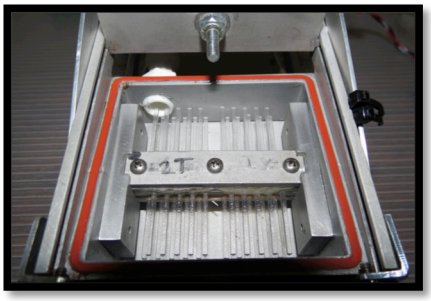


Figure 9: Each chamber contains forty rods coated with silicone grease for impact sampling in the stratosphere.

4.2 Post Flight Analysis

HASP was launched at 8:57 MDT on Monday, September 2, 2013. Float altitude was reached at 12:56 MDT and maintained for 10 hours and 29 minutes. At 21:25 MDT the HASP 2013 flight was terminated. The HASP impact occurred at 8:10 UTC on 9/3/13 at 33.96 N, 112.98 W. The microbiological samples were placed on ice by midday and were stored on ice during the return to the lab in Fort Sumner on Thursday, September 5, 2013.

4.2.1 Limits of Detections

Each rod has a sampling area of 3.5 x 10^{-5} m^2 and each chamber contains 40 rods. Both chambers sampled air at float altitudes. The minimum number of cells required to achieve a signal is presented in (Table 7).

Table 7: Minimum requirements for HADES to achieve the level of detection 1 σ above the background. † Assuming 0.1% culturability using standard enrichment media techniques. *Priority level 1 was performed upon return of the samples to the base. Priority level 2 was processed within the first week and priority level 3 was completed upon return to LSU.

Technique	1 σ Limit of Detection Rod^{-1}	Priority Level*
ATP	8.0 x 10^2 attomoles (8.0 x 10^{-16} moles) ATP	1
SYBR gold	5.0 x 10^2 DNA containing cells	3
Culturing†	1.0 x 10^3 viable cells	2

4.2.2 Total Cell Concentration

To determine the total number of cells present at float altitudes samples were enumerated using a DNA specific stain. This method will allow the quantification of live and dead cells, but is unable to penetrate the spore coat of bacterial endospores. The rods dedicated to microscopic analysis were stored at -20° C and transported back to LSU. Cells were stained with a 1:1 solution of antifade and SYBR Gold (Molecular Probes, Inc., cat. no. S-11494) and visualized at 1000X using an Olympus bx51epiflourescence microscope. Rods from the control chamber were counted in parallel to determine the background contamination in the sample. For each rod, 60 fields of view were counted. The area of each field of view is ~28 x 10^5 µm^2. The number of cells on a rod was estimated by dividing the total rod area of the sample by the area counted and scaling accordingly. The average number of DNA containing cells rod^{-1} from the samples was 1.4 x 10^3 with a Poisson standard deviation of 37. The controls carried a background concentration of 4.6 x 10^2 cells rod^{-1}, with a Poisson standard deviation of 21 (Figure 10). To calculate the total number of cells in a cubic meter of stratospheric air, the collection efficiency of the rods must be known. The collection efficiency of the rods is predicted to be dependent on the size of the particles in the atmosphere. Experiments are currently underway to determine the collection efficiency of the rods for particles 10-0.7 microns in diameter.

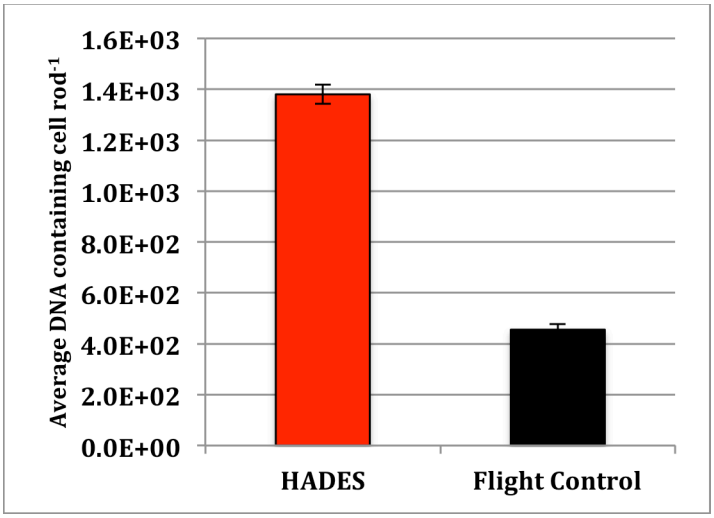


Figure 10: The total number of cells collected during float was quantified using epifluorescence microscopy. The samples were greater than 3 σ above the control rods (n=3).

4.2.3 Quantification of ATP

The measure of microbial ATP in is our most sensitive assay to estimate viable biomass recovered from atmospheric samples. The amount of light produced, measured in relative light units (RLUs), in the reaction was directly proportional to the concentration of ATP in the sample.

The samples were analyzed using ATP Biomass Kit HS (Biothema, Inc cat no. 266-112). Purified ATP (100 nmol L^{-1}) supplied by the manufacturer, was diluted into sterile filtered, autoclaved deionized water (DIW) to create a standard curve. Rods were removed from the chamber and placed into equal volumes of sterile filtered, autoclaved (DIW) and the ATP eliminating reagent provided by the manufacturer to eliminate extracellular ATP. The samples were incubated for 17 minutes at room temperature before extracting the intracellular ATP according to manufacturer's instructions. Over the course of the campaign, 92 flight control rods were measured to determine the amount of background ATP (Figure 11). The average concentration of ATP flight control rod^{-1} was 6.2 x 10^{2} attomoles (6.2 x 10^{-16} moles) ATP with a Poisson standard deviation of 25 attomoles (2.5 x 10^{-17} moles). The 60 HADES sample rods carried an average 2.0 x 10^{3} attomoles (2.0 x 10^{-15} moles) of microbial ATP with a Poisson standard deviation of 45 attomoles (4.5 x 10^{-17} moles). The HADES samples achieved greater than a 3σ signal above the controls indicating the presence of metabolically active microorganisms in the stratosphere (Figure 12).

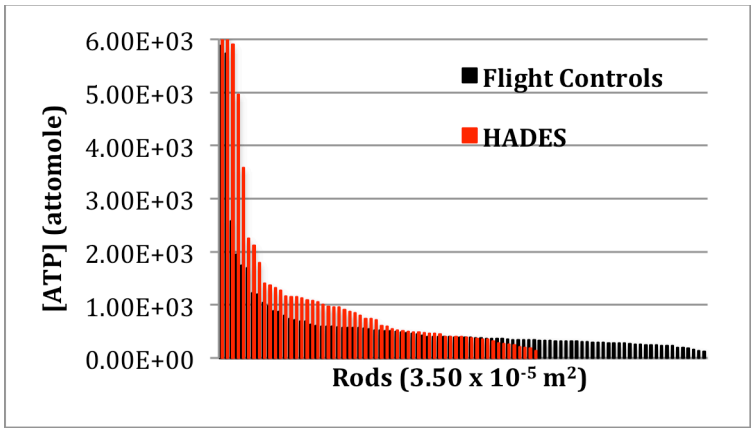


Figure 11: Concentration of ATP from individual rods (Controls, n=92, HADES n=60).

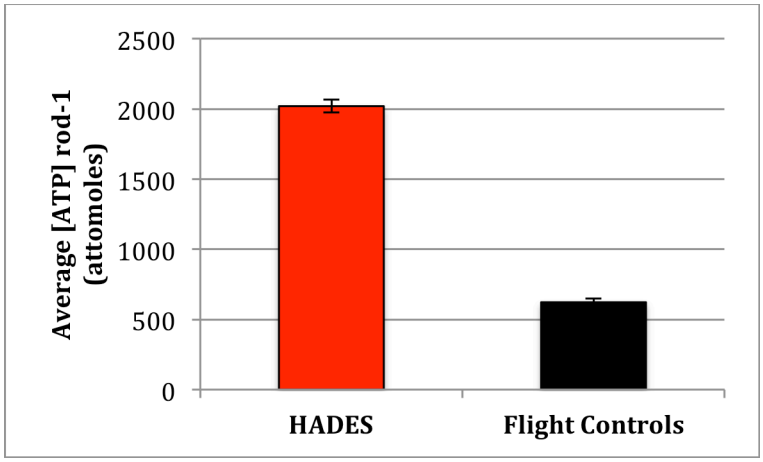


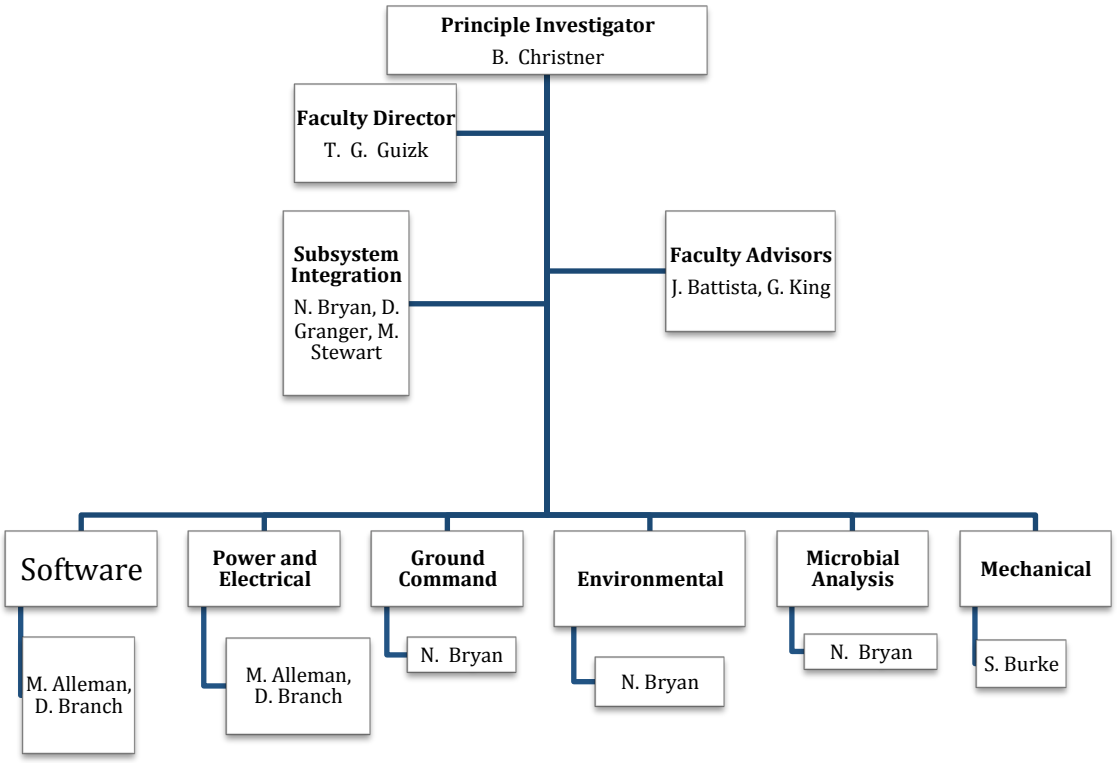
Figure 12: The average mole concentration of ATP rod^{-1} from the HADES payload. The samples were greater than 3 σ above the control rods (n=60, n=92).

4.2.4 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 (3-10, 12, 13). The majority of the isolates are endospore-forming *Bacillus,* but fungal isolates, as well as a single Gram-negative *Staphylococcus,* have also been reported (3-10, 12, 13). In an attempt to verify these results, we conducted culturing experiments. Five rods from the controls and the samples were placed into R2A liquid media on Thursday, September 5, 2013, and incubated aerobically at 4° C. Subsamples were routinely plated on solid media and monitored for growth. As of December 11, 2013 there have been no isolates recovered from the samples or flight controls.

5.0 Team Organization

The payload for HASP 2013 was developed and operated under the support of the NASA EPSCoR MARSLIFE project at Louisiana State University. The faculty advisors were B. Christner (Science Advisor) and T. G. Guzik (Payload Advisor). B. Ellison, D. Granger, and M. Stewart served as technical staff mentors. Project manager Noelle Bryan led the team of S. Burke, M. Alleman, and D. Branch (undergraduates). After completing the project, students gained 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.



6.0 References

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