MTL#725 - *Salivary Markers*

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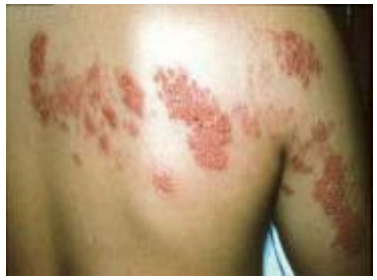
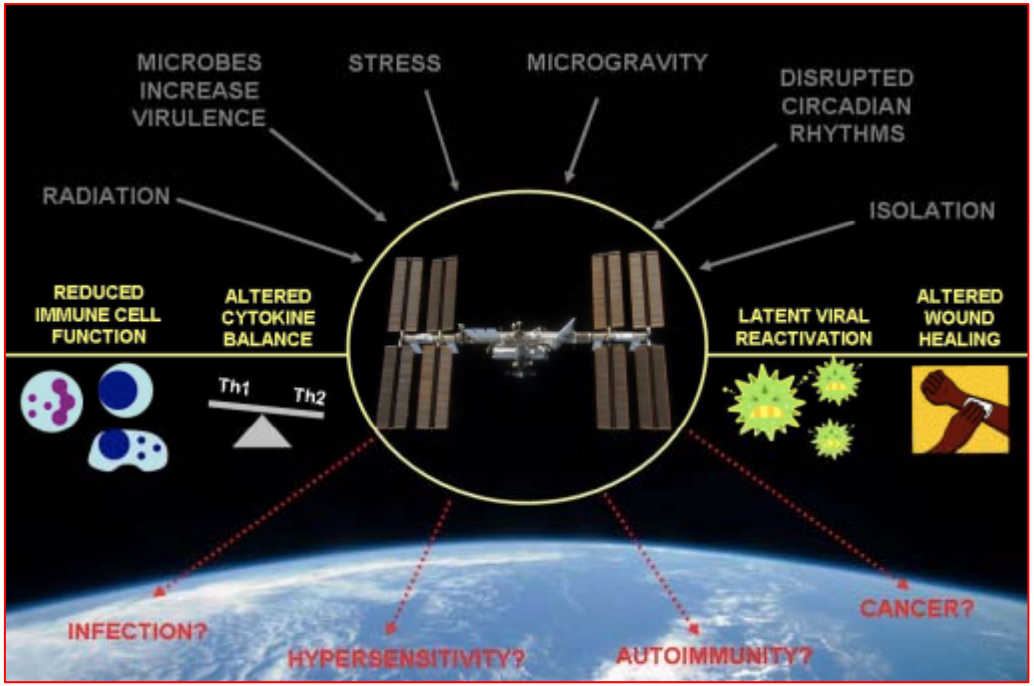
Spaceflight and the Immune System

- Immune system dysregulation has been documented during and after spaceflight, but it is not known if these changes increase infection susceptibility or pose a significant health risk to crewmembers
- Infectious episodes reported among crewmembers aboard the Mir station (i.e. conjunctivitis, acute respiratory and dental infections)
- Many microorganisms have been identified in space vehicles including *Escherichia coli*, *Salmonella enterica*, *Bacillus subtilis*, *Staphylococci*, *Micrococci*, and *Coryneform*
- Crewmembers have been found to shed live Varicella Zoster Virus (VZV) and other latent viruses both in-flight and on return to Earth



- Impaired immunity coupled with increased pathogen exposure could trigger a number of adverse events that could compromise crew safety and mission objectives.

The Effects of Spaceflight on Immunity

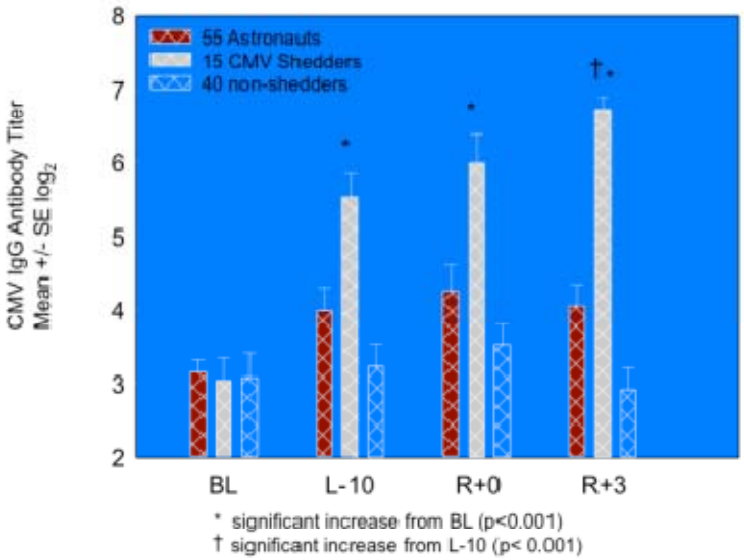


Important to determine if long-duration spaceflight impairs immunity before planetary exploration class missions can be considered

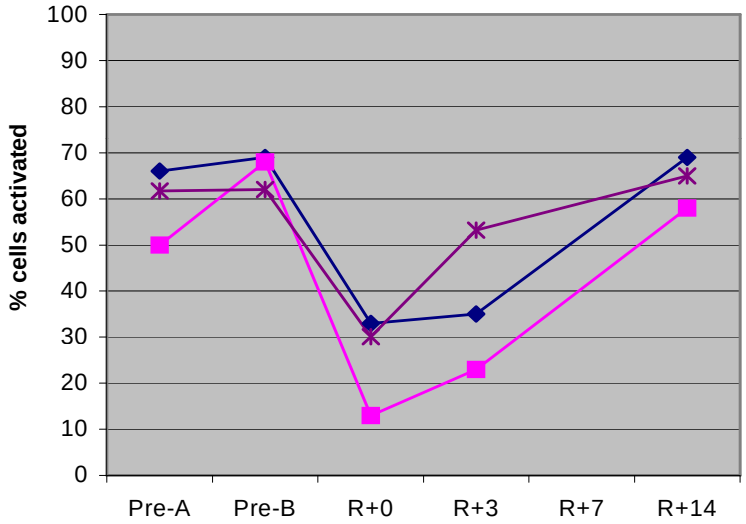
- Difficult to control for the many confounding factors known to impact the immune system
- Pre and post-flight changes perhaps due to the stressors associated with landing and re-adaptation to gravity
- Most in-flight immune data are limited to short-duration spaceflight missions
- Small subject numbers used for in-flight research, logistical difficulties associated with the transportation of specialized equipment to space and viable sample return
- Studies investigating the impact of spaceflight on the innate arm of the immune system are scarce

Virus Load Increases while Immune Cell Function Decreases during Spaceflight

CMV (cytomegalovirus) Reactivation after spaceflight



T-cell Function

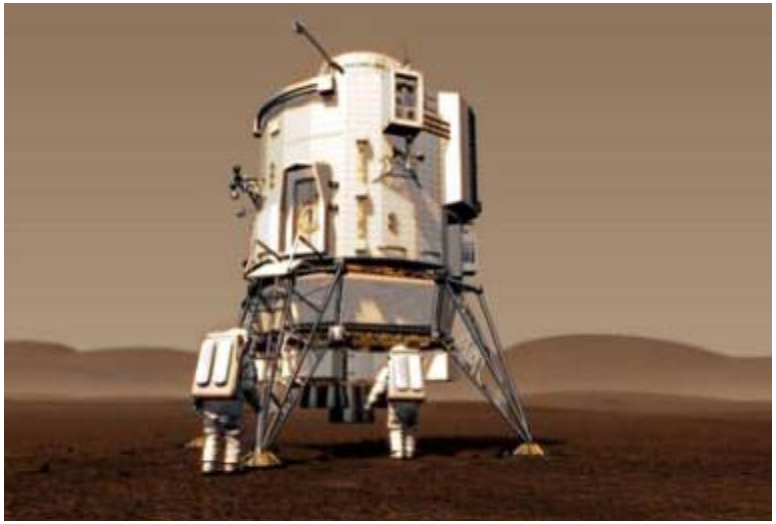


Few In-Flight data exist on immune function, particularly in relation to:

1. long duration spaceflight (i.e. ISS mission)
2. Innate host immune defense

POTENTIAL CONSEQUENCES OF *PROLONGED* IMMUNE DYSREGULATION DURING *EXPLORATION-CLASS* SPACEFLIGHT

- Increased incidence of infection
- Allergy/hypersensitivity reactions
- Consequences of viral reactivation
- Impaired tumor surveillance, increased incidence of malignancies
- Impaired wound healing



Any adverse clinical event related to immune dysfunction would likely have an immediate mission impact.

Recent Spaceflight Immune Studies

Russian Med-Ops/ CYTOKINE DSO-501 (ISS)			L-60		L-10									R+0		R+7	R+14			
Latent Virus/ DSO-493 (SHUTTLE)	L-180				L-10		saliva	saliva						R+0	saliva	saliva	saliva R+14			
Immune Function/ DSO-498 (SHUTTLE)					L-10									R+0				R+21		
Japan Immunology/ DSO-206 (SHUTTLE)			L-60	L-30										R+0	R+3	R+7		R+30		R+90
Epstein Barr/ DSO-500 (SHUTTLE)		L-120	L-65		L-10	L-3/2								R+0	R+3		R+14		AME/ R+120	
Epstein Barr/ E129 (ISS)	AME/ L-180		L-60		L-10	L-3/2								R+0	R+3		R+14			AME/ R+180
Immuno- ESA/ (ISS/SOYUZ)				L-30				L+90 to L+120				R-15 to R-7		R+1		R+7		R+28		
Integrated Immune/ SDBI-1900 (SHUTTLE)	AME**/ L-180				L-10**								R-1	R+0**			R+14			
Integrated Immune/ SMO-015 (ISS)	AME**/ L-180			L-30**			MD 8-10			MID*			R-1	R+0**				R+30**		
	PRE-FLIGHT						***IN-FLIGHT***							***POST-FLIGHT***						

Very few studies have obtained samples In-Flight.
Integrated Immune was the first study to comprehensively examine immune system changes In-Flight, although this study focused mostly on adaptive immunity

Salivary Markers Specific Aims:

- **Project Goal:** This project will address the PRD Risk title: Risk of Adverse Health Event Due to Altered Immune Response and the IRP Gap – M1: Does spaceflight alter immune function?

- **Specific Aim#1:** Longitudinally examine the impact of long-term spaceflight (up to 6 months) on salivary and cellular markers of innate immune function and latent viral reactivation

- **Specific Aim#2:** Examine the relationship between changes in salivary and cellular markers of innate immune function and changes in other stressors associated with the spaceflight environment (i.e. circadian desynchronization, sleep loss/disruption, mood state disturbances, stress, infection incidence).

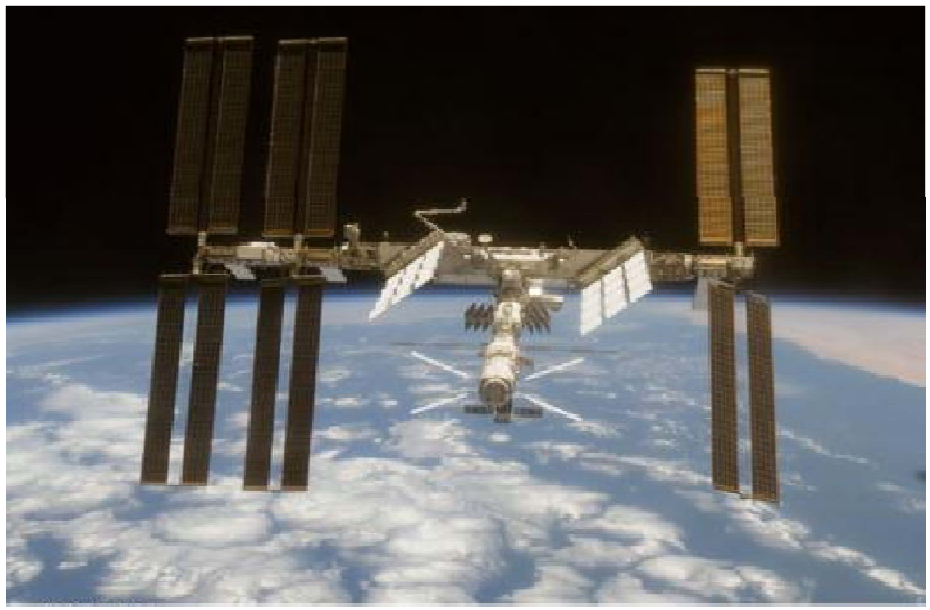
Objectives of *Salivary Markers*:

Address current lack of data regarding immune status *during* flight, particularly in relation to innate immunity

Determine the in-flight status of antimicrobial immunity, latent viral reactivation, cellular innate immune function and physiological stress

Delineate the long-term effects of spaceflight from the effects of acute stress on immune function

Determine the clinical risk related to immune dysregulation for exploration class spaceflight



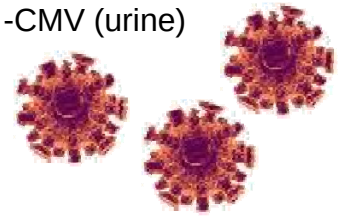
Determine the appropriate monitoring strategy for spaceflight-associated immune dysfunction, that could be used for the evaluation of countermeasures

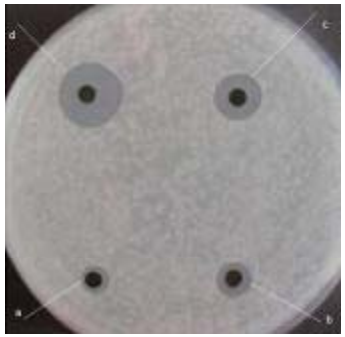
Salivary Markers Specific Assays

Salivary Antimicrobial Proteins/peptides

Salivary Peptide/Protein	Origin	Function
α-defensins HNP 1-4	Neutrophils, gingival sulcus, salivary duct cells	Antibacterial, antifungal, antiviral
β-defensins (HBD-1-3)	Epithelia, salivary ducts	Antibacterial, antifungal, antiviral.
cathelicidin (LL-37)	Neutrophils, gingival sulcus, salivary glands and ducts	Antibacterial
Histatins	Salivary ducts/glands	Antifungal
lysozyme	Neutrophils, salivary ducts	Antibacterial
lactoperoxidase	Salivary glands	Antibacterial
lactoferrin	Neutrophils, salivary glands	Antibacterial, antifungal, antiviral
Immunoglobulin A	Salivary glands	Immune cell activation

Latent Viral Reactivation:
-EBV (saliva)
-VZV (saliva)
-CMV (urine)

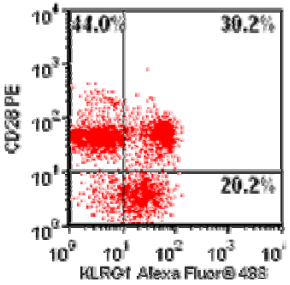


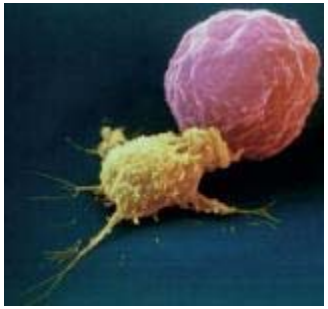


Antibacterial capacity of saliva:
-E. coli inhibition assay

Flow Cytometry

Detailed leukocyte phenotype assays:
-T-cells
-NK-cells
-Monocytes
-B-cells
-Dendritic cells

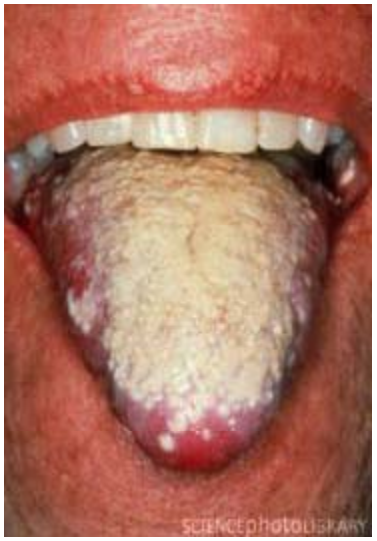


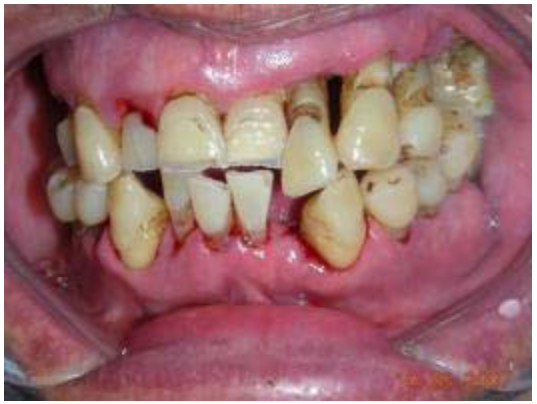


Cellular Functional assays:
-NK-cell cytotoxicity
-Neutrophil degranulation
-TLR ligand stimulations
-Mitogenic T-cell stimulations

Salivary Antimicrobial proteins

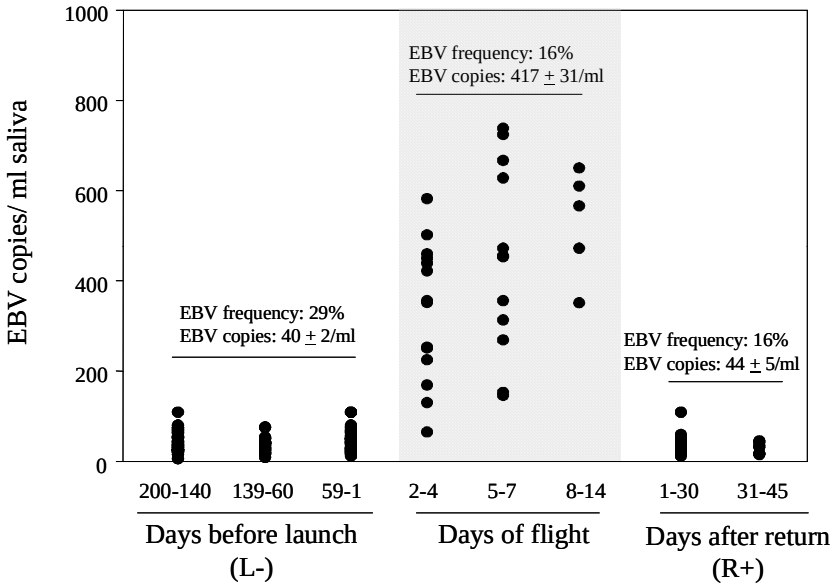
- Evolutionary conserved component of the innate immune system
- Subdivided into subgroups based on their amino acid composition and structure
- Generally between 12 and 80 amino acids
- Have been demonstrated to kill a broad range of pathogens, including Gram Negative and Gram positive bacteria, enveloped viruses, fungi and malignant cells.
- Concentration and secretion of specific SAPs can change in the presence of infection and may be predictive of immunocompromise
- e.g. low concentrations of lysozyme and lactoferrin in saliva have been linked with oral thrush (candidiasis), periodontitis (gingivitis) and rhinovirus



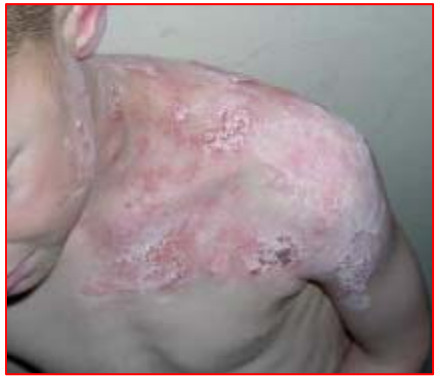


Latent Viral Reactivation

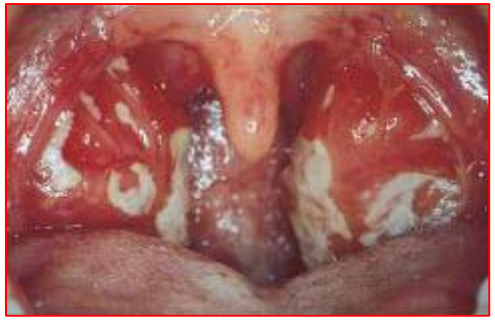
- There are 8 known herpesviruses that infect humans and establish lifelong latency
- Latent viral reactivation has consistently been observed among crewmembers before, during and after space flight
- Latent viral reactivation coupled with immunosupression could be clinically relevant in crewmembers



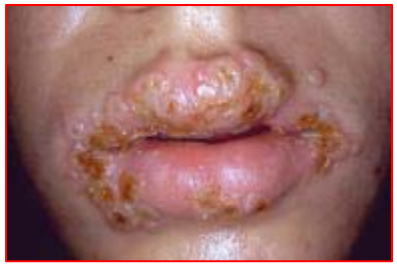
CMV
Mostly Asymptomatic



VZV
Shingles

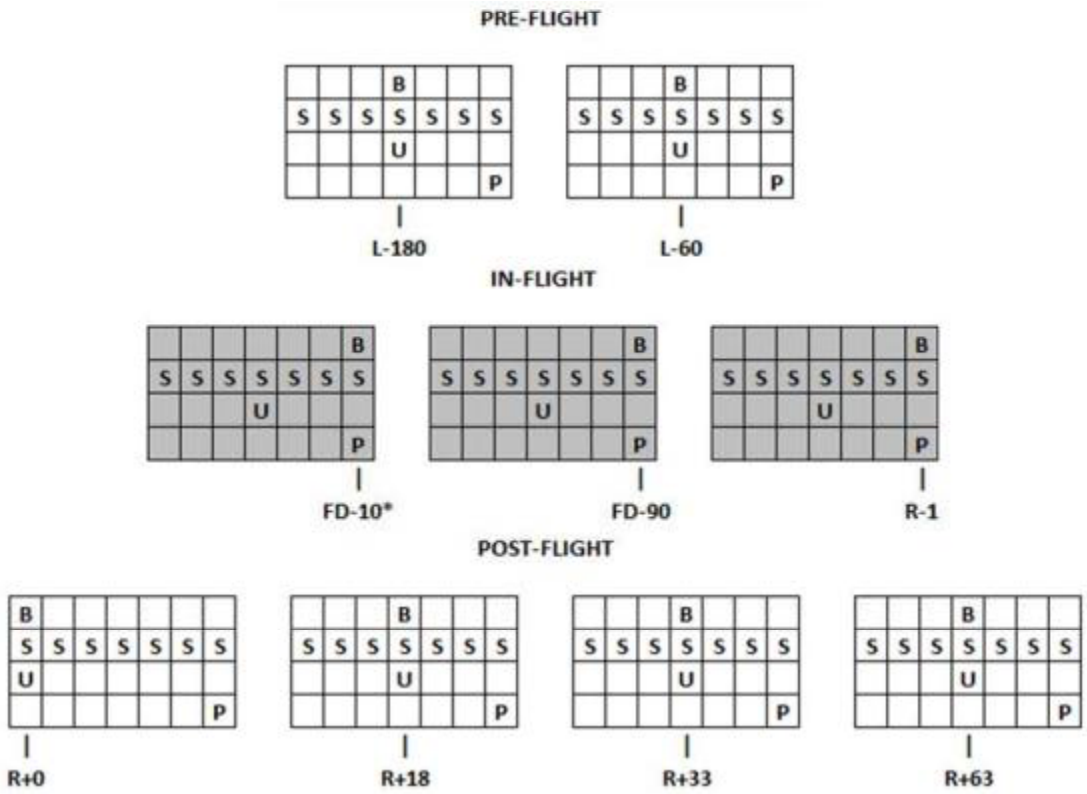


EBV
Infectious Mononucleosis



HSV
Cold Sores

Experiment Design Overview



KEY:

B: Single blood collection (29ml) collected

S: Saliva sampling (3x oral swab)

U: Single 24h urine collection.

P: Health assessment

* *On FD10, only 19ml of blood will be collected and frozen*

Saliva Sampling

Inert polymer cylindrical swab (10 mm x 30 mm). Soft and comfortable (doesn't taste too bad either)

Placed under the tongue for a defined period of time (usually 2-minutes). Saliva is passively absorbed in the swab

21 saliva samples collected over a 7-day period (3/day, all in the morning)

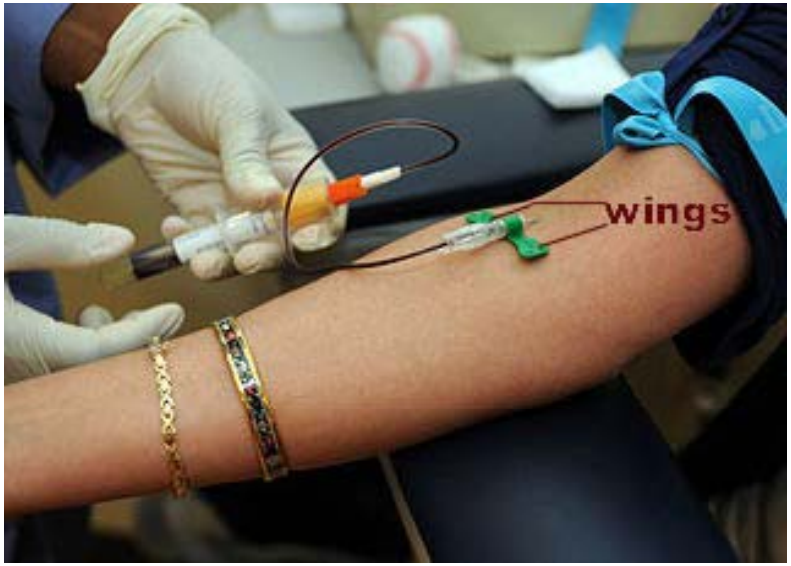
Samples collected at:
Pre-flight: L-180, L-60

In-flight: early (FD-10), mid (FD-90), and late (R-1)

Post-flight: R+0, R+18, R+33, R+63

One of the three daily saliva samples is a timed collection. This is so we can determine salivary flow rate

Blood Sampling



Three blood tubes (total of 29ml) will be collected from a single needle stick at each of the 9 sampling schedules with the exception of FD when only 19ml of blood will be collected and frozen

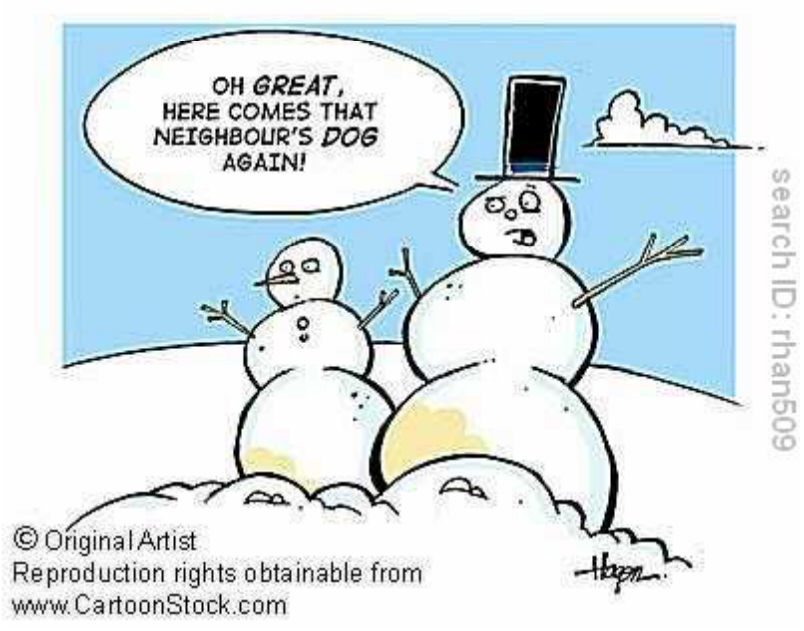
9 intravenous blood samples collected over the entire duration of the study

Samples collected at:
Pre-flight: L-180, L-60

In-flight: early (FD-10), mid (FD-90), and late (R-1)

Post-flight: R+0, R+18, R+33, R+63

Urine Sampling



9x 24h urine samples collected over the entire duration of the study

Samples collected at:
Pre-flight: L-180, L-60

In-flight: early (FD-10), mid (FD-90), and late (R-1)

Post-flight: R+0, R+18, R+33, R+63

Scheduled in conjunction with the blood draws

Health Assessment

Each assessment should take approximately 10-minutes to complete. Scheduled at the end of the 7-day saliva sampling period

"pen and paper" test designed to monitor adverse medical events, impairments in wound healing, sleep quality and mood state

9 assessments will be made over the entire duration of the study:
Pre-flight: L-180, L-60
In-flight: early (FD-10), mid (FD-90), and late (R-1)
Post-flight: R+0, R+18, R+33, R+63

Of particular concern are conditions related to immunology, such as allergies, rashes, hypersensitivities, infections and wound healing. Recording such events will allow us to correlate them to any observed changes in biological immune markers

Questions?