National / International – Cross Public / Private
Proposed Collaboration

PILOT STUDY ON THE INVESTIGATION OF TEAR FLUID BIOMARKERS AS AN INDICATOR OF OCULAR, NEUROLOGICAL, AND IMMUNOLOGICAL HEALTH IN ASTRONAUTS

Collaborators

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PILOT STUDY ON THE INVESTIGATION OF TEAR FLUID BIOMARKERS AS AN INDICATOR OF OCULAR, NEUROLOGICAL, AND IMMUNOLOGICAL HEALTH IN ASTRONAUTS – Year 1

Project Goal

- Pursue technology development to establish collection and assessment of tears to identify relevant biomarkers for spaceflight.

Project Description

- Tear sampling is non-invasive and used as a guide in understanding pathologies, including ocular and systemic inflammatory conditions. Tear biosamples are localized to the eye, central nervous system (CNS), and the brain.
- Tear biomarkers are found to be altered in various conditions, including inflammatory diseases, ocular diseases, metabolic disease and allergies *(Hagan et al., 2016).*
- Tear biomarkers may reflect conditions affecting the CNS *(Salvisberg et al., 2014)*. The concordance rate between tear biomarkers versus cerebrospinal fluid (CSF) for Multiple Sclerosis is approximately 83% *(Devos et al., 2001)*.
- Given the physiological dysregulation and clinical incidence which persist during spaceflight, including ocular and inflammatory alterations, we anticipate tear fluid biomarkers will have relevance for exploration class missions.

Approach

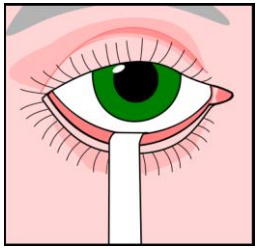
- Tear collection is performed ('TearFlow' or similar, i.e. Schirmer strip, microcapillary).
    1. Develop and validate tear collection, desiccation, reconstitution;
    2. Validate assessment of stress hormones, cytokines, viral DNA in reconstituted tears;
    3. Stability study for desiccated tear samples;
    4. 'Normal range' generation for relevant biomarkers in tears.

Project Objective

- Compared to standard liquid collection, to assess both alterations due to stimulatory effect by the strips, or loss of protein due to binding on the strips.
- Current SANS (VIIP) measurements restricted to 'form and function', this technology would allow another dimension of eye assessment to inform crew of ocular alterations.
- Follow-on research may include assessment of clinical disease/clinical correlations, human space analog conditions, and astronauts prior to-, during-, and after- exposure to the spaceflight environment.

Strategic Alignment

This project aligns with HAT targets: Long Duration Space Medical Care, Long Duration Spaceflight Behavioral Health and Performance, Microgravity Biomedical Countermeasures for Long Duration Space flight and Microgravity Biomedical Countermeasures-Optimized Exercise.



Tear Biomarker Collection

Pilot Study Development

Project: A Pilot Study on the Investigation of Tear Biomarkers as an Indicator of Human Health

ID	Task	Start Date	End Date	Month 1	2	3	4	5	6	7	8	9	10	11	12
1	Project start date			▲											
2	Project kick off meeting				▬										
3	Identify and procure materials					▬	▬								
4	Collect, process and analyze samples (materials testing, assay development, stability study, etc.)						▬	▬	▬	▬	▬	▬			
5	Data Analysis Visualization and clustering								▬	▬	▬	▬	▬		
6	Protocol Refinement & Report									Profile	▬	▬	▬		Report
7	Close out Project														▲

Leveraging JSC Experience

- Dry chemistry and rehydration technology development (urine);
- Dry saliva storage for cortisol (ISS astronauts);
- Viral DNA detection by qPCR.
- Cytokine analysis and quantification

Leveraging Glasgow Caledonian University, Scotland, UK Experience

- Cytokine analysis and quantification using a magnetic multiplex bead immunoassay (Milliplex, Merck Millipore, and UK) and the Luminex 200 system (Luminex Corp, USA)

Leveraging NIH - NAID Experience

- Non-human analogies, data mining – bioinformatics - visualization, and study design – non-human

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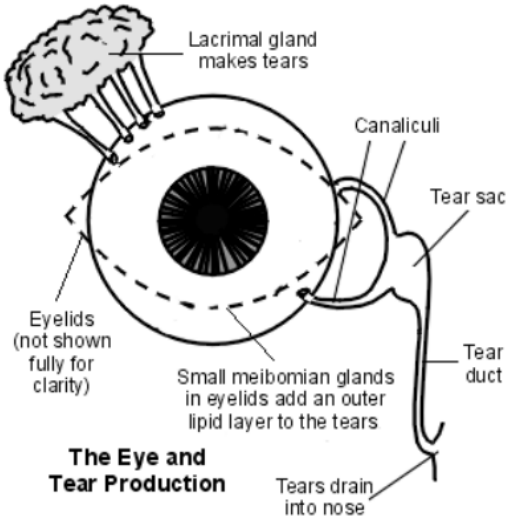
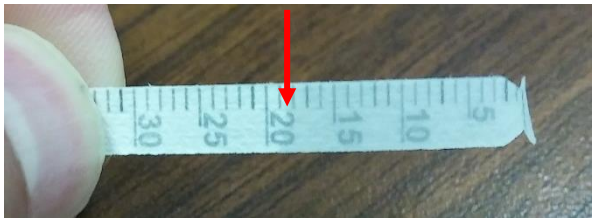
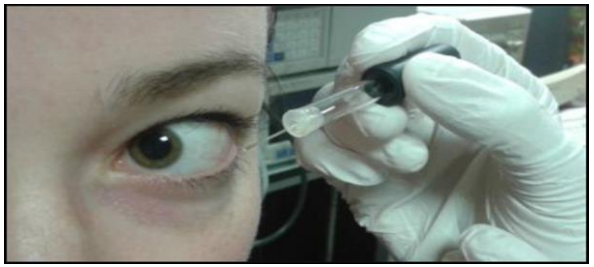
Technical Summary



Tear Collection Protocols





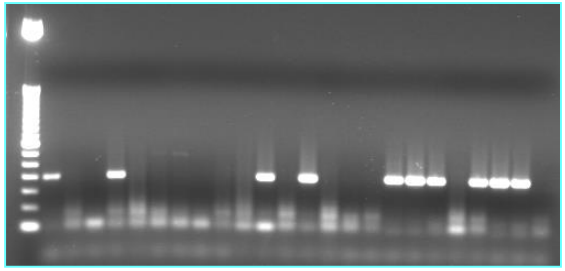
Left: Strip-based tear collection.
Right: Tear Fluid sampling using a glass microcapillary (Microcap)
Source: Tear Fluid Biomarker Profiling: A Review of Multiplex Bead Analysis SUZANNE HAGAN, PHD, AND ALAN TOMLINSON, DSC, PHD, FCOPTOM

Validation and Stability

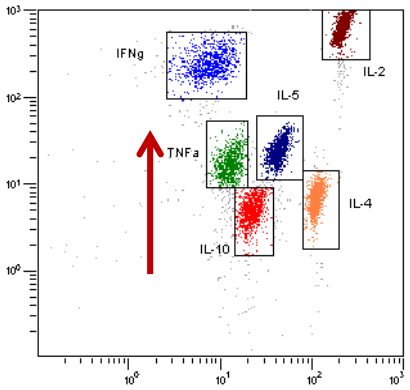
- Types of collection hardware
- Dried vs fresh sample
- Reconstitution options
- Biomarker stability to 1 year

Biomarkers

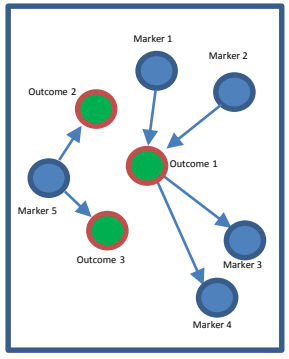
- **Stress hormones** - Cortisol, prolactin, ACTH (Immunoassay)
- **Cytokines** – Pro-, Anti-inflammatory, Th1/Th2/Th17 (Multiplex bead array)
- **Latent Herpesvirus** – HSV-1, VZV (Quantitative PCR)



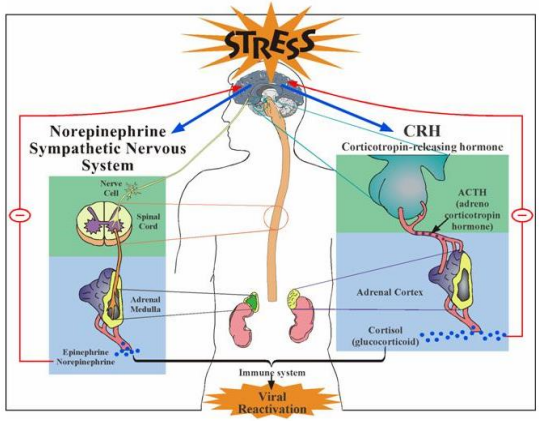
PCR for Latent Herpesviruses



Multiplex analysis of cytokines

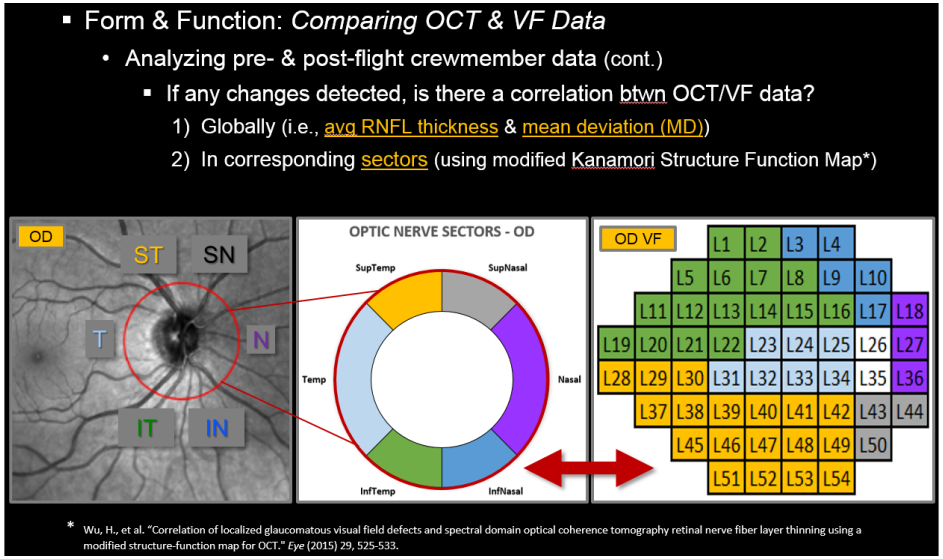


Data Visualization, Clustering, Analysis & Mapping



Relevance to Crew Health (SANS/VIIP)

SANS (VIIP)



Tear biomarkers analysis potentially opens a window to explore….

- Other dimensions of ocular health
- Conditions of the CNS
- Neurological state of human health

- Cause of SANS (VIIP) remains unknown. We do not understand to whom or why SANS happens…
- Biomarkers in saliva, plasma correlate well with dysregulated astronaut physiology (existing ISS data)
- Basal tears are a fluid directly related to ocular disease, CNS health and neurological diseases *(Hagan et al, Zhou et al)*.
- New innovative approach, no one has looked at Tear biomarkers to inform SANS (VIIP)
- Requires innovative technology development, validation (this proposal)
- Proposal will develop protocol to sample tears, enable dried long-term storage, reconstitution and assessing biomarker stability. PI team has experience in dry chemistry.
- Selected biomarkers directly correlate with stress, inflammation, physiology which could impact SANS (VIIP)
- Developed protocols will ID biomarkers, provide technology to assess in astronauts/analogs, have stability for in-flight studies
- High likelihood of year one success

Reference

[1] Hagan S, Martin E, Enríquez-de-Salamanca A. Tear fluid biomarkers in ocular and systemic disease: potential use for predictive, preventive and personalised medicine. EPMA J, 2016; 7:15.

[2] Mader TH, Gibson CR, Pass AF, Kramer LA, Lee AG, Fogarty J, Tarver WJ, Dervay JP, Hamilton DR, Sargsyan A, Phillips JL, Tran D, Lipsky W, Choi J, Stern C, Kuyumjian R, Polk JD. Optic disc edema, globe flattening, choroidal folds, and hyperopic shifts observed in astronauts after long-duration space flight. Ophthalmology. 2011;118(10):2058-69.

[3] Hagan S, Tomlinson A. Tear fluid biomarker profiling: a review of multiplex bead analysis. Ocul Surf. 2013;11(4):219-35.

Questions ?