UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 17, 2019 Mary Gates Hall

# Online Proceedings

## POSTER SESSION 1

**Balcony, Easel 102**

*11:00 AM to 1:00 PM*

### LAVA: A Simple Visualization Tool for Longitudinal Analysis of Viral Alleles

*Michelle Jade Lin, Senior, Biology (Molecular, Cellular & Developmental)*
*Mentor: Alex Greninger, Laboratory Medicine*

With their small genomes, fast evolutionary rates, and clinical significance, viruses have long been fodder for studies of whole genome evolution. One common need in these studies is the analysis of viral evolution over time through longitudinal sampling. This analysis is relevant to basic scientists to understand viral evolution in different model systems, to drug manufacturers to understand antiviral resistance development, and to clinical researchers to understand viral evolution within hosts across space and time. However, there exists no simple tool to automate such analyses. We created a simple command-line visualization tool called LAVA (Longitudinal Analysis of Viral Alleles) originally to survey HPIV1 and HPIV2 adaptations to culture. Our hypothesis was that the culturing process selects for mutations in viral entry proteins (hemagglutination-neuraminidase and fusion) that are never seen during growth in humans. Previously done manually, LAVA cut down the workload for analysis of minor allele variants significantly, and furthermore visualized the results to instantly show that the primary regions of HN and F in which many of the mutations took place were different than those found in the previously studied HPIV3. Further development on LAVA allowed it to grow into a critically useful bioinformatics tool in virology. LAVA allows dynamic and interactive visualization of viral evolution across the genome, over time, and within three-dimensional protein structure. Results are easily shared via a single HTML file that also allows interactive analysis based on read depth and allele frequency. LAVA requires minimal input and runs in minutes for most use cases. LAVA is programmed mainly in Python and is compatible with Mac and Linux machines. LAVA is a user-friendly command-line tool for generating, visualizing, and sharing the results of longitudinal viral genome evolution analysis. Instructions for downloading, installing, and using LAVA can be found at https://github.com/michellejlin/lava.

## POSTER SESSION 1

**Balcony, Easel 117**

*11:00 AM to 1:00 PM*

### The Effect of Potassium on Inflammasome Activation

*Claire Lin, Senior, Biochemistry*
*UW Honors Program*
*Mentor: Susan Fink, Laboratory Medicine and Pathology*
*Mentor: Andreas den Hartigh, Laboratory Medicine, UWMC*

Inflammasomes are multimeric protein complexes involved in innate immune responses. Inflammasomes include a protein sensor, such as NLRP3 or pyrin, linked to the enzyme caspase-1 via the adaptor protein, ASC. Active caspase-1 is responsible for release of the cytokine interleukin (IL)-1ßand triggering inflammatory cell death. Inflammasomes are crucial in defense against pathogens. However, excess inflammasome activation is linked to diseases such as Alzheimer's, atherosclerosis, and other inflammatory conditions. Although inflammasomes are linked to several diseases, we do not fully understand how inflammasomes are activated. We are researching the role that potassium plays in inflammasome activation. To detect inflammasome activation, I measured released IL-1ßusing enzyme-linked immunosorbent assays (ELISAs). I determined whether inflammasome activation requires potassium efflux by measuring IL-1ßreleased from cells stimulated in high extracellular potassium, which prevents potassium efflux. I found that IL-1ßrelease triggered by NLRP3 inflammasome activators is prevented when cells are stimulated in high extracellular potassium. However, IL-1ßrelease triggered by the pyrin inflammasome was not affected by high extracellular potassium. From these results, we conclude that the NLRP3 inflammasome is dependent on potassium efflux from the cell, whereas the pryin inflammasome is not. The implications of our research are two-fold. First, our findings argue against a long-standing hypothesis that high extracellular potassium blocks ASC binding. Both NLRP3 and pyrin need ASC, but our data show that only NLRP3 is affected by potassium concentration. This suggests that potassium affects NLRP3 activation at an unknown point. Second, understanding the role of potassium in regulating inflammasome activity provides a potential therapeutic target. There are drugs that regulate ion concentrations by controlling ion channel activity. Knowing whether an inflammasome pathway is potassium efflux dependent could be beneficial in

limiting excess inflammasome activation that is linked to a variety of human diseases.

## POSTER SESSION 1

**Balcony, Easel 116**

*11:00 AM to 1:00 PM*

**Optimizing and Quantitating Immunofluorescent Detection of Hepatitis C Virus Proteins to Evaluate the Efficacy of the Broad-Spectrum Antiviral Drug Arbidol**

*Jessica Graham, Fifth Year, Medical Laboratory Science*
*Mentor: Stephen Polyak, Laboratory Medicine*
*Mentor: Jessica Wagoner, Lab Medicine*

The threat of influenza, Ebola, Lassa, and Zika (ZIKV) viruses are unprecedented in their current scale and spread, causing enormous global health and economic burdens. The need for effective drugs which are safe for use in humans, including during pregnancy and in children, is critical to addressing this worldwide threat. The antiviral drug Arbidol (ARB) is used clinically in Russia and China as an anti-influenza drug. ARB binds to the influenza hemagglutinin protein to block entry of the virus into human cells. ARB also blocks infection of cells by Hepatitis C virus (HCV), Ebola, Lassa, and ZIKV primarily by suppressing viral entry and potentially by inhibiting post-entry events such as viral replication. The purpose of this project is to optimize and quantitate immunofluorescent detection of the HCV NS5A protein to evaluate the efficacy of ARB against HCV replication. Various dilutions of primary and secondary antibody will first be tested to optimize detection of HCV NS5A in HCV replicon cells. The assay will be miniaturized in 96 well plates. Next, an automated microscope (Biotek Cytation 1) will be evaluated as a method for quantification of immunofluorescent detection by imaging wells for HCV NS5A. HCV replicon cells will then then be treated with various doses of ARB and the antiviral efficacy of treatment quantitated. Defining the various ways by which ARB suppresses different viruses may expand the drug's use and, thereby, inclusiveness for addressing multiple global viral threats.

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## SESSION 1P

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## MCNAIR SESSION - BIOLOGICAL MANIPULATIONS TO DEVELOP MEDICAL AND ENVIRONMENTAL INTERVENTIONS

*Session Moderator: Barbara Juarez, Psychiatry*

**MGH 295**

*12:30 PM to 2:15 PM*

* Note: Titles in order of presentation.

**Analysis of B Cell Gene Expression during Herpes Simplex Virus Type 2 Reactivation**

*Vy Khanh Pham Nguyen, Senior, Microbiology, Biology (General)*
*McNair Scholar, UW Honors Program*
*Mentor: larry Corey, LAB Med, Fred Hutch*
*Mentor: Anton Sholukh, VIDD, Fred Hutch*

Herpes simplex virus (HSV) infects mucosal epithelial cells and can cause recurrent, painful ulcers. HSV is not curable and establishes lifelong latency in host neuronal ganglia. Herpes affects more than 400 million people globally, with an increased risk of genital ulcer disease, HIV acquisition, and transmission of HSV-2 to partners or neonates. Persons with altered T cell immunity have been reported to have prolonged and more frequent lesions, suggesting that the adaptive immune responses are associated with HSV clearance. Additionally, there is evidence of innate cell and T lymphocyte recruitment to the infection site to help clear the lesion. While there is extensive evidence on the role of T cells in these lesion infiltrates, little is known about B cell activation and antibody response during HSV reactivation. The study of B cells and antibodies can be used in developing a HSV vaccine since all licensed vaccines induce robust antibody responses. Antibodies block virus entry and mediate antibody-dependent cell-mediated cytotoxicity (ADCC). That is why a better understanding of local antibody responses against HSV-2 is needed to develop a successful vaccine. My project examines whether HSV antibody-producing B cells are found in areas where HSV-2 reactivates and the potential role that these antibody-producing cells play in host clearance of the virus. To examine this question, ddPCR using B-cell-specific probes were used to identify different B-cell subtypes responding to the HSV infection. Results to date have shown high variability with few emergent patterns. In the future, additional samples will be examined using new protocols for better cDNA synthesis, and more positive and negative controls to increase the accuracy of the data.

## POSTER SESSION 2

**MGH 258, Easel 191**

*1:00 PM to 2:30 PM*

**Development and Validation of a Bivalirudin Assay Utilizing Plasma-Diluted Thrombin Time**

*Kathleen Burke, Fifth Year, Medical Laboratory Science*
*Mentor: Wayne Chandler, Laboratories*

Extracorporeal membrane oxygenation (ECMO) is used to maintain adequate blood-gas exchange in patients experiencing cardiac or cardiopulmonary failure. Close therapeutic drug monitoring of anticoagulants is required to prevent thrombosis within the ECMO circuit or in vivo bleeding from mucosal membranes. The goal of this research was to implement a laboratory developed test for bivalirudin quantification

based on plasma diluted thrombin time (dTT). The assay was run on the Diagnostica Stago STA compact. Two calibrations were performed each with R2=0.99. The analytical measurement range was 0.0 to 2.9 ug/mL with a limit of detection of 0.1 ug/mL. Clotting times strongly correlated with the current standard of measurement. Although further testing must be performed, using this calibrated plasma diluted thrombin time assay would improve efforts for safe therapeutic drug monitoring of bivalirudin.

# SESSION 2F

## ADENOVIRUSES AND MALARIA VACCINE

*Session Moderator: James Mullins, Microbiology*
**MGH 242**
*3:30 PM to 5:15 PM*
* Note: Titles in order of presentation.

**The Acute Challenge Model: Assessing Pre-Erythrocytic *Plasmodium* T Cell Antigens for Malaria Vaccine Development**
*Irene Cruz Talavera, Senior, Anthropology: Medical Anth & Global Hlth, Microbiology*
*Levinson Emerging Scholar, Mary Gates Scholar*
*Mentor: Sean Murphy, Laboratory Medicine & Pathology*
*Mentor: Brad Stone, Laboratory Medicine, Center for Emerging and Reemerging Infectious Diseases*

For many years, concerted efforts to combat malaria through the use of antimalarial drugs, bed nets, and other public health measures led to marked reductions in morbidity and mortality. Unfortunately, progress has stalled. Reductions in malaria have leveled off and even reversed in certain areas (WHO, 2017). As of 2016, there were 216 million cases and 445,000 deaths annually due to *Plasmodium* infections (WHO, 2017). To regain momentum and accelerate malaria eradication efforts, an effective and durable vaccine is needed. The Murphy Laboratory focuses on developing novel pre-erythrocytic (PE) malaria vaccines that can effectively stop the *Plasmodium* sporozoite (spz) before the clinically symptomatic blood stage begins. Identification and inclusion of multiple different protective *Plasmodium* antigens is thought to be crucial to developing a broad immune response and durable protection against this intracellular parasite. To test and define protective antigens, the Laboratory developed an "Acute Challenge" (AC) model in order to sensitively measure T-cell responses that are completely or partially protective. In this model, DNA vaccines encoding *Plasmodium yoelii* proteins are delivered by gene gun to induce CD8+ T-cell responses in BALB/c mice. At the peak of the immune response, we challenge the mice with luciferase-expressing P. yoelli sporozoites and measure the parasite burden and protection using IVIS imaging. A known protective epitope derived from *P. yoelii* circumsporozoite protein (CSP) induces a potent and protective response in this system. My project is to utilize the AC model to assess *P. yoelli* candidate antigens, of unknown protective potential, that are putatively exported or secreted from the parasite-containing vacuole into the host cell cytoplasm. Confirmed protective antigens will then be assessed for their localization and defined T-cell epitopes. The results will be used to create vaccines designed to maximize such responses and target the responding T-cells to the liver.

# POSTER SESSION 3

**Balcony, Easel 120**
*2:30 PM to 4:00 PM*

**Method Validation of Hemoglobin A1c on the Vitros 4600**
*Cindy Au, Senior, Medical Laboratory Science*
*Mentor: Rhona Jack, Laboratory, Seattle Children's Hospital*

Hemoglobin A1c (HbA1c) is an important marker to aid in the diagnosis and monitoring of diabetes, as it is a measurement of average blood glucose concentration over the previous two to three months. The current instrument used at Seattle Children's Hospital to measure glycated hemoglobin is the DCA$^{TM}$ Vantage Analyzer. The DCA$^{TM}$, being a point of care (POC) device, can only run one sample at a time and requires a significant amount of tech time to run a batch of samples. With that, there is a desire to validate HbA1c on the Vitros 4600 as it would improve utilization of tech time and potentially provide more accurate and precise results. A correlation study was first done by running 30 different samples across the Analytical Measurement Range (AMR $<$14%) on the Vitros 4600 and compared to the original values run on the DCA$^{TM}$ using the statistical program in EP Evaluator. The overall bias of the two methods was -3.265%, but after closer analysis, the bias $<$9% HbA1c was -0.509%, while the bias $>$9% HbA1c was -5.026%. Another method comparison was done by running high HbA1c samples (8-14%) on the DCA$^{TM}$ and Vitros 4600 compared against ion exchange HPLC, which is the gold standard for the measurement of HbA1c. The Vitros 4600 had a better correlation with HPLC (bias of 1.075%) on the high end for HbA1c compared to the DCA$^{TM}$, suggesting the DCA$^{TM}$ actually has a high-end bias with higher HbA1c values compared to HPLC. Thus, by using the Vitros 4600, there can be more accurate results for HbA1c levels especially when managing diabetes, while also increasing productivity by having the ability to run multiple samples compared to a POC device.

## POSTER SESSION 4

**Balcony, Easel 105**

*4:00 PM to 6:00 PM*

**Assessment of Peripheral Blood and Bone Marrow Specimen Storage Viability by DNA Quantification and Fragment Analysis**

*Mark Bayuga, Senior, Medical Laboratory Science*
*Mentor: David Wu*
*Mentor: Shannon Nesbitt, Molecular Hematopathology*

Currently at the UW Molecular Hematopathology lab, peripheral blood and bone marrow samples stored at room temperature and at 4C are used to perform molecular testing for clinical diagnoses. The current maximum storage length for both specimen types is established at 4 days, and samples received that are older than 4 days must have degradation and DNA quality assessed, a specimen redraw is requested, or the specimen is even rejected. To test the possibility of extending sample storage life by analyzing DNA quantity and quality of samples older than 4 days, I collected peripheral blood and bone marrow specimens and I stored them respectively at either room temperature or storage temperature conditions. I then extracted DNA from the samples on set days throughout a 14-day period, where I then quantified the DNA by spectrophotometry. Other technologists within the lab then qualitatively assessed the extracted DNA samples by fragment analysis. DNA concentrations appear stable through Day 14 and Day 11 for PB and BM, respectively, well beyond the current 4 day age limit for samples. Temperature did not seem to contribute to decline in DNA concentrations, though did seem to affect DNA quality. It would be beneficial to extend storage beyond 14 days to see when quantity and quality eventually do decline. There is good reason to believe sample holds and quality assessment can now be reliably deferred for samples older than 4 days, at least until the 14 day age mark for peripheral blood specimens and the 11 day age mark for bone marrow specimens. The quantity and quality of PB and BM samples did not decline significantly when held for longer than 4 days, nor did temperature or storage time up to 14 days significantly affect sample viability.

## POSTER SESSION 4

**Balcony, Easel 120**

*4:00 PM to 6:00 PM*

**Optimization, Calibration, and Standardization of the Becton Dickinson FACSymphonyTM Flow Cytometer for the HIV Vaccine Trials Network (HVTN)**

*Maxwell (Max) Krist, Senior, Medical Laboratory Science, Microbiology*
*Mentor: Stephen De Rosa, Laboratory Medicine*

The HIV Vaccine Trials Network (HVTN) has flow cytometry labs at both the Fred Hutchinson Cancer Research Center (FHCRC) and the Cape Town HVTN Immunology Lab (CHIL) which both perform testing on HIV vaccine clinical trial samples. There can be a significant level of variability in the performance of flow cytometers, potentially leading to inconsistent data. The large degree of intra- and inter-instrument variability necessitates a quality assurance (QA) protocol be followed to standardize new flow cytometers to the instruments currently in use in both HVTN laboratories to ensure accurate, consistent results between instruments. Using a modified version of Perfetto et al.'s protocol which utilized sets of standardized calibration beads as well as singly stained CD4 T-cells, we successfully optimized, calibrated, standardized, and qualified a new BD FACSymphonyTM (Becton-Dickinson, San Jose, CA) flow cytometer for future use at CHIL. The successful completion of this QA protocol ensures that this instrument will be able to collect accurate and reproducible data for HIV vaccine clinical trials. It is iimportant to guarantee the quality of the data collected during these clinical trials so that the effectiveness of candidiate HIV vaccines can be properly assessed.