including a 2013 outbreak where CHIKV was introduced to the Americas. Despite the large number of cases and severe disease caused by CHIKV, there are no licensed vaccines or specific therapeutics. While there has been extensive analysis of the role that viral proteins play in CHIKV replication and pathogenesis, less attention has been paid to the role of RNA secondary structure in the viral lifecycle. To define the secondary structure of the CHIKV RNA genome, we used selective 2'-hydroxyl acylation analyzed by primer extension and mutational profiling (SHAPE-MaP). SHAPE-MaP combines chemical probing data with computer modeling to generate experimentally informed RNA secondary structure models of long RNAs with single nucleotide resolution. Our analysis found numerous RNA secondary structures throughout the genome, and identified 23 highly structured regions within the CHIKV genome. Five of these structures are known RNA structures important for viral replication, while 18 regions represent novel RNA structures that are undescribed in the literature. To define whether these novel RNA structures play a role in CHIKV replication or pathogenesis, we are mutating each structured region and testing these viruses for defects in replication, host range, and pathogenesis. As a positive control, we disrupted the 5' conserved sequence element (CSE), known to enhance replication in other alphaviruses, along with a large stem loop (SL3) that is immediately 5' of the CSE. Our data indicate that SL3 functions in concert with the 5'CSE to promote CHIKV replication in mammalian and mosquito cells. These studies provide new evidence that stable RNA secondary structures are distributed throughout the CHIKV genome, and demonstrate the importance of RNA structure during CHIKV replication.

W47-6

SPR AS A METHOD FOR SCREENING ANTI-INFLUENZA NEUTRALIZING ANTIBODIES IN CRUDE HUMAN PLASMA

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We applied Surface Plasmon Resonance (SPR) technology to develop a method of screening for potency and quantification of anti-influenza antibodies in minimally processed human plasma samples and immunoglobulin (IGIV) products. We found that specific antibodies in human plasma or IGIV can inhibit binding between hemagglutinin recombinant protein and receptor-analogous glycans and do so in concentration-dependent manner. We ranked the inhibitory activity of plasma samples from multiple donors and observed a good correlation between SPR assay measurements and conventional hemagglutination inhibition assay (r=0.89). The SPR method was also applied to screening of the amount of specific anti-influenza antibodies in pre- and post-2009 H1N1 pandemic IGIV lots produced by different manufacturers. In addition, SPR was used to test inhibition of intact A/California/07/2009 (H1N1) virus binding to 2,6 or 2,3-linked glycans. In summary, SPR technology can provide high-throughput, time-saving and semi-automated alternative to conventional assays such as hemagglutination inhibition or microneutralization in situations where screening of large number of donor plasma samples may be required.

W47-7

UTILIZING YEAST TO IDENTIFY GENETIC INTERACTORS OF VIRAL PROTEINS AND ANTIVIRAL THERAPEUTICS

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Viral proteins must interact intimately with the host cell machinery for replication and pathogenesis. While yeast have been used extensively to study cell biology, there has been comparatively little use of this model system to investigate how viral proteins interact with a cell. We have found that expression of proteins from various viruses can cause defective yeast growth. We hypothesize that the growth defect is a result of the viral protein interacting with and disrupting cellular pathways. Therefore, the defect can be leveraged to identify novel genetic interactors of viral proteins in a eukaryotic cell. These genetic interactors could potentially be host-targets for antiviral intervention. Moreover, drug screens can be performed directly in the yeast, allowing for an unbiased identification approach to find compounds targeting either cellular or viral proteins. Work has focused on the Middle East respiratory coronavirus (MERS-CoV) ORF4a protein. ORF4a has been expressed in the yeast knockout library collection and a suppressor screen has been used to identify potential genetic interactors of the viral protein. Hits have been identified from the screening and work is ongoing to test the role of mammalian homologues in viral infection using CRISPRi and other approaches. To date, five viruses across multiple families have been tested and all have been found to have at least one protein capable of inhibiting yeast growth, this system may therefore provide a broadly applicable tool to analyze the interaction of viral proteins with eukaryotic cells.

W47-8

A NUCLEASE PROTECTION ELISA ASSAY FOR COLORIMETRIC AND ELECTROCHEMICAL DETECTION OF NUCLEIC ACID

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Nucleic acid testing (NAT) assays like quantitative polymerase chain reaction (qPCR) are the preferred method used to diagnose viral diseases like Zika virus (ZIKV). And while qPCR offers excellent sensitivity and specificity, its complexity and instrumentation requirements limit its clinical use to specialized diagnostic laboratories. Because ZIKV circulates in regions with few diagnostic laboratories, sample volume is often overwhelming, increasing the turn-around time. Reducing assay complexity and cost increases the accessibility of NAT for diagnostic use in regions where laboratory resources are limited. S1 nuclease protection has been used for over forty years to map genomic and messenger RNA elements, detect cellular and viral microRNAs, and even to monitor algal species in the environment. Although it has not been used in a clinical context, the technique demonstrates high specificity and has served as an effective replacement for Northern blotting and PCR techniques in laboratory research. Briefly, the nuclease protection ELISA (NP-ELISA)

mixes and hybridizes probe oligos with a nucleic acid sample. Because S1 nuclease is specific for single stranded nucleic acids, only hybridized double-stranded products remain after digestion. Remaining protected probe is immobilized to a neutravidin plate via a 3' biotin linkage and HRP-conjugated antibody is added to bind to the 5' digoxigenin linkage. Although HRP reactions are typically visualized colorimetrically, the enzymatic oxidation of its 3,3',5,5'-Tetramethylbenzidine (TMB) substrate can also be detected electrochemically to enhance sensitivity. Using complementary oligos as a model system, the electrochemical assay demonstrates high specificity and detection ranges comparable to authorized qPCR assays. Because ZIKV is an RNA virus, the system was also tested with specific and nonspecific viral RNA. The NP-ELISA directly and specifically detects ZIKV RNA eliminating the need for reverse transcription. The NP-ELISA is a good candidate for an alternative NAT tool with fewer reagents, inexpensive equipment, and potential for automation.

W47-9

EFFECTIVELY MONITORING VIRUS REPLICATION USING NEURAMINIDASE ACTIVITY-BASED IART

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Influenza A(H3N2) viruses are important respiratory pathogens that have been afflicting humans since 1968. The human antibody response exerts selective pressure on influenza viruses by targeting surface glycoproteins hemagglutinin and neuraminidase (NA), causing their rapid evolution. Contemporary A(H3N2) viruses have lost the ability to efficiently agglutinate red blood cells (RBCs) *in vitro*, which precludes them from testing in the hemagglutination (HA) assay. An alternative assay is needed for determining when to harvest virus for antigenic, antiviral, and other tests.

Previously, we demonstrated that the rapid influenza Antiviral Resistance Test (iART), a prototype system developed by Becton, Dickinson and Company for research use only (BARDA Contract HHSO100201300008C), can detect antiviral resistant viruses in clinical specimens and isolates. In this study we evaluated whether iART could be used in lieu of the traditional HA assay to monitor virus replication by measuring NA activity. Thirty clinical specimens containing A(H3N2) viruses were used to inoculate MDCK-SIAT1 cells. At 24hpi, virus load in culture supernatants was assessed by HA, TCID50, real-time RT-PCR, digital RT-PCR, and iART assays. Correlations were drawn between the assays. Not surprisingly, the Ct values (real-time RT-PCR) and vRNA copy numbers (digital RT-PCR) showed a perfect inverse correlation (r=-1.00). The vRNA copy numbers and NA activity (iART) also showed a very good correlation (r=0.98). On the other hand, HA titers with guinea pig RBCs were either low or undetectable, despite majority of supernatants having infectious titers ≥6.5 log10TCID50, which is sufficient to perform most infectivity-based assays. Importantly, the correlation between TCID50 and NA activity was strong (r=0.91), suggesting that NA activity is a good predictor of when to harvest virus for use in infectivity-based assays. These results demonstrate that in addition to drug-susceptibility testing, iART provides a simple, reliable, and fast method for monitoring virus replication in cell cultures.

W47-10

TRANSFORMATION OF THE CHLOROVIRUS CA-4B USING CRISPR-CAS9

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Having the ability to carry out molecular genetic modifications of the large dsDNA chloroviruses, with genomes of 290 to 370 kb, would help elucidate the function of both known and unknown, novel virus-encoded proteins. These plaque-forming viruses replicate in certain unicellular, eukaryotic chlorella-like green algae and are present in aqueous environments throughout the world. However to date, only a few algal species and essentially none of their viruses have been genetically manipulated due to inefficient genome editing methods creating a transformation bottleneck. Therefore, the development of heterologous gene expression tools for the viral chlorella-like green algal hosts would be an important accomplishment. Here we report that the CRISPR-Cas9 editing technology can be used to generate stable targeted gene mutations in the chlorovirus CA-4B. Delivery of preassembled Cas9 protein-gRNA ribonucleoproteins (RNPs) resulted in frameshift mutations in the CA-4B-encoded gene *a064r*, which encodes a glycosyltransferase involved in glycosylation of its major capsid protein independent of the host's biosynthetic machinery. This novel heterologous gene expression platform in chlorella-chlorovirus counterparts using the CRISPR-Cas9 technique will accelerate the exploration of these microalgae and their viruses for a broader range scientific investigations and biotechnological applications.

W48-1

VPS34 LIPID KINASE IS CO-OPTED TO GENERATE PI3P FOR CONSTRUCTION OF VIRAL REPLICATION COMPARTMENT

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Viral replication compartment is formed to produce progeny virus and to protect the virus against host antiviral responses. Similar to many other RNA viruses, *Tomato bushy stunt tombusvirus* (TBSV) rewires various cellular protein trafficking pathways and lipid metabolic pathways through different strategies to multiply within host cells. Phosphatidylinositol 3-phosphate (PI3P) is one of the important phospholipids in eukaryotic cell membranes, and it is involved in many critical cellular processes. Yet, it is not known whether PI3P plays any role in TBSV replication. In current study, we discovered that TBSV co-opts cellular Vps34 lipid kinase to facilitate the construction of viral replication compartment in yeast model host and plants. Recruitment of Vps34 by viral replication proteins leads to the enrichment of PI3P in the viral replication compartment. Depletion of cellular PI3P level with chemical inhibitors, PI3P phosphatase, or sequestering endogenous PI3P with PI3P binding proteins, results in the decreased level of dsRNA replication intermediates within TBSV replication compartment, and reduced accumulation of viral (+)RNAs. Inhibiting the kinase activity of Vps34 makes TBSV dsRNA more sensitive to the RNAi machinery and other restriction factors. *In vitro* reconstitution of the TBSV replicase in yeast extracts lacking Vps34 showed poor replicase activity, demonstrating critical function for Vps34 during replicase assembly. Furthermore, the replication of the mitochondria-replicating *Carnation Italian ringspot tombusvirus* and other (+)RNA viruses is compromised upon blocking Vps34 kinase activity. Altogether,