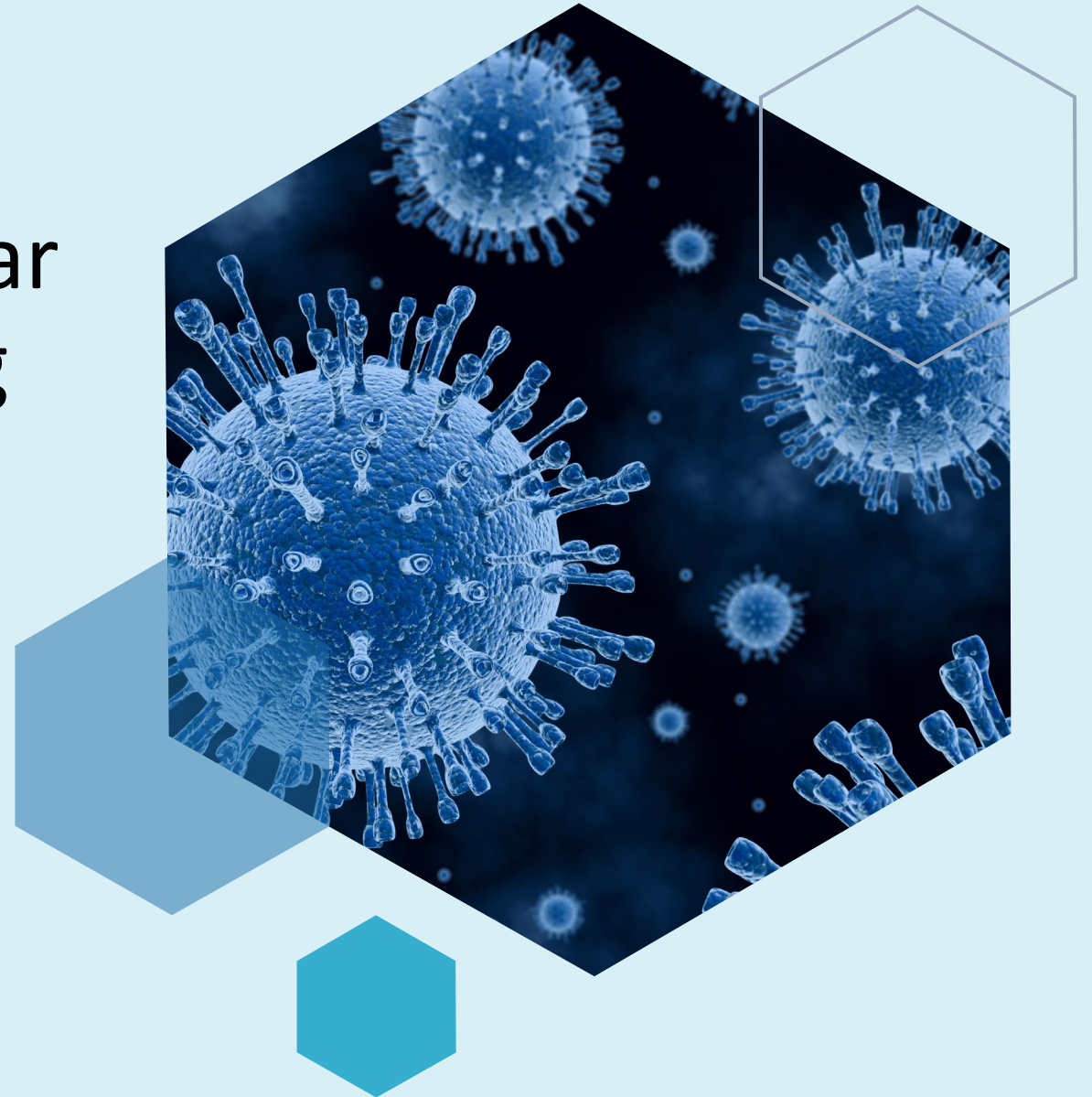


# Reflections on Our First Year as an Influenza Sequencing Center

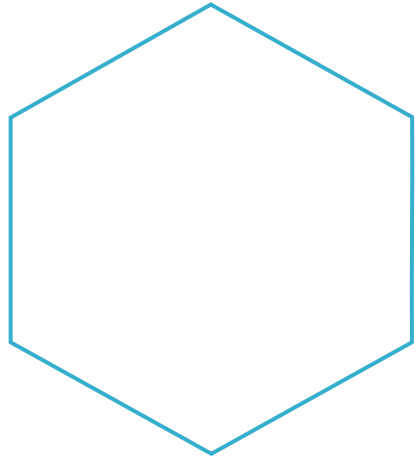
Bonnie Oh, PhD

Microbial Genomics  
Team Lead

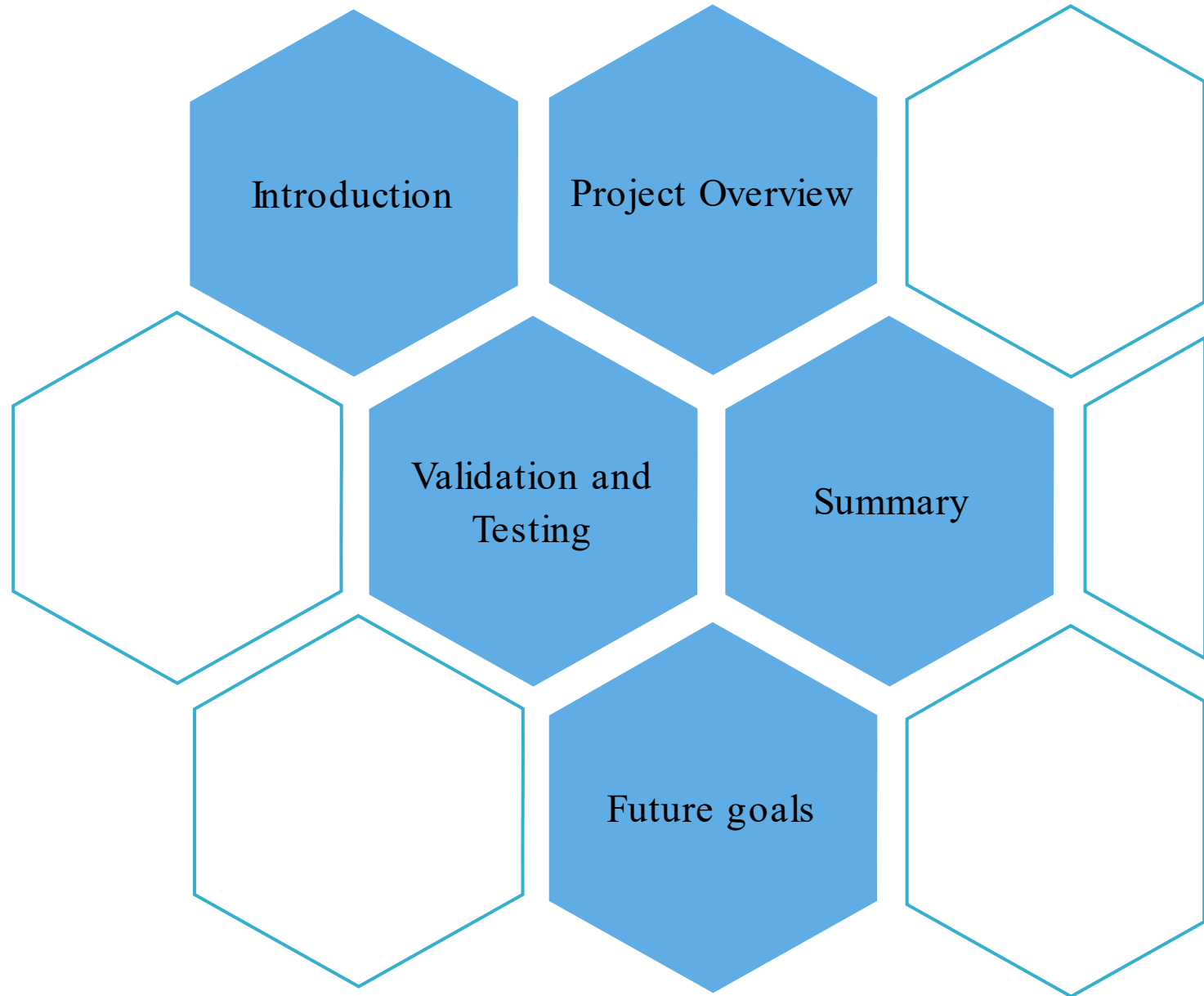


Texas Department of State Health Services

Public Health Laboratory Division, Genetic Sequencing Branch

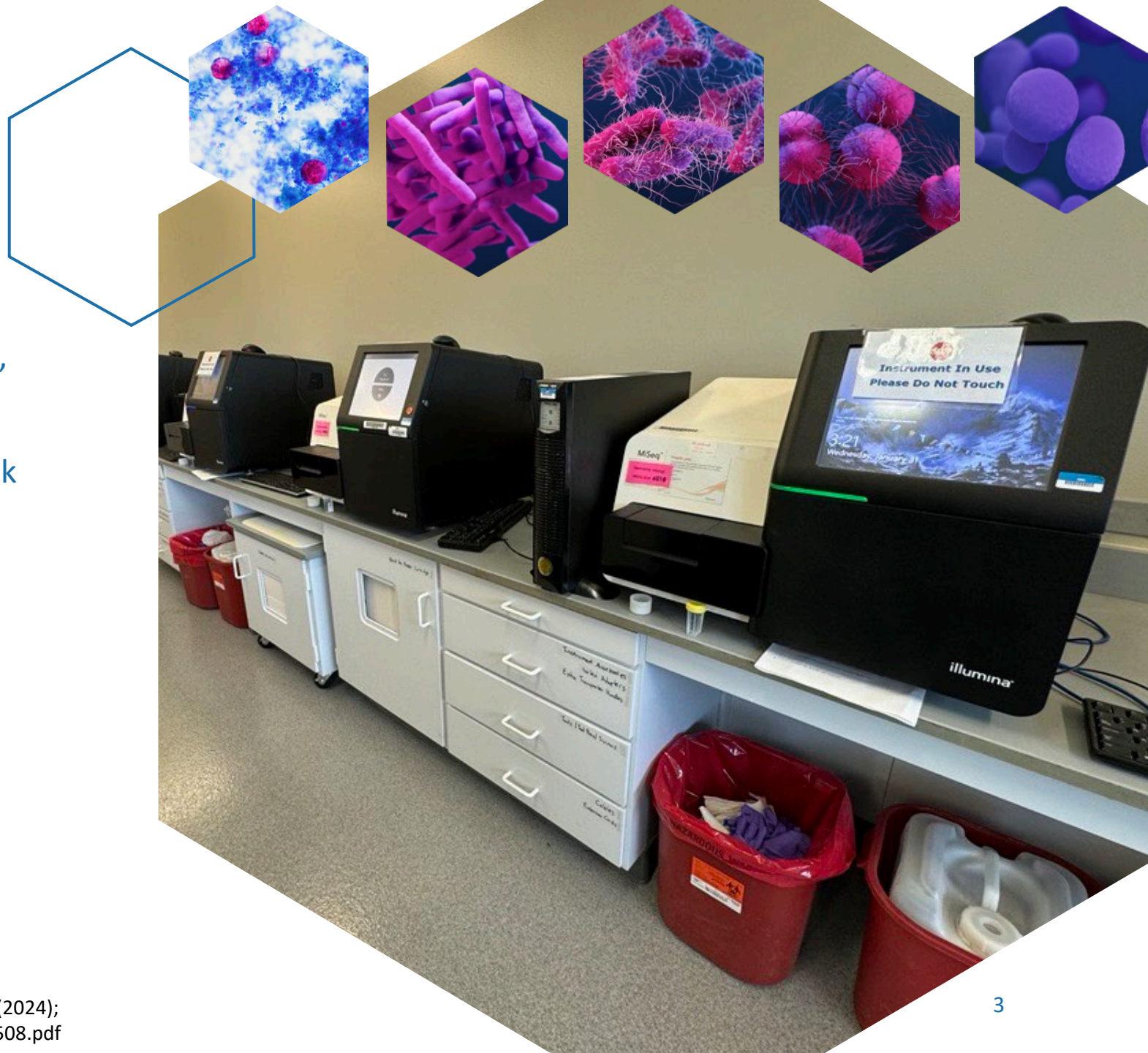


# Agenda



# Introduction

- The Genetic Sequencing Branch (GSB) performs Next-Generation Sequencing (NGS) testing on pathogenic bacteria, fungi, parasites, and viruses.
- We work with DSHS Epidemiologists to track outbreaks and identify variants of interest.
- We partner with CDC, FDA, and Texas Universities.
- Influenza Sequencing Center (ISC)
  - We work with the Clinical Virology team to sequence Influenza A and B.
  - Sharing data with the CDC



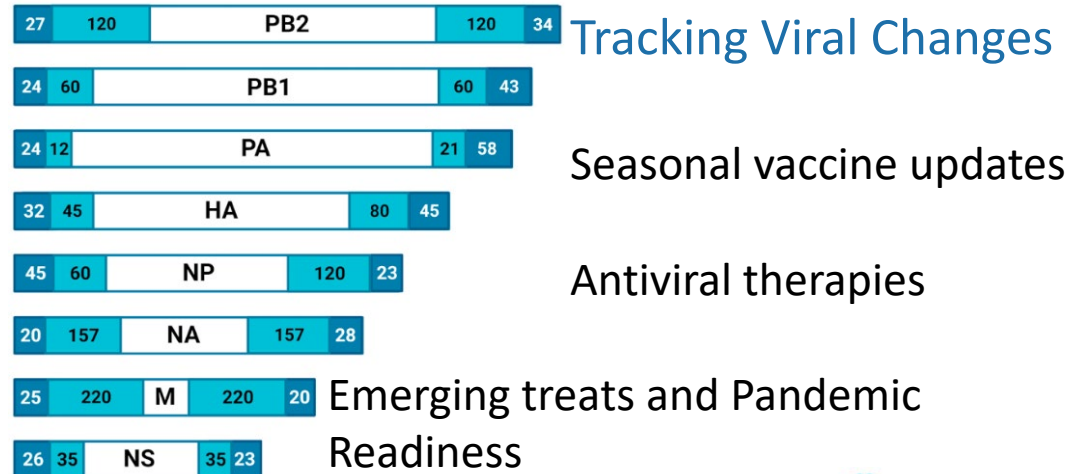
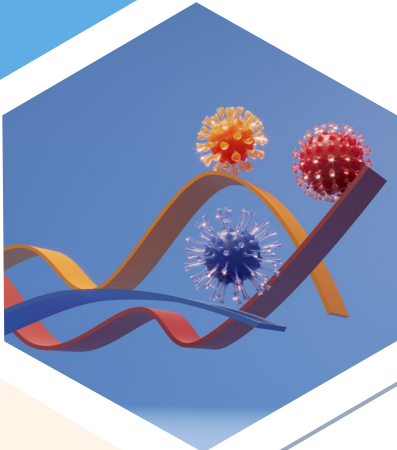


# Importance of Influenza Surveillance

## Antigenic Drift and Shift

**Drift:** small changes over time

**Shift:** major change or recombination



Tracking Viral Changes

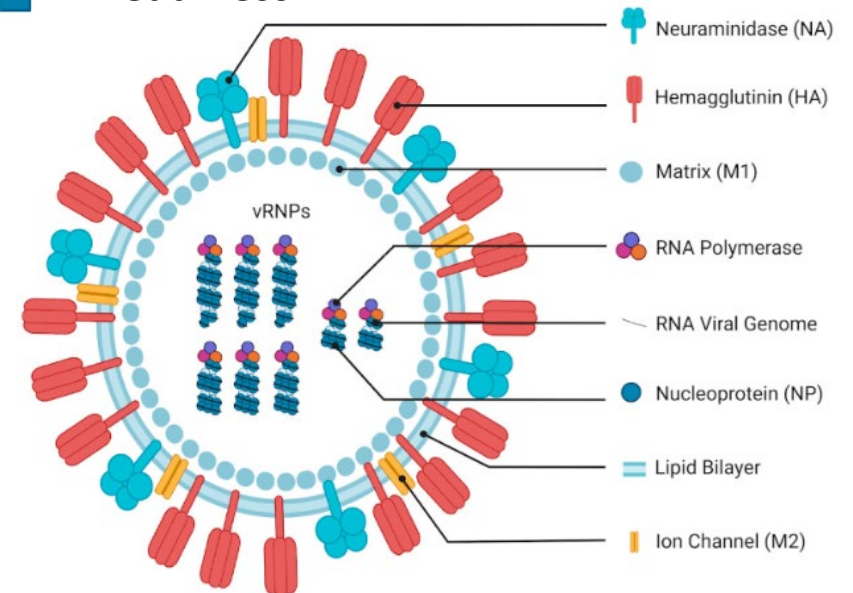
Seasonal vaccine updates

Antiviral therapies

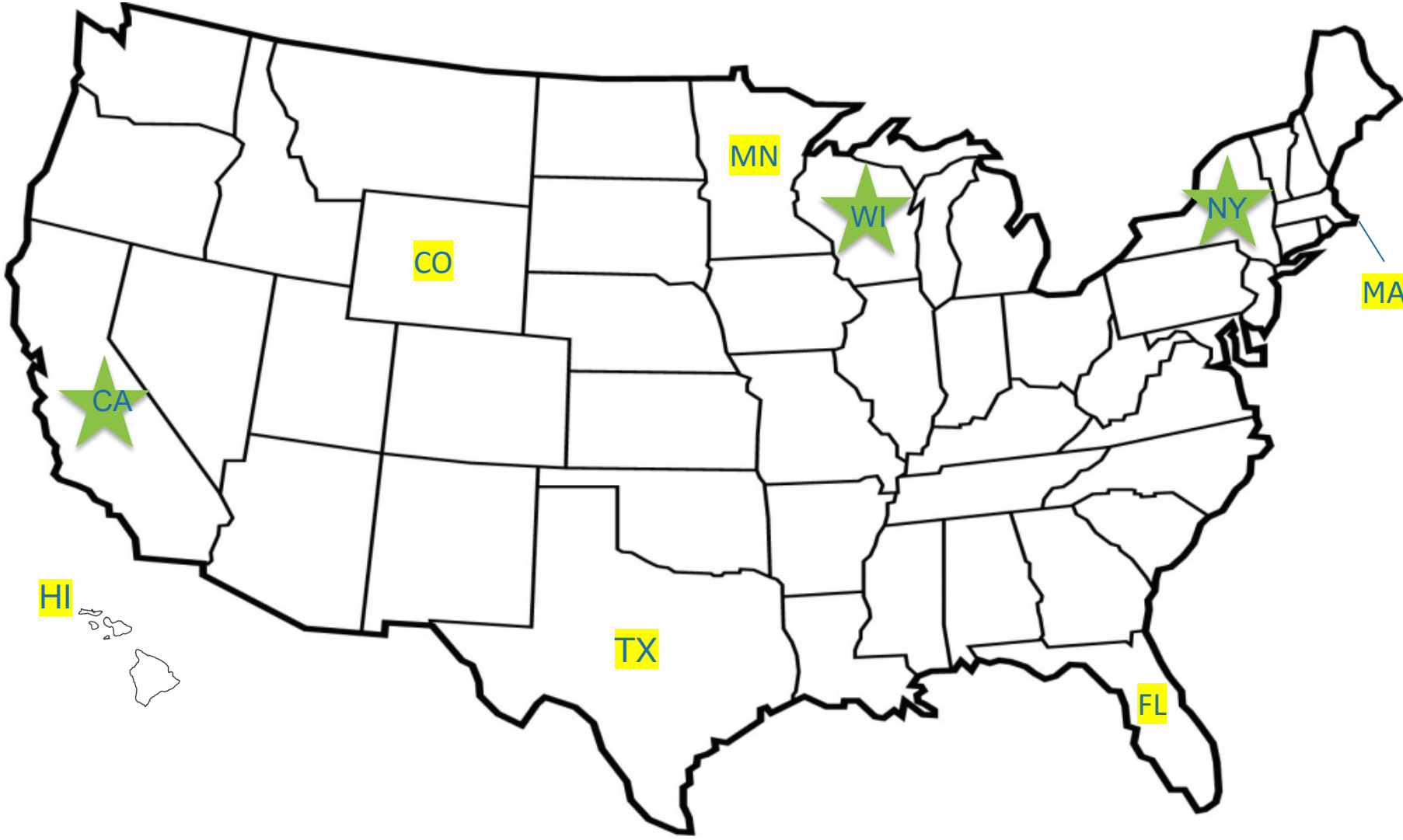
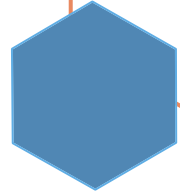
Emerging treats and Pandemic Readiness

## CDC Estimates\* for U.S. 2023-2024 Flu Season

- 35 million illnesses
- 400,000 hospitalizations
- 25,000 deaths



# National Influenza Reference Centers & Influenza Sequencing Centers



## NIRC

- perform NGS on ~1000 samples/year
- viral culture and isolation

## ISC

- perform NGS on ~500 samples/year
- geographically diverse, high-travel states

# Project Overview

Influenza Sequencing Centers perform next generation sequencing of Influenza A and B for surveillance, vaccine strain selection, and detecting antiviral resistances.



## ISC Award

Texas acts as a geographically diverse transit hub for Influenza surveillance data.

Awarded January 2023



## IT and LabWare Set Up

Set up automatic AWS connections from sequencing instrument to APHL AIMS cloud.

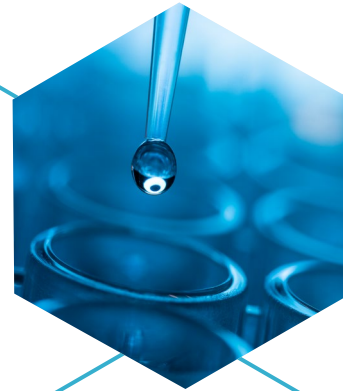
Updated PHLIP messages to CDC.

Staff access to Clarity LIMS, AWS Workspace, and Tableau.



## Bench Staff Training

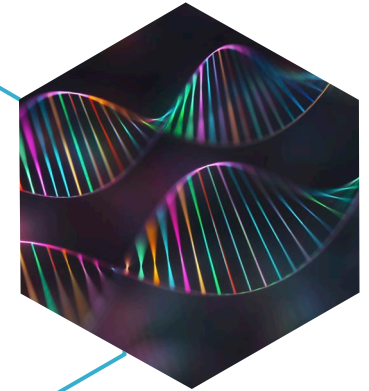
Two lab staff attended training at CDC.



## Proficiency Testing

Tested dilution series (Limit of Detection, LOD) of Flu A/B positive controls.

Tested internal samples.



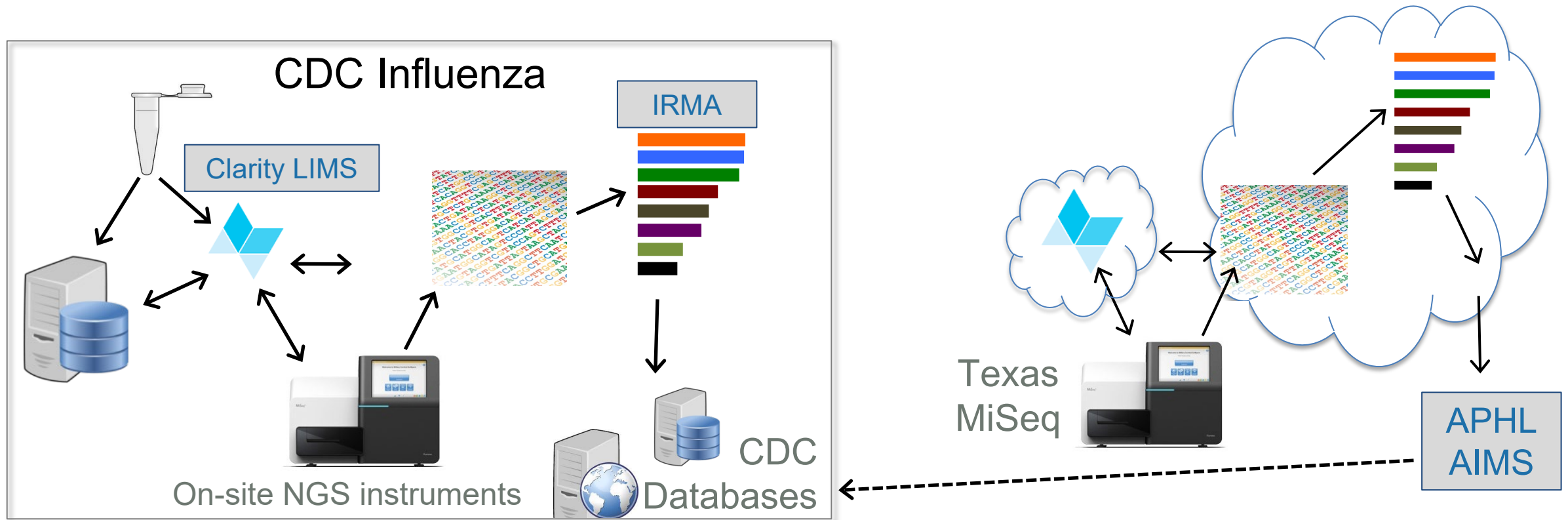
## Sequencing and Submission

Sequencing, submission of metadata to Clarity LIMS, checking consensus sequences for errors/deletions.

Submission of sequences to CDC's Influenza database.

# Project Overview: Informatics Set-Up

Texas Mirrored Workflow Setup for National Influenza Reference Centers



**IRMA:** Iterative Refinement Meta-Assembler; genome assembly, variant calling, phasing of RNA viruses

**Clarity LIMS:** track reagent information, sample metadata, sample progress/re-queueing

**(APHL AIMS) APHL Informatics Messaging services:** cloud-based e-data sharing



## Sending to California NIRC:

- ✓ Shipments every two weeks
- ✓ Texas specimens stored in VTM
- ✓ Tested PCR positive with Ct Value  $\leq 28$
- ✓ DOC no older than 14 days, preferable  $< 7$  days
- ✓ No Flu A + Flu B co-infections
- ✓ Maximum number of sample requested biweekly:
  - 6 Flu A (H3N2) positives
  - 4 Flu A(H1N1)pdm09 positives
  - 4 Flu B/Victoria positives

## ISC:

- ✓ No samples already being sent to CDC/NIRC
- ✓ Co-infections with SARS-CoV-2 allowed, but must be annotated on G-2V submission form



# Validation Testing

## Proficiency Test #1:

Dilution series of 3 Flu Controls: H1N1, H3N2, and Bvic

- Unable to detect all segments at higher dilutions
- Some of our negative controls showed contamination (>1000 reads)



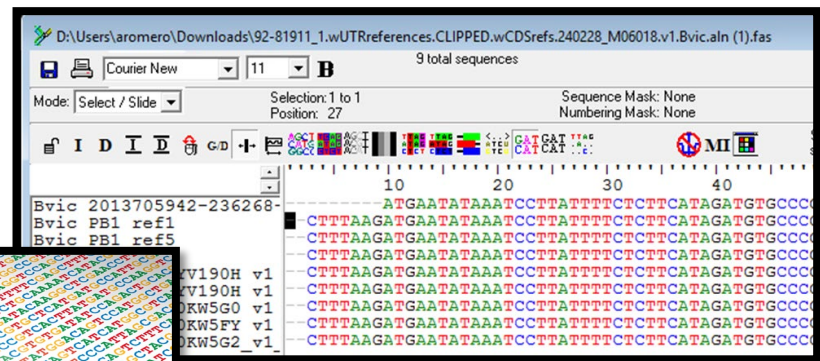
## Proficiency Test #2:

24 previously tested samples with good quality results.

We were given the green light to start sequencing in Jan 2024.

# Sequencing Workflow

Includes updating progress in Clarity LIMS with each step

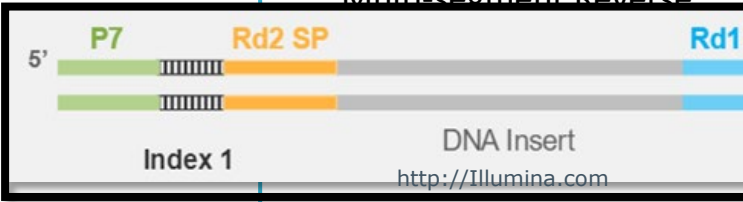


## Exonuclease

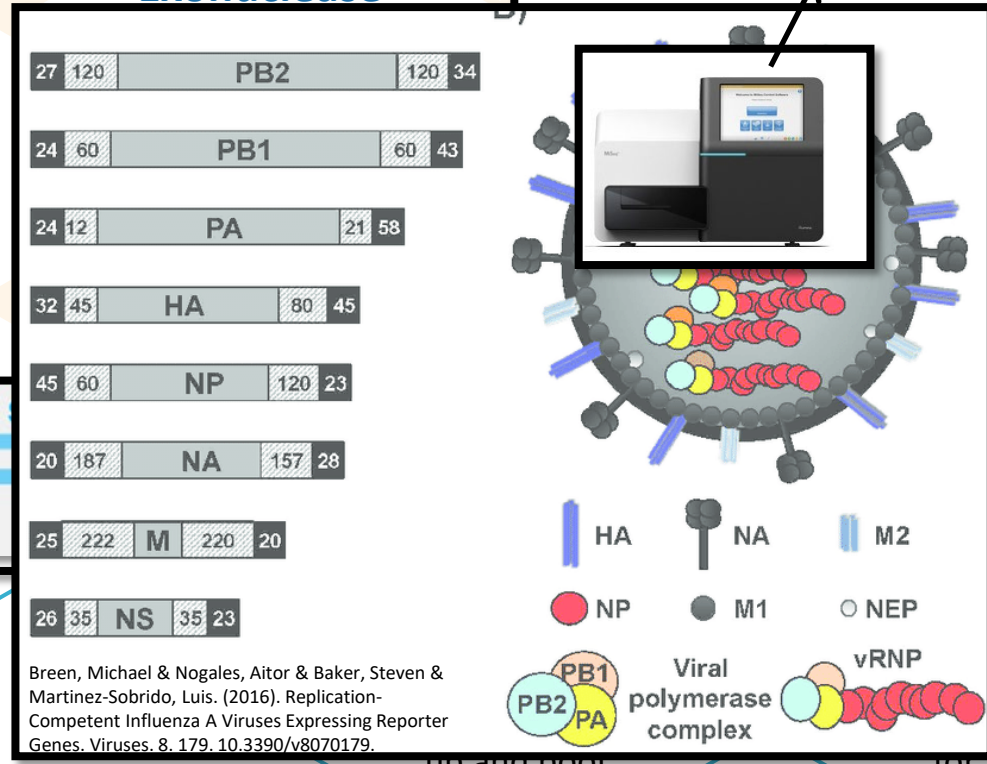
10.1 Typical ... of the MRT-PCR as seen on the QIAxcel Advance system.

## RNA → DNA

Multi-segment Reverse



8 segments



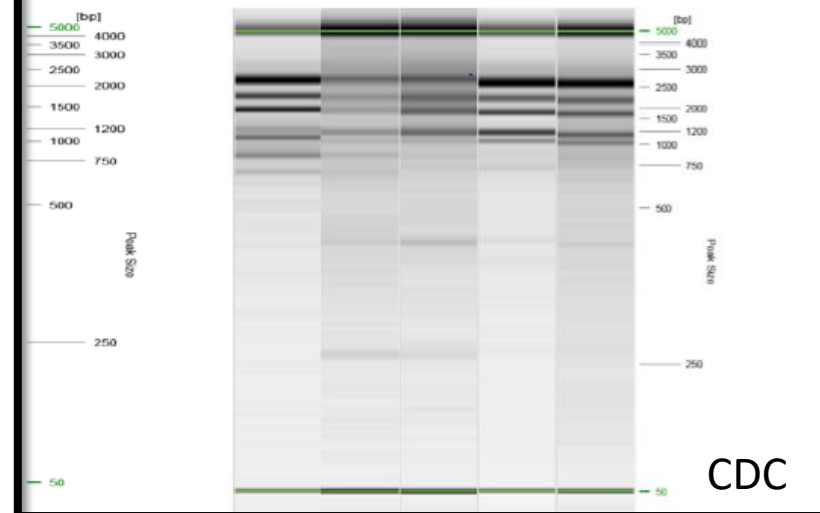
Breen, Michael & Nogales, Aitor & Baker, Steven & Martinez-Sobrido, Luis. (2016). Replication-Competent Influenza A Viruses Expressing Reporter Genes. *Viruses*. 8. 179. 10.3390/v8070179.

up and pool

for analysis

mer set)

Figure IB. MRT-PCR products (FLU B Virus-GA2 primer set)



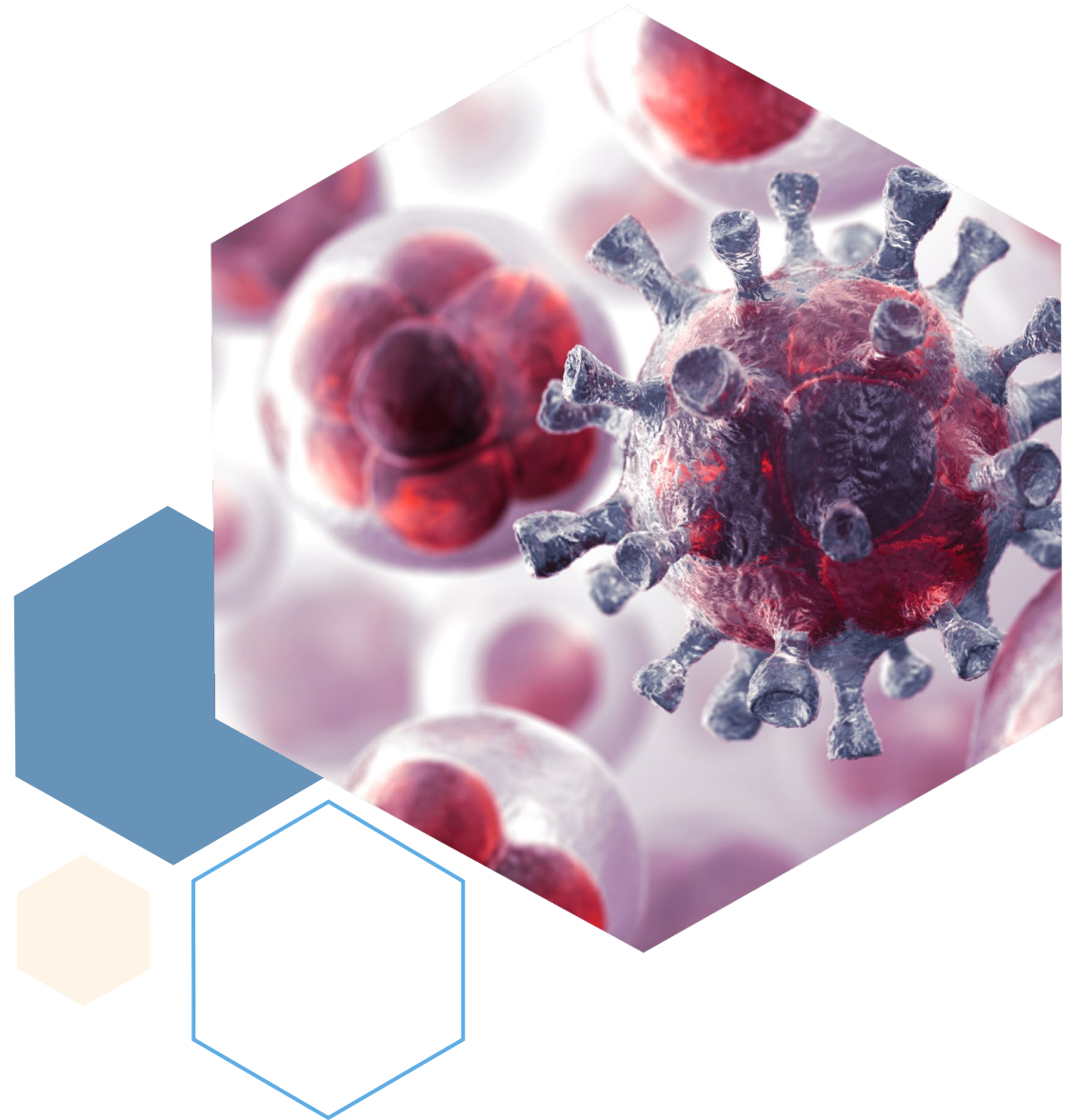
CDC

Automatically with CDC/APHL

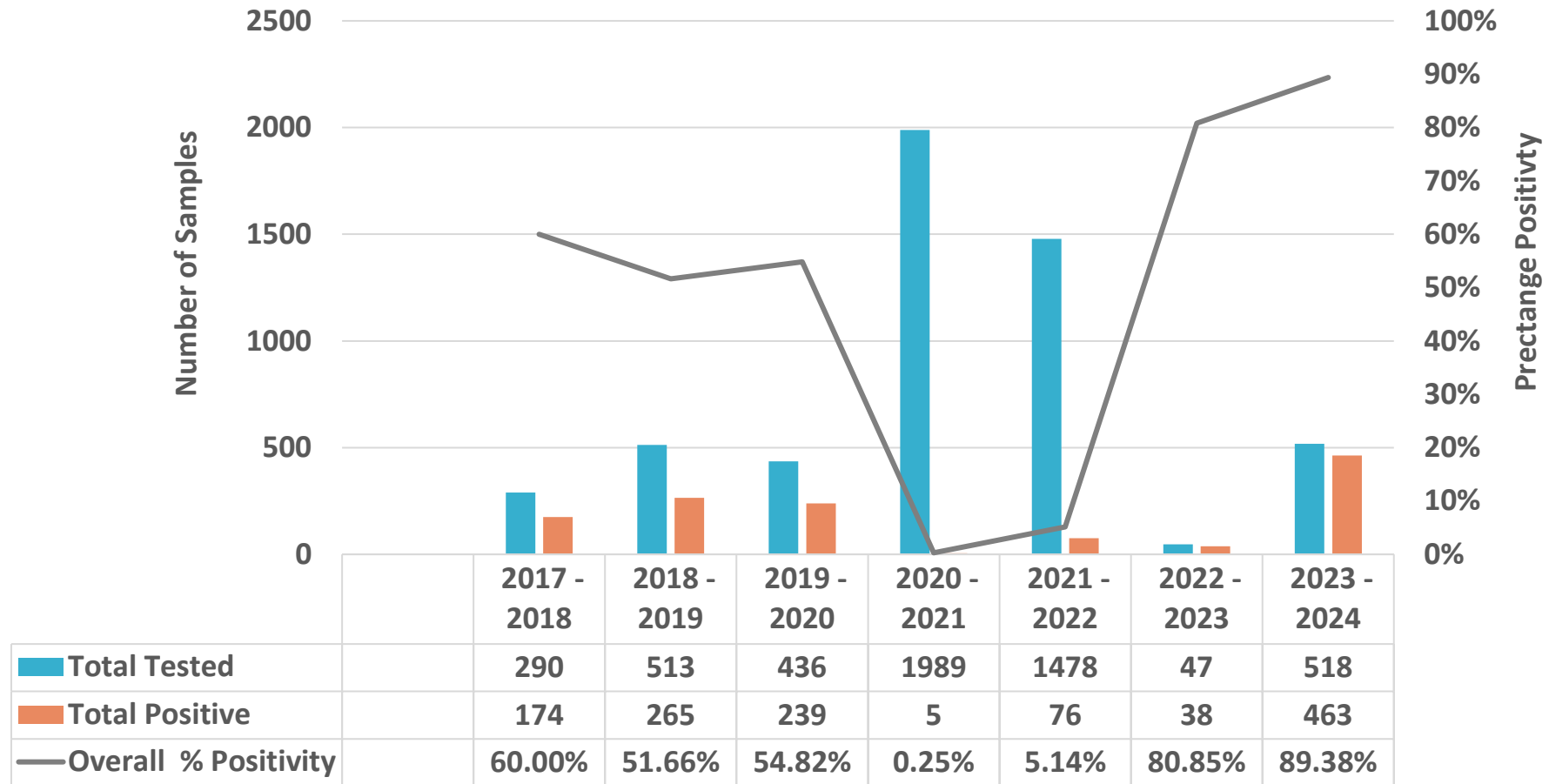
# 2023-2024 Flu Testing Summary

The Lab received 518 samples for Flu testing

- Tested 483 positive Flu samples.
- 96 samples sent to CA NIRC.
- 53 samples sequenced in-house.

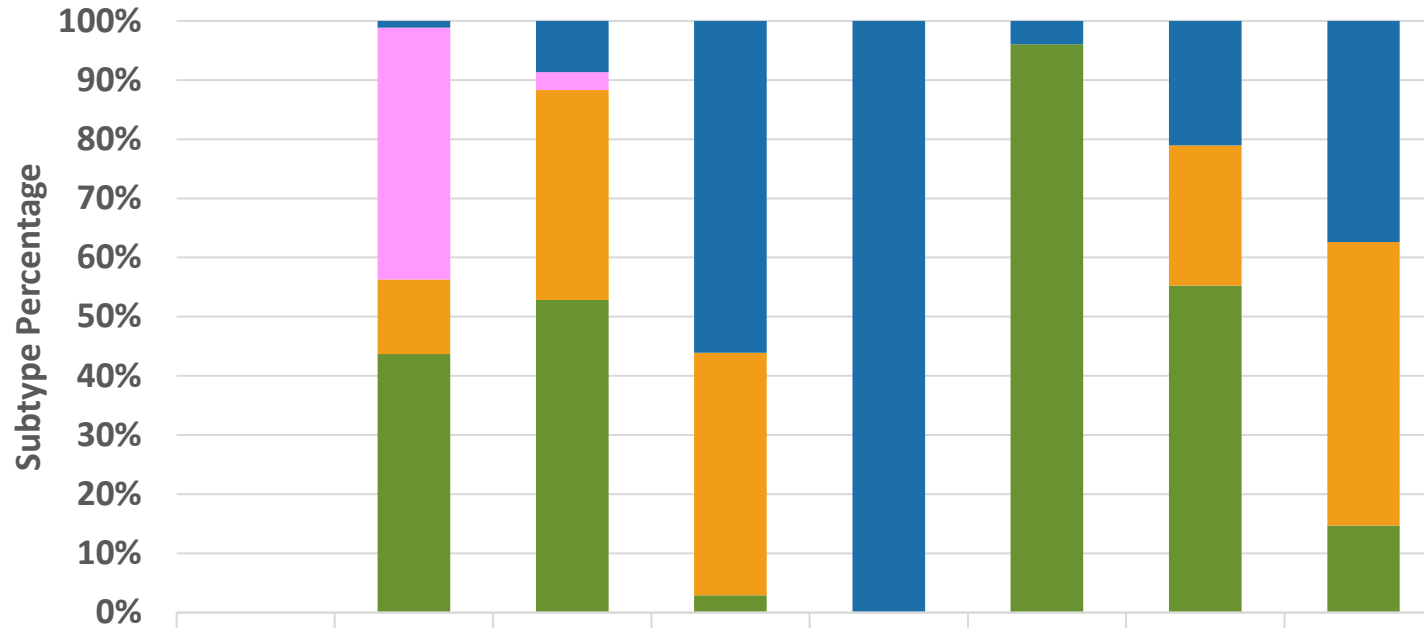


# DSHS Positivity Rate During Flu Seasons

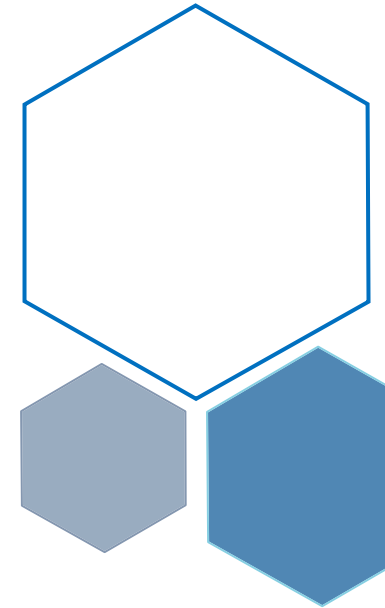
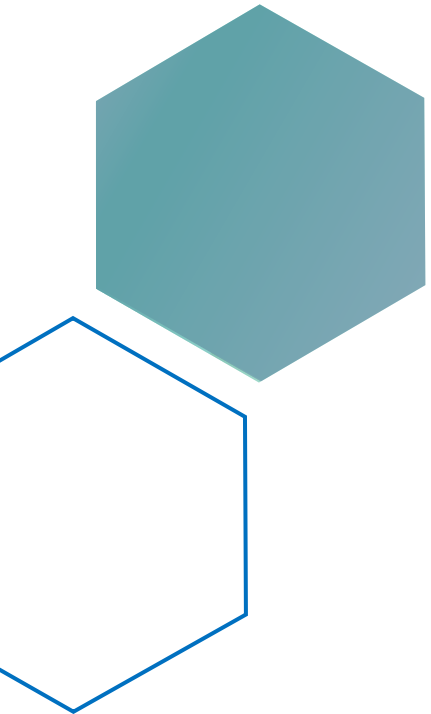




# Flu Subtype Breakdown Over Time



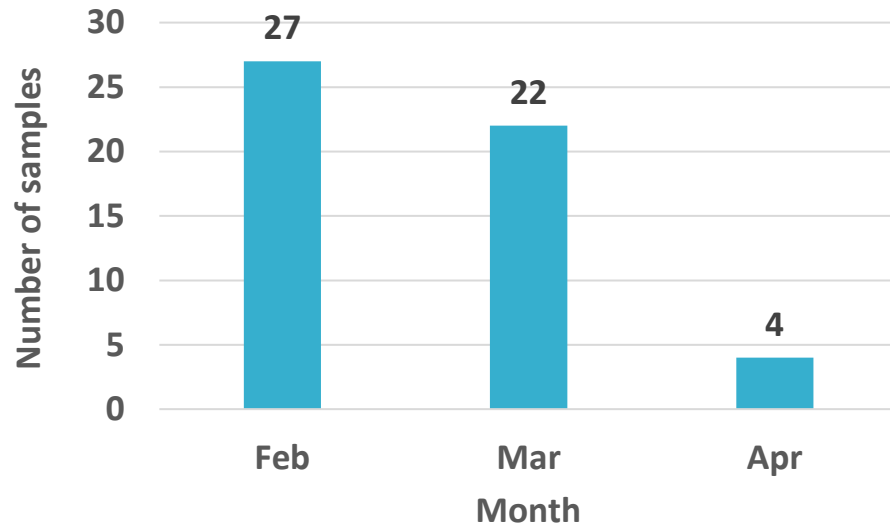
	2017 - 2018	2018 - 2019	2019 - 2020	2020 - 2021	2021 - 2022	2022 - 2023	2023 - 2024
<b>B/Victoria</b>	2	23	134	5	3	8	173
<b>B/Yamagata</b>	74	8	0	0	0	0	0
<b>A(H1N1)pdm09</b>	22	94	98	0	0	9	222
<b>A(H3N2)</b>	76	140	7	0	73	21	68



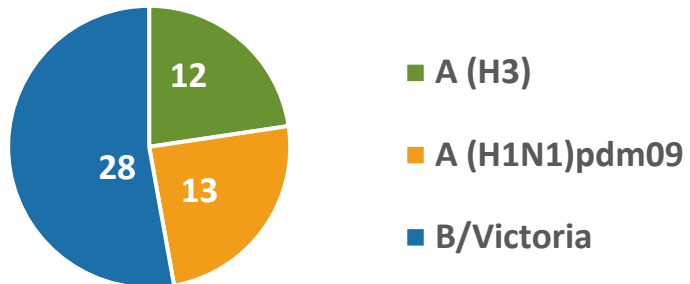
# Sequencing Statistics

We sequenced 53 samples over 7 sequencing runs

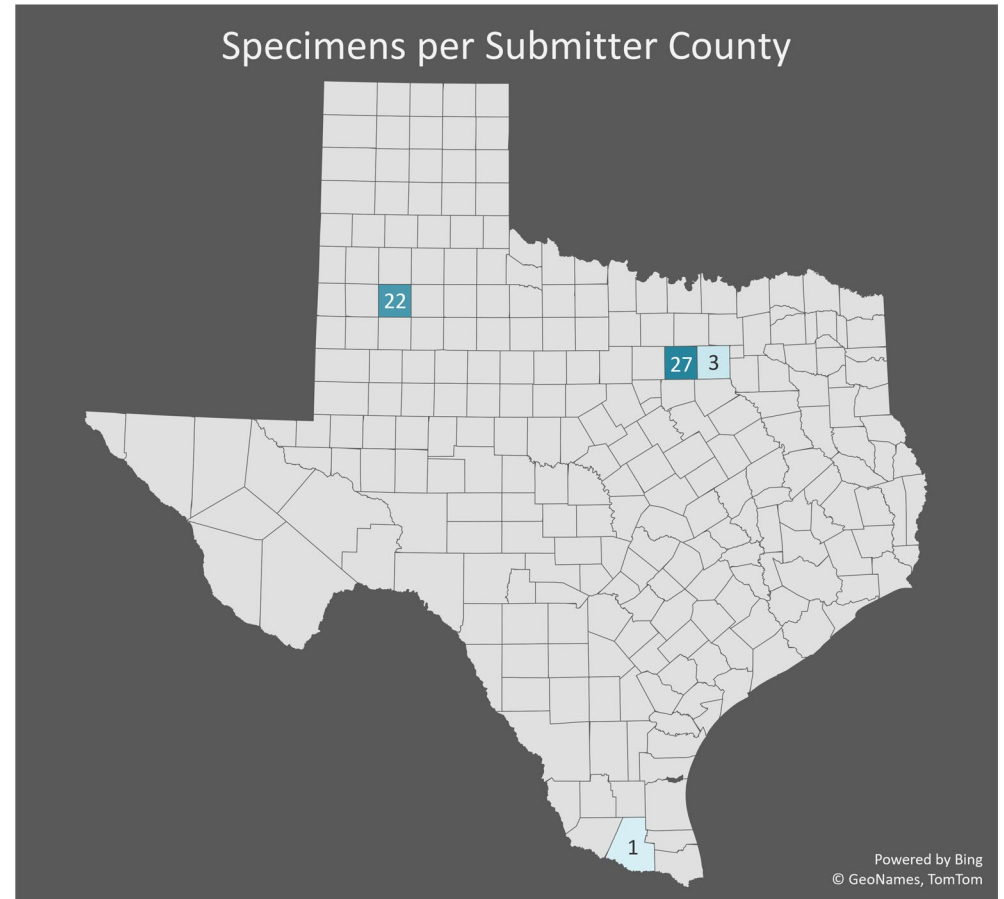
### Samples Sequenced by Month



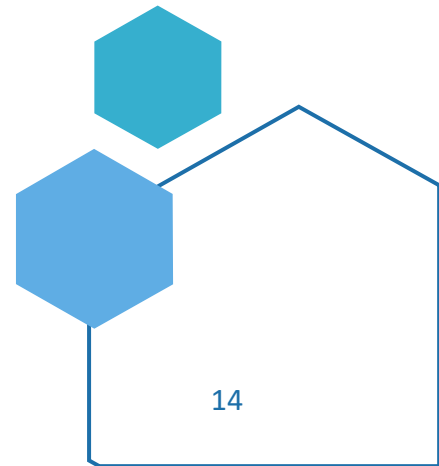
### Number of Samples by Subtype



### Specimens per Submitter County



Submitter County	Specimens
Tarrant County	27
Lubbock County	22
Dallas County	3
Hidalgo County	1



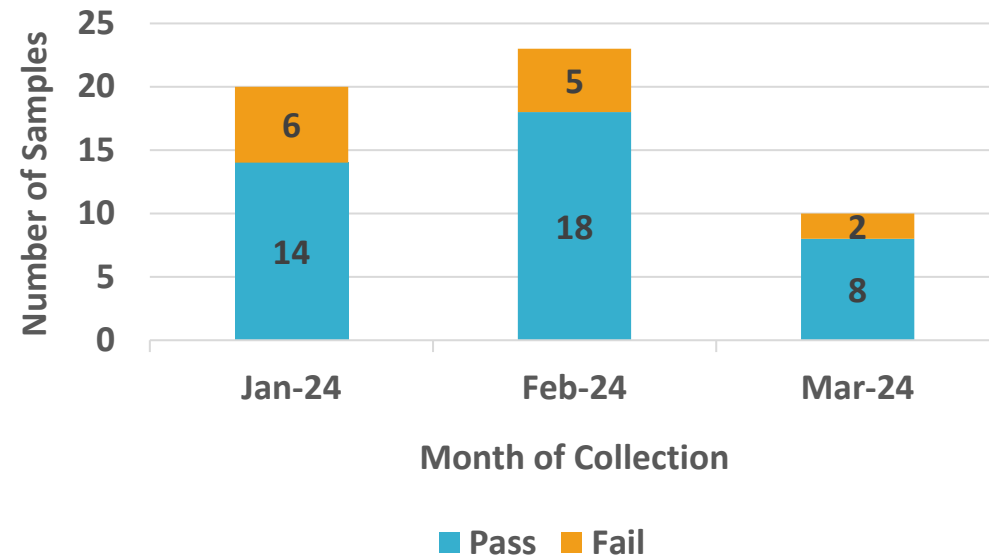


## GISAID: Global Initiative on Sharing All Influenza Data

❖ Established in 2008 for open access sharing of influenza data and has expanded to an epidemic and pandemic virus database.

- EpiFlu™
- EpiCoV™
- EpiRSV™
- EpiPox™
- EpiArbo™

GISAID Submissions of TX ISC Data





Registered Users | **EpiFlu™** | EpiCoV™ | EpiRSV™ | EpiPox™ | EpiArbo™ | My Profile

EpiFlu™ | Search | Back to results | Worksets | Upload | Batch Upload | CLI Upload | Settings | Analysis | Help

 AudacityInstant	 BLAST	 Emerging Variants	 EpiCharts	 FluSurver	 PrimerChecker (beta)
 Search & Browse	 Submission Tracker	 HA Mutation Surveillance	 Subtype / Clade Frequency		
 Vaccine Reference Sequences	 Phylodynamics Global	 Phylodynamics Global	 Phylodynamics Global	 Phylodynamics Global	 Phylodynamics Global

**Analysis Update (2024-07-03)**

 Clade progression in all sequences	 Regional distribution of subtypes	 H5N1 Bird Flu Circulating in Dairy Cows	 Influenza Hemagglutinin mutation surveillance	 Emerging variants by Spread (page 1)	 Emerging variants by Spread (page 2)
 Emerging variants by Spread (page 3)	 analysis update.pdf				



# Common Reasons Samples Cannot be Sent to NIRC or be Sequenced In-House



## DOC timeline not met

- Biggest issue is the 2 weeks from DOC time limit



## 21 samples UNSAT for testing

- Specimen received cold >72 hrs from collection
- Sample leaked in transit
- Specimen and/or Form discrepancies

# Future Goals

## Successes

Implementation of APHL AIMS IT set up for data sharing

PHLIP messaging for samples tested to CDC

53 samples sequenced

Next ISC Contract: Oct 2024-June 2025

## Room for improvement

Goal of 500 samples sequenced in-house

Increase recruitment of samples sent to Lab by 2-fold

We intend to apply for next round of NIRC and ISC applications (2025-2030)



*Thank you*

[Bonnie.Oh@dshs.texas.gov](mailto:Bonnie.Oh@dshs.texas.gov)

[WGS.DSHS@dshs.texas.gov](mailto:WGS.DSHS@dshs.texas.gov)

*Special Thanks to:*

- ❖ Clinical Virology Team
- ❖ LabWare Team
- ❖ Bioinformatics Team
- ❖ Astrid Romero, Michael Jost, Shivangi Vayla
- ❖ Esther Lee
- ❖ DSHS Epis
- ❖ CDC and APHL