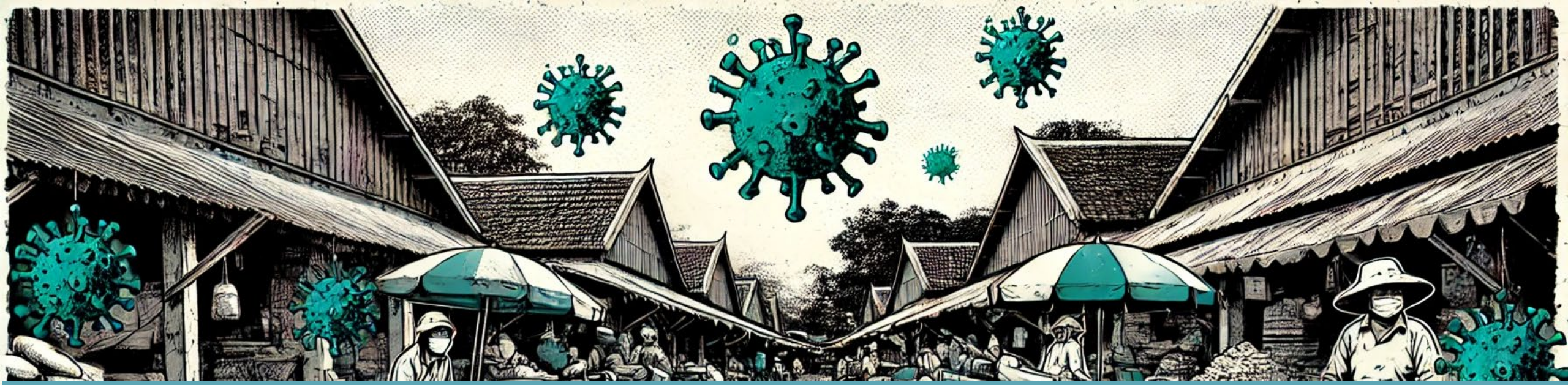


# Overcoming Blind Spots in Pathogen Detection: Novel Sample Collection and Diagnostics for Endemic and Emerging Pathogen Surveillance


**Dr. Erik Karlsson**

Head of Virology Unit
Director, National Influenza Center
Director, WHO H5 Reference Laboratory
Institut Pasteur du Cambodge

Institut Pasteur du Cambodge

PASTEUR NETWORK

INSTITUT PASTEUR DU CAMBODGE - Virology Unit | 1996-2024

# 28 years of surveillance at the animal-human interface

# What are we already looking for? Some examples…

AGRICULTURAL DISEASES

Newcastle Disease
Avian
Coronaviruses
**Influenza**

BAT-BORNE DISEASES

Nipah
Filoviruses
Coronaviruses
Paramyxoviruses
Astroviruses
**Influenza**

RODENT-BORNE DISEASES

Arenavirus
Hantavirus
Hepatitis E
**Influenza**

VECTOR-BORNE DISEASES

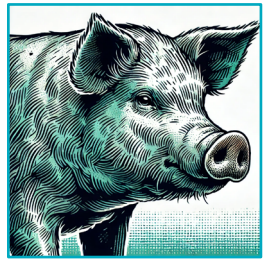
Zika
Dengue
Chikungunya
Malaria
Rikettsial
Pathogens

COMPANION ANIMALS

Rabies
**Influenza**

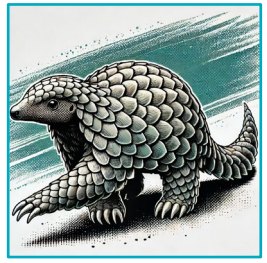
HUMAN DISEASES

Retroviruses
Enteroviruses
Papillomavirus
Hepatitis
**Influenza**



SWINE DISEASES

African Swine Fever
Japanese Encephalitis
**Influenza**



OTHER ZOONOTIC, EMERGING, AND UNKNOWN PATHOGENS

mPox
Acute Hepatitis
Wildlife Diseases
**Influenza**

# Early Warning: we need to get ahead of endemic and emerging diseases at high-risk interfaces

## ANTICIPATE

Ability to **understand risks** of disease emergence and determine potential hotspots

## DETECT

Ability to **quickly detect** emerging diseases to prevent and/or slow down potential spread

## PREVENT

Ability to **actively reduce** and/or protect against diseases before they emerge

## RESPOND

Ability to quickly react and **break the chain of transmission**

High-risk interfaces require active surveillance.

...but active surveillance is costly and time consuming


...and often it is not always in the easiest of places

## Environmental Samples present a viable option to improve **Early Warning** for avian influenza at high-risk interfaces

The ability to use environmental samples for in-field diagnostic surveillance and discovery would not only increase surveillance capacity, but also contribute to our understanding of new or emerging pathogens at high-risk interfaces


Cages
Wash/
Waste
Water
Feathers
Surfaces
Air

# IBMS

# LBMs


ABBATOIRS


FARMS

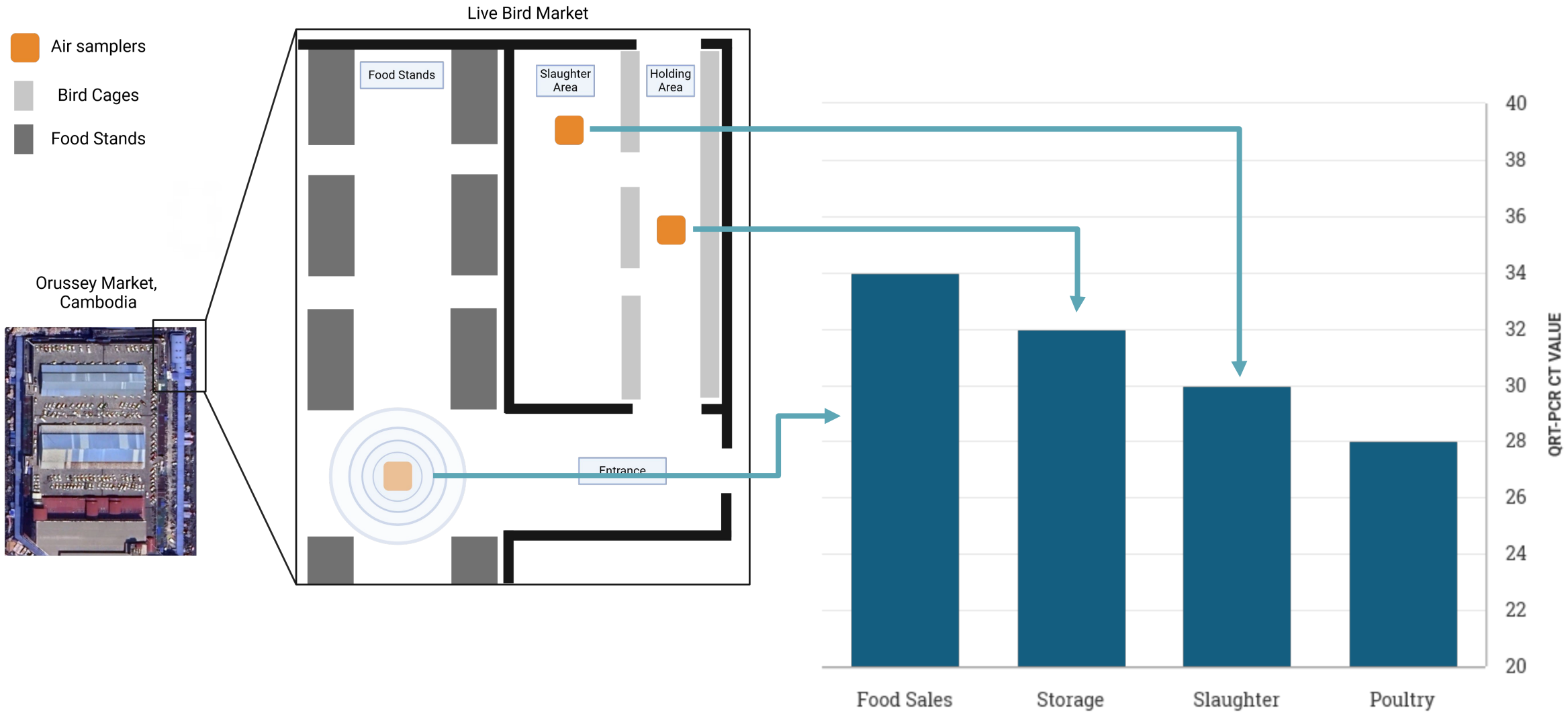

CAVES


Sampling in wild and captive animals

...and even remote

# Does it work?

# Detection of AIV in paired poultry and air samples in a Cambodian LBM by qRT-PCR




HORWOOD ET AL *ZPH*
*HTTPS://DOI.ORG/10.1111/ZPH.13009*

BUT IT'S NOT JUST ABOUT THE SAMPLES…

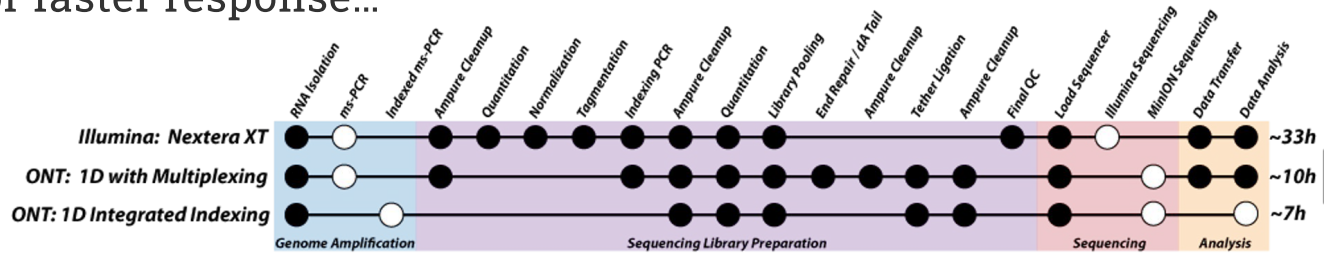
# Development of rapid, field-forward diagnostics

ONE HEALTH IN ACTION

# Integrated Indexing for Rapid Genomic Surveillance in Humans and Poultry

# Integrating indexing and real-time bioinformatics to decrease turnaround times

## Enabling rapid genomic surveillance allows for faster response…




### Tailed priming enables one step RT-PCR indexing

Incorporation of A-tailing and end repair in PCR

Single final step with ONT ligation reagents

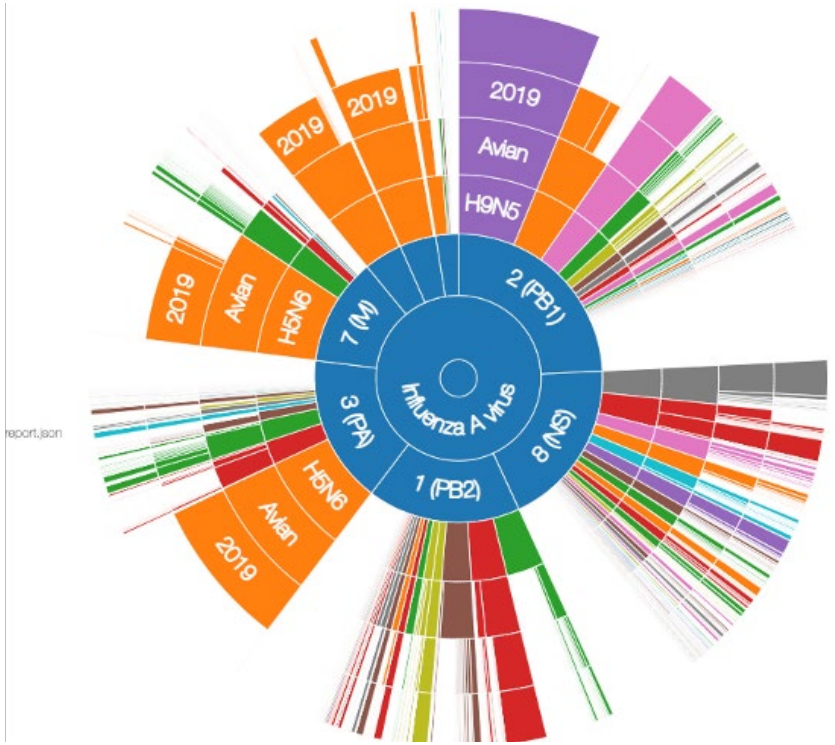
### Bioinformatics are laptop-deployable and cloud-enabled

- Enables ultra-fast response timelines
- Reduces analysis bottlenecks
- Does not require internet connectivity

### Raw data visualized by subtype in real-time

- Real-time subtype information
- Quick evaluation of historic reassortment events
- Explorable visualization to identify potential host origins

### Can be expanded to other amplicon-based systems and even to metagenomics

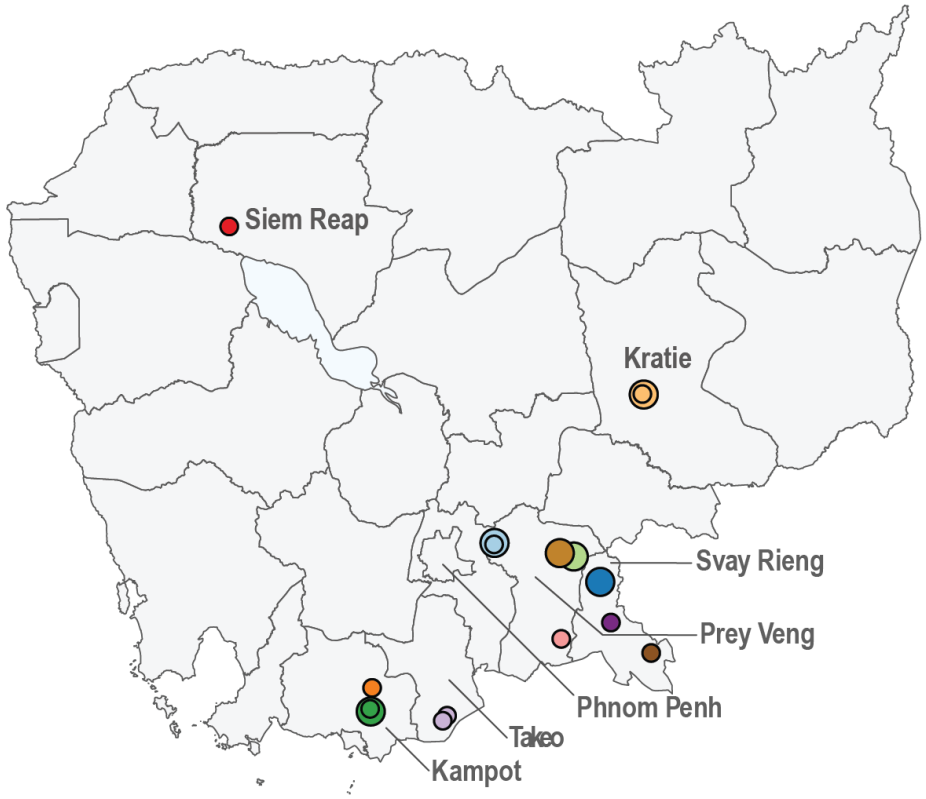


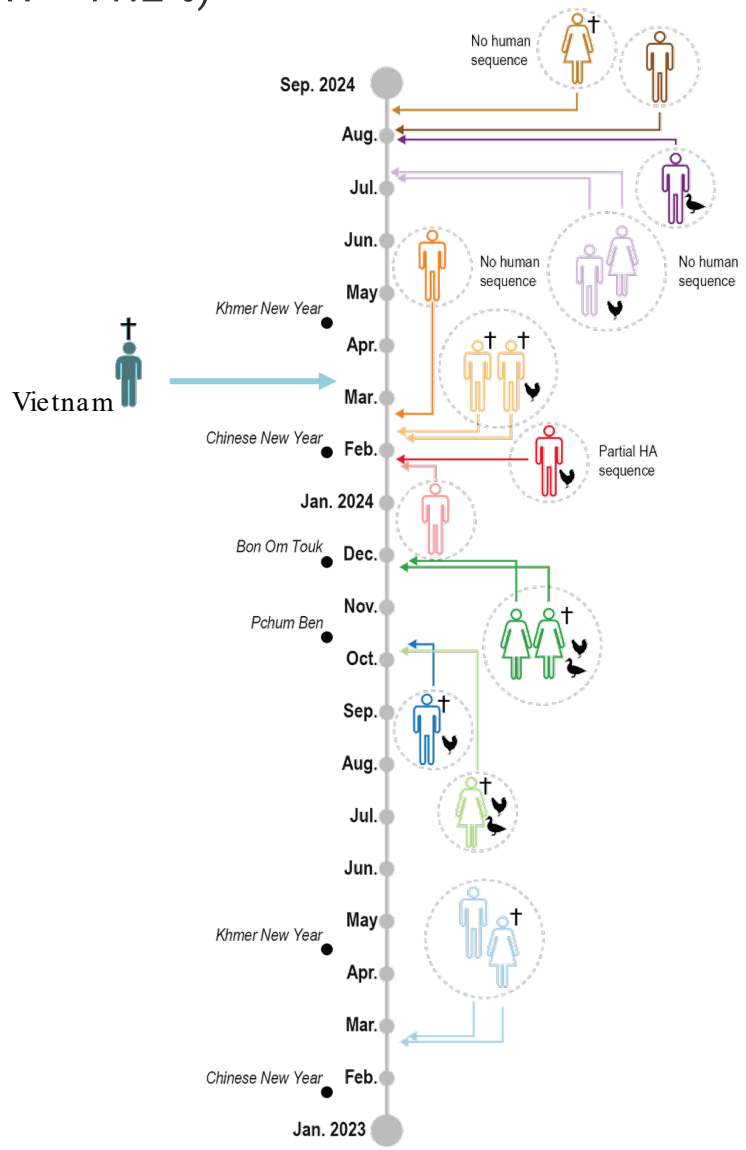
..and we can do it anywhere, anytime, for anything...

Nanopore technology allows us to do rapid genomic surveillance in real-time with just a laptop

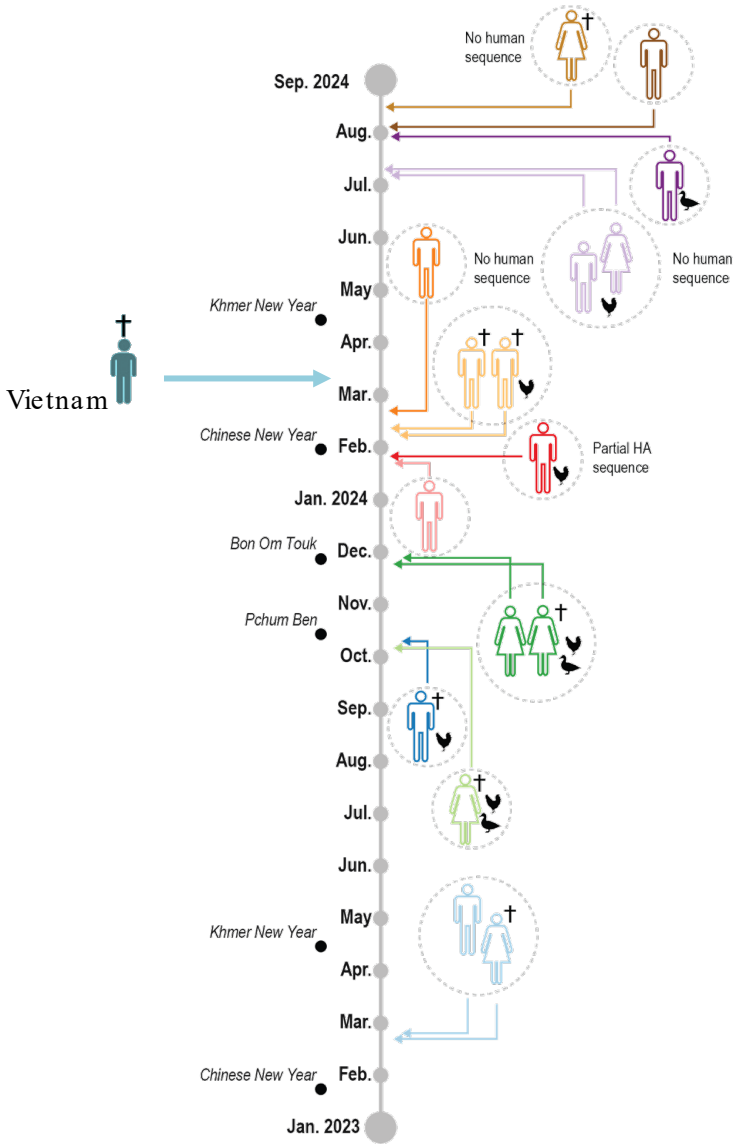
# Human A/H5 cases in the Greater Mekong Subregion 2023-2024

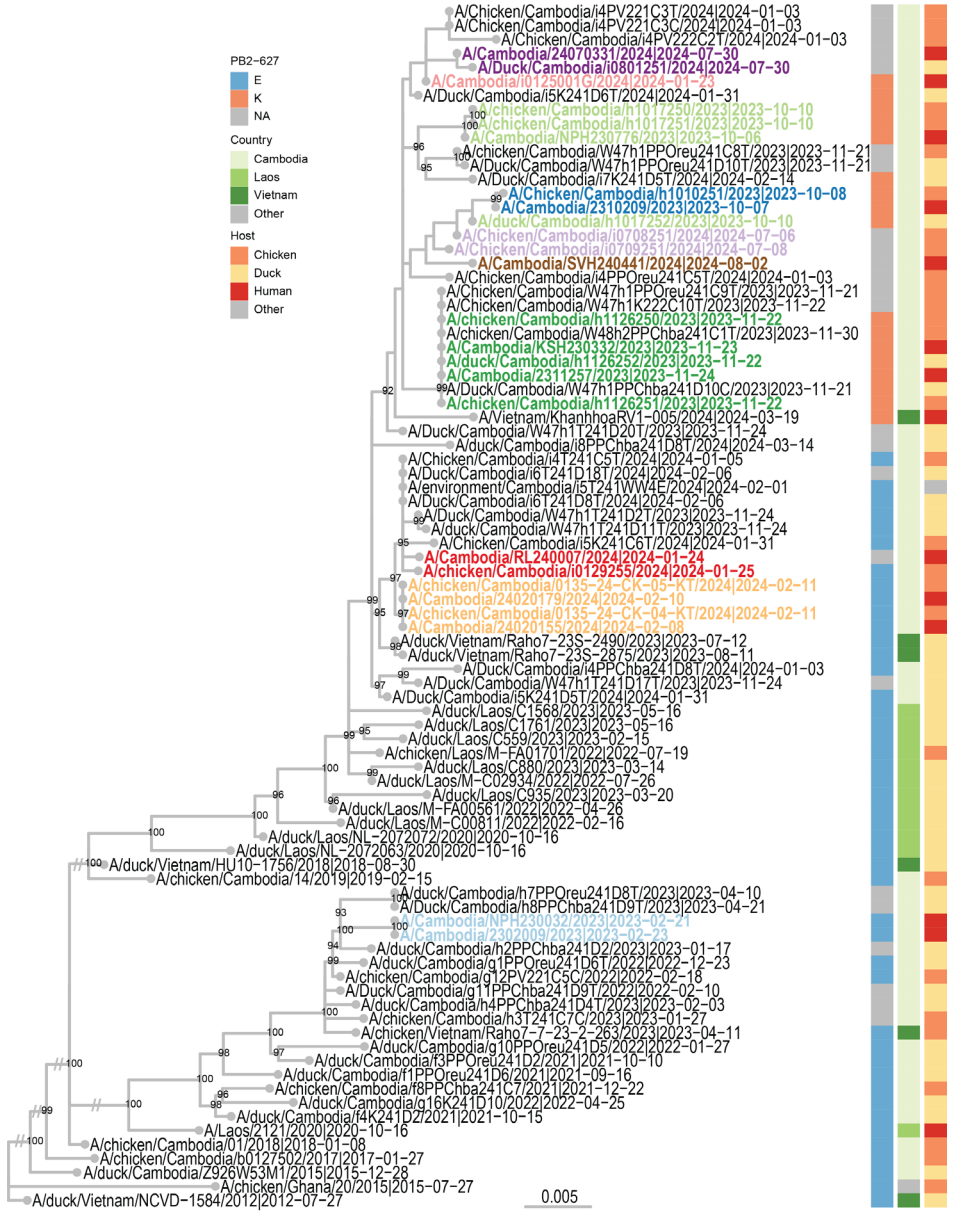
17 cases of A/H5 in the GMS since Feb 2023, 7 deaths (CFR = 41.2%)
In Cambodia: 16 cases, 6 deaths (CFR = 37.5%)



# Human A/H5 cases in the Greater Mekong Subregion 2023-2024



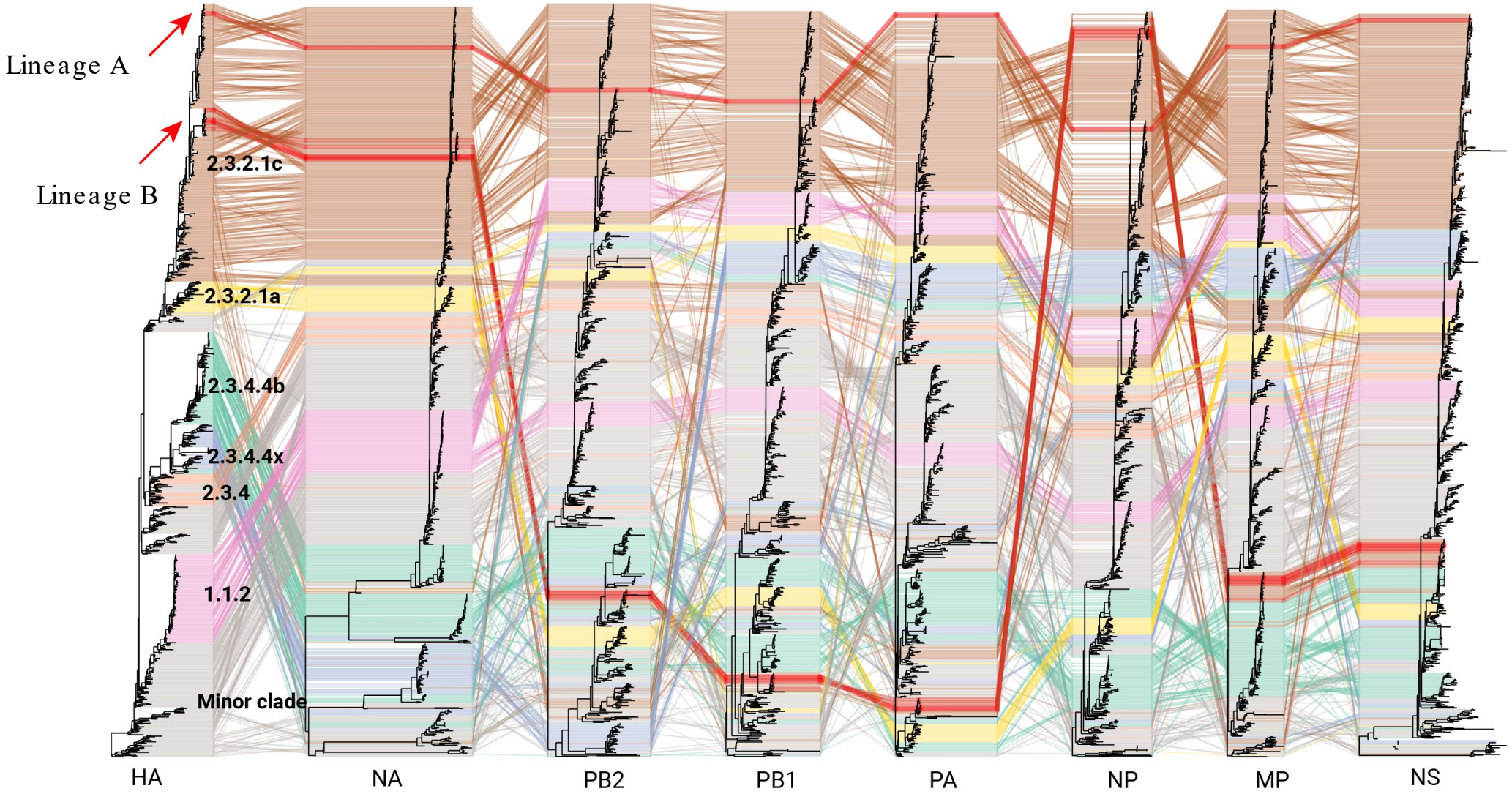

**All cases can be genetically linked to infected poultry (when available) indicating direct poultry-to-human transmission with no evidence of human-to-human spread

Lineage B

Lineage A

# Viruses in Lineage B are reassortments between 2.3.2.1c and 2.3.4.4b

## 2.3.4.4b is known to reassort into numerous genotypes

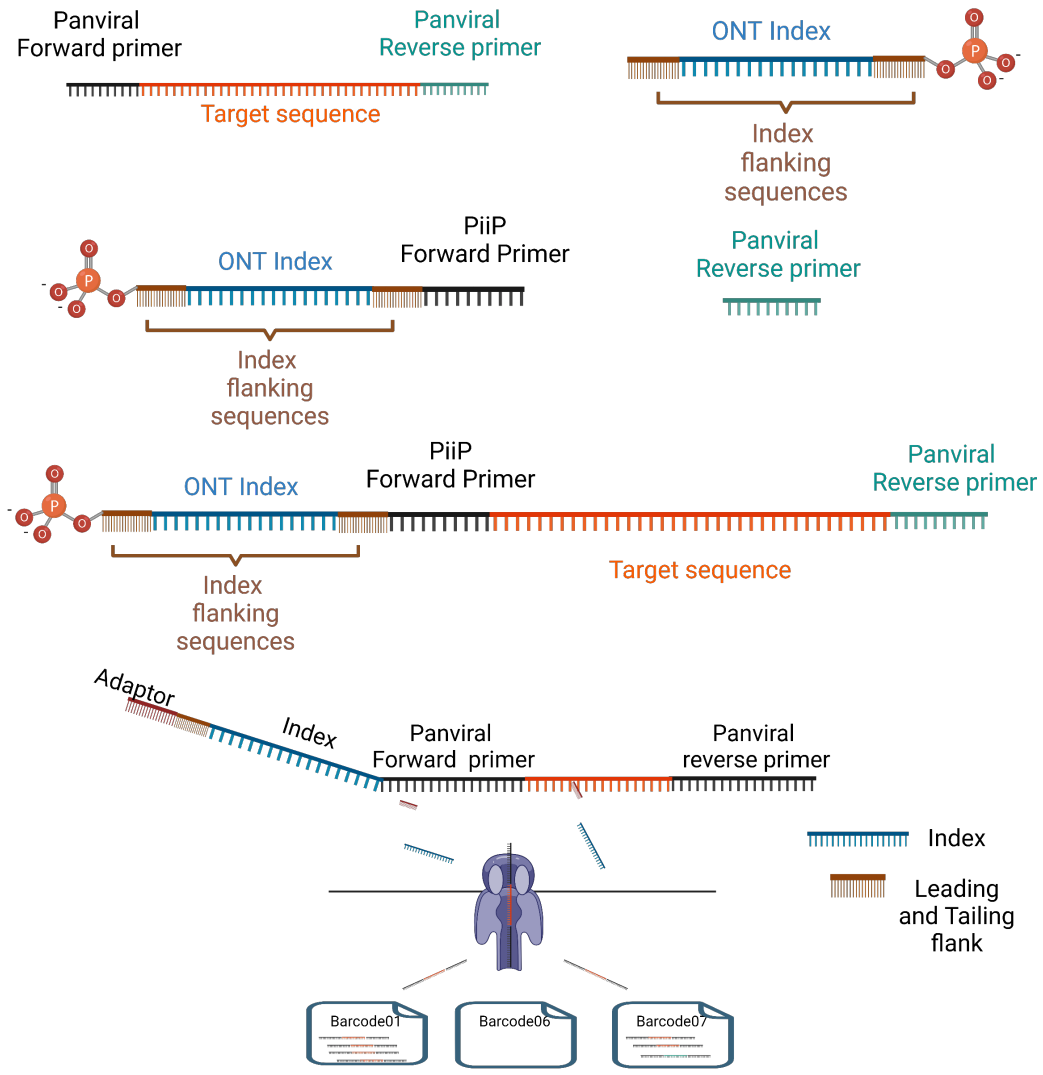


MOVING FURTHER

# Multiplex integrated index pan-viral family assays

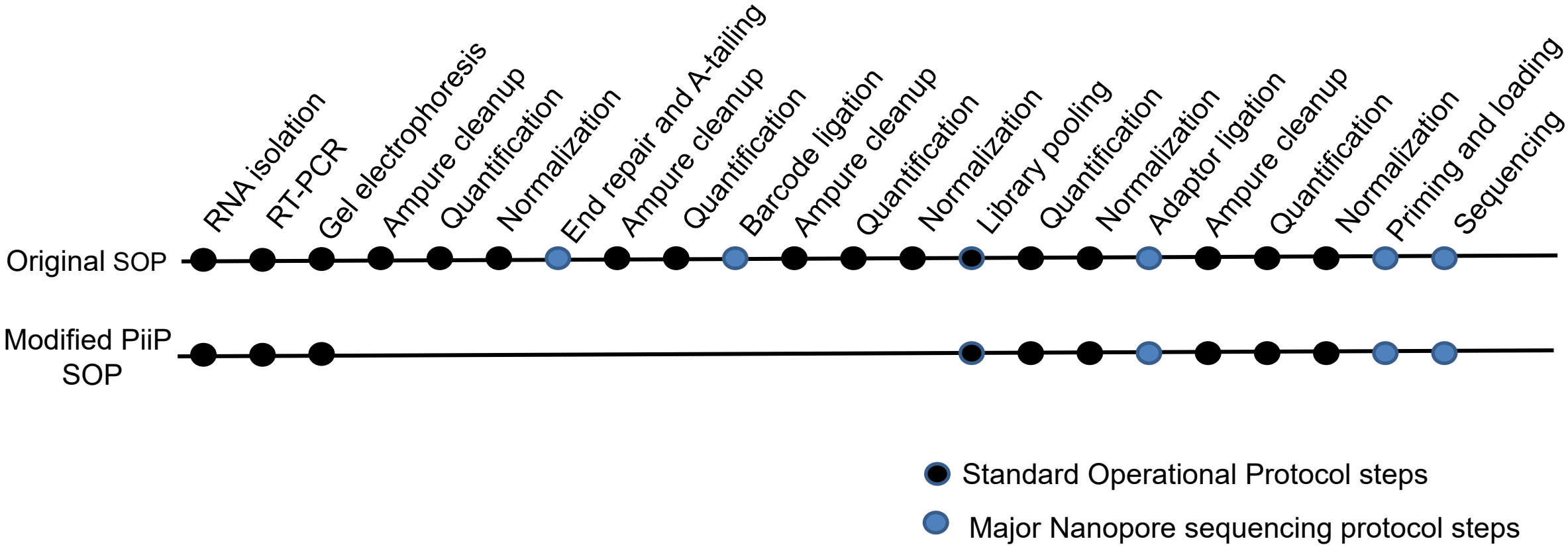
# Generation of Viral Pan-family Integrated Index Primers (PiiP)

## Integration of IAEA/FAO Pan-family primers with integrated Nanopore indexed barcodes

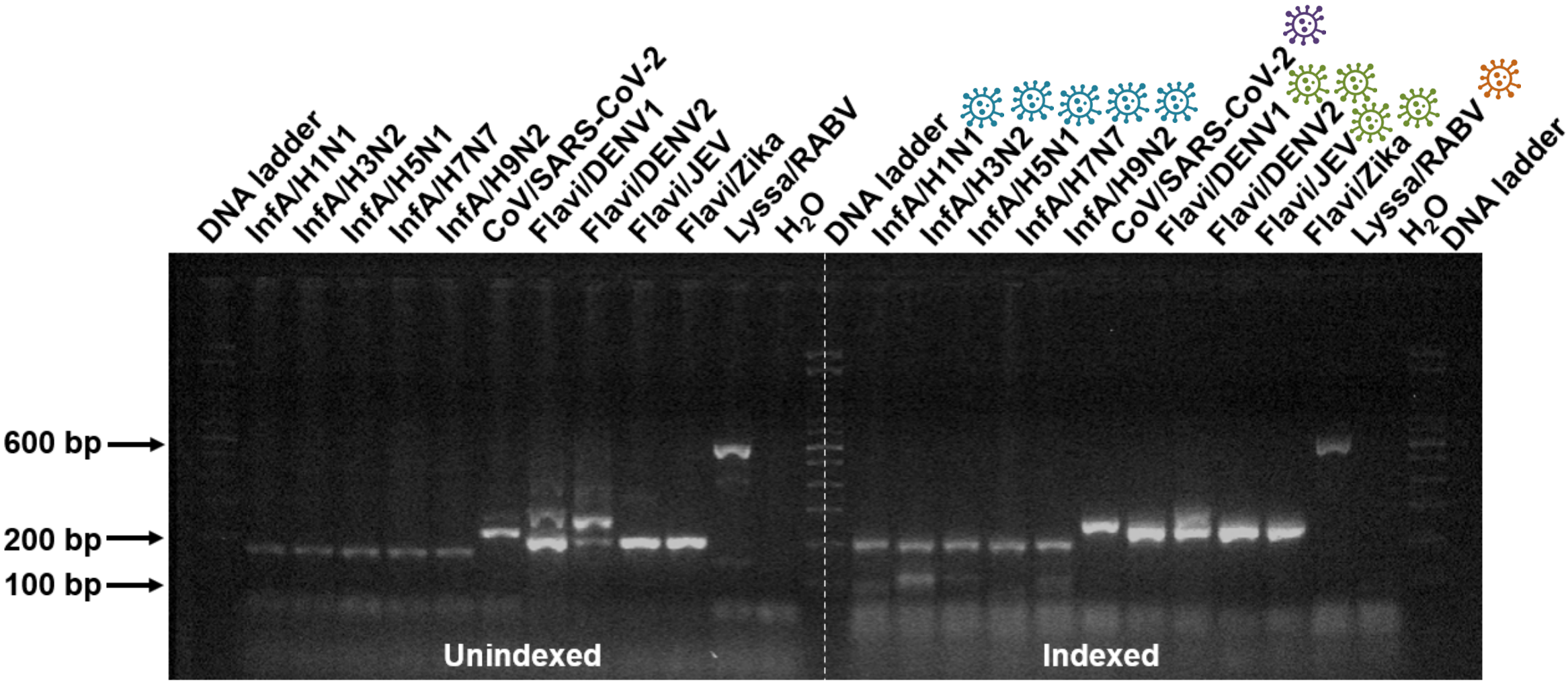



# Generation of Viral Pan-family Integrated Index Primers (PiiP)

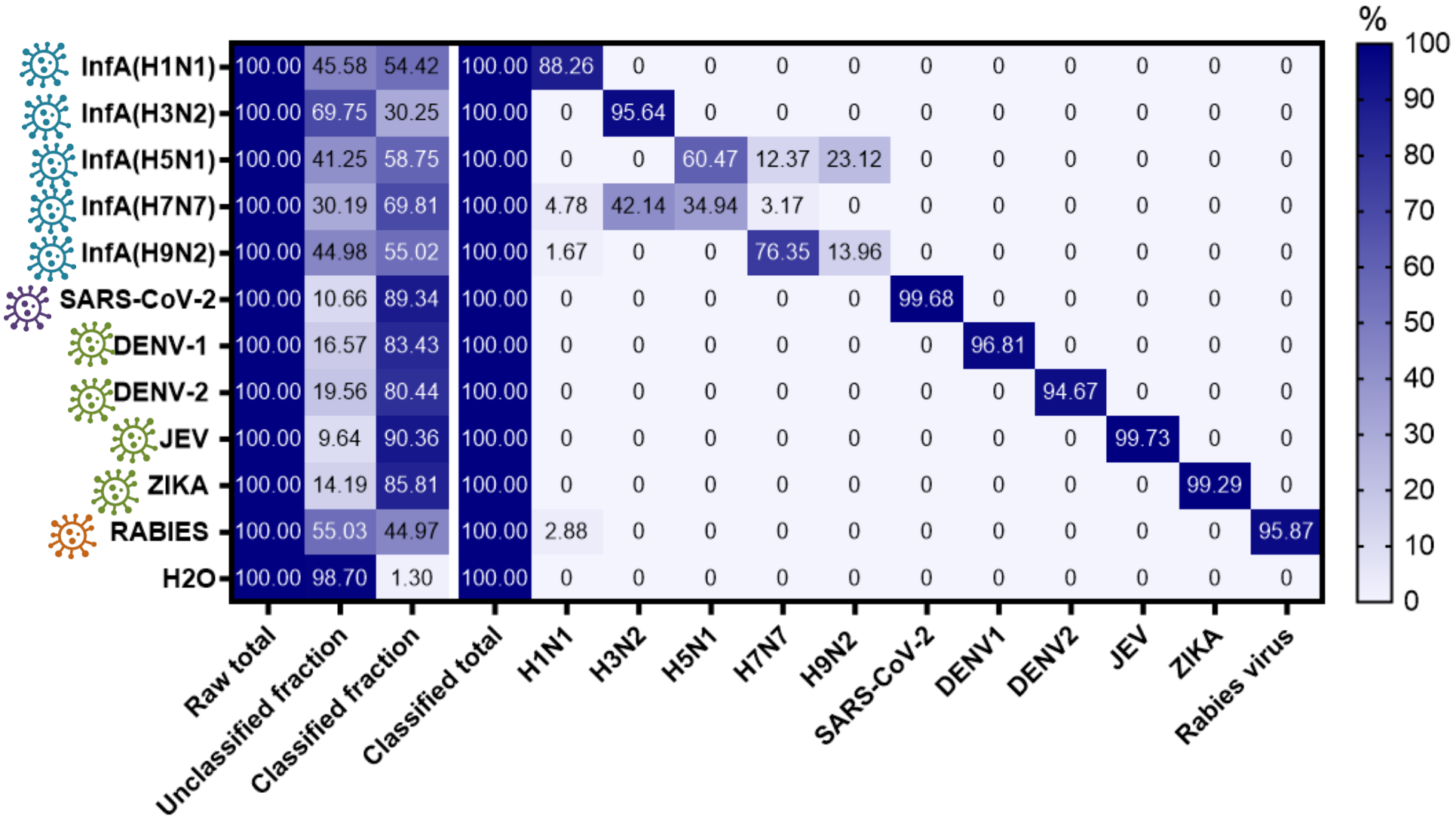
Saves time AND money…

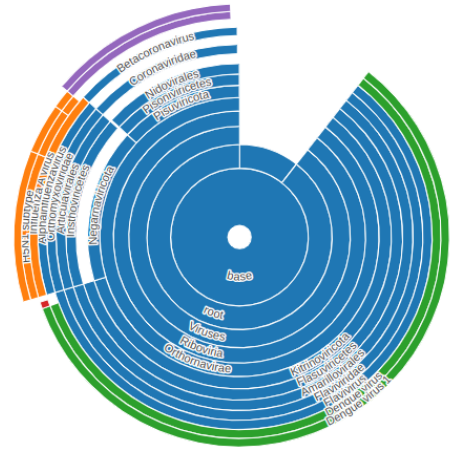


# Will PiiP primers work for multiple viral families in the same tube?



# Will PiiP primers work for multiple viral families in the same tube?




**Legend**

Total Reads 71096
at Rank Code: S

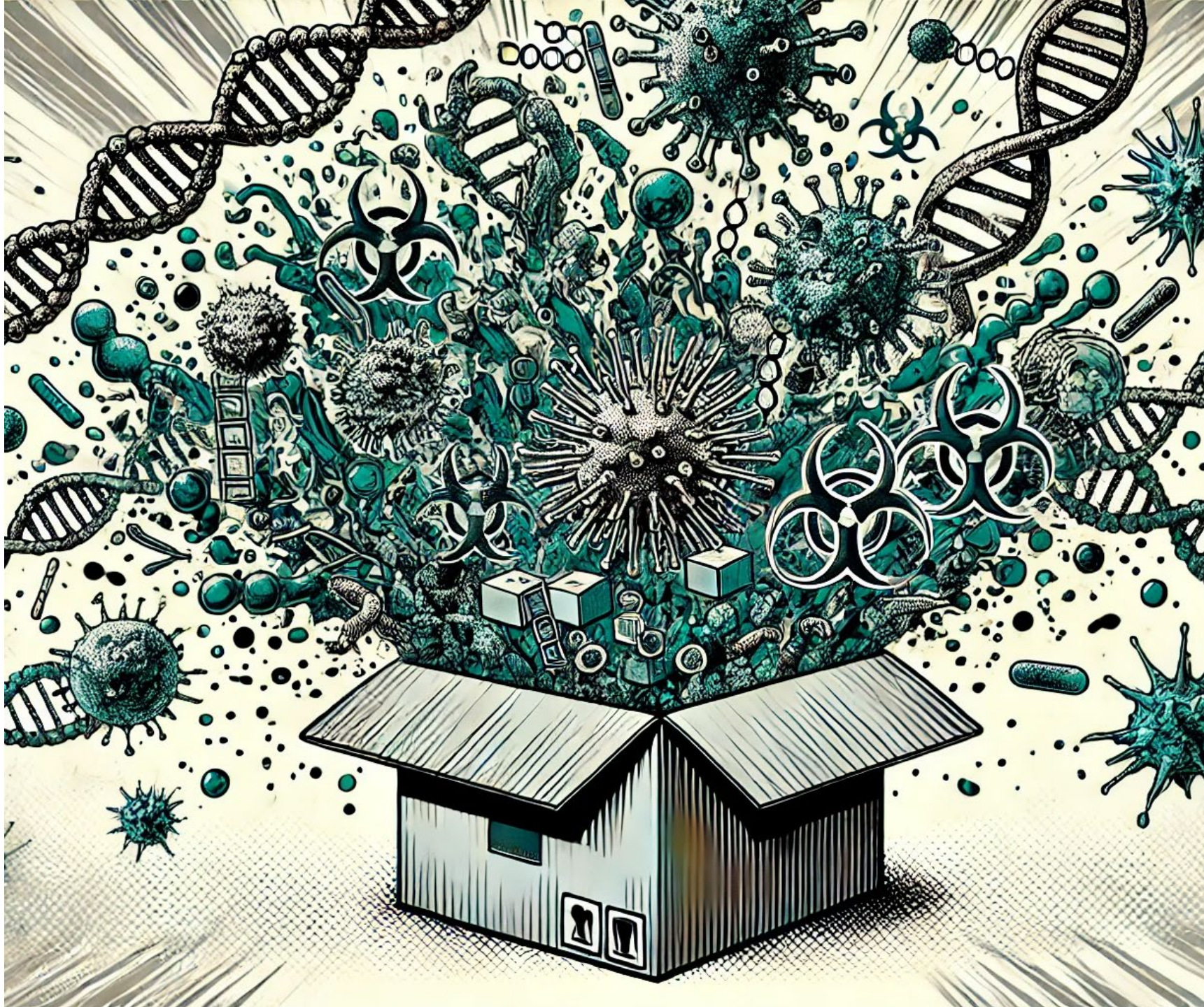
Sample: MIX_NB11

- Dengue virus (58.923%) (47126 fragments classified) taxid: 12637
- Influenza A virus (16.270%) (13013 fragments classified) taxid: 11320
- Severe acute respiratory syndrome-related coronavirus (13.184%) (10
- Cell fusing agent virus (0.516%) (413 fragments classified) taxid: 3165


Le 10 Février 1995
L'INSTITUT PASTEUR DU CAMBODGE
a été inauguré par
SON ALTESSE ROYALE LE PRINCE NORODOM RANARIDDH
Premier Président du Gouvernement Royal du Cambodge
et par
MONSIEUR BERNARD DEBRE
Ministre de la coopération de la République Française
...and it can all be done in the field

MOVING EVEN FURTHER...

# Can we combine ES with Metagenomics for even more rapid information



# **Metagenomics** to increase the utility of Environmental Sampling at high-risk interfaces

Metagenomic surveillance can uncover known and novel threats, provide Early Warning, and give a comprehensive picture of microbial ecology as it relates to infectious disease emergence and transmission. Integrating metagenomics into surveillance systems could improve prediction and prevention of zoonotic outbreak

## UNBIASED

Reduces bias compared to pathogen-specific diagnostics

## PATHOGEN DISCOVERY

Can identify all microbes present in a sample, including novel or unexpected pathogens - enables early detection of pathogens of concern and new zoonotic threats before outbreaks oc

## BROAD

Can identify geographic hotspots, seasonal patterns, and directionality of pathogen transmission between animals and human

## DYNAMIC

Can obtain information on both pathogens and hosts within an environement

## FUNCTIONAL

Can identify microbial genes involved in pathogenesis, resistance, and persistance

# Can NGS on Environmental Samples perform equal or better to "gold standard" PCR?

Can we use NGS to detect viruses in ES from the live bird market?

Is NGS comparable to qRT-PCR on ES and poultry samples from live bird markets?

Are ES samples able to effectively replicate what is detected in poultry in the live bird market?

Can we use NGS to detect viruses in ES from the live bird market?

**YES**

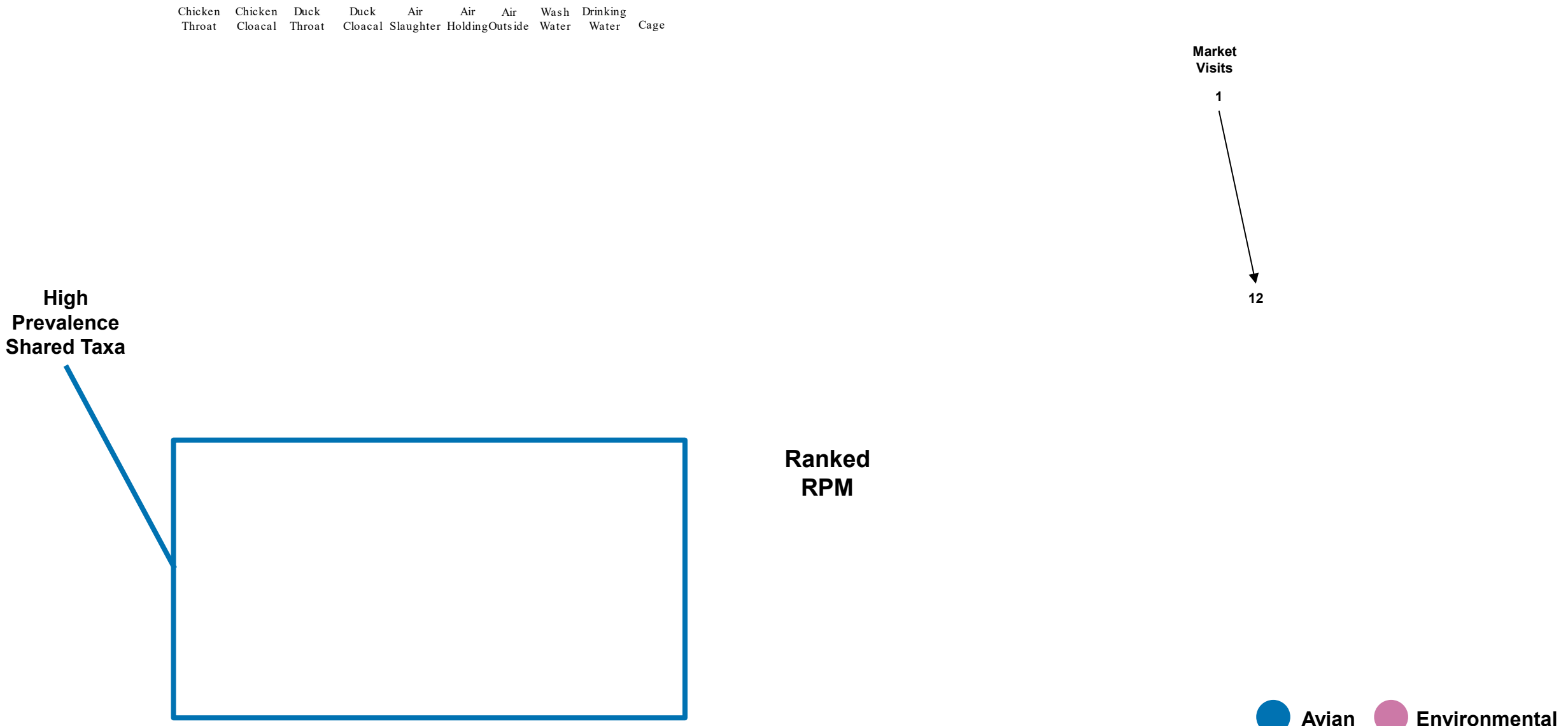
Is NGS comparable to qRT-PCR on ES and poultry samples from live bird markets?

**YES** – with caveats: need to define standards, how should the data be interpreted?, segmented viruses, etc…

Are ES samples able to effectively replicate what is detected in poultry in the live bird market?

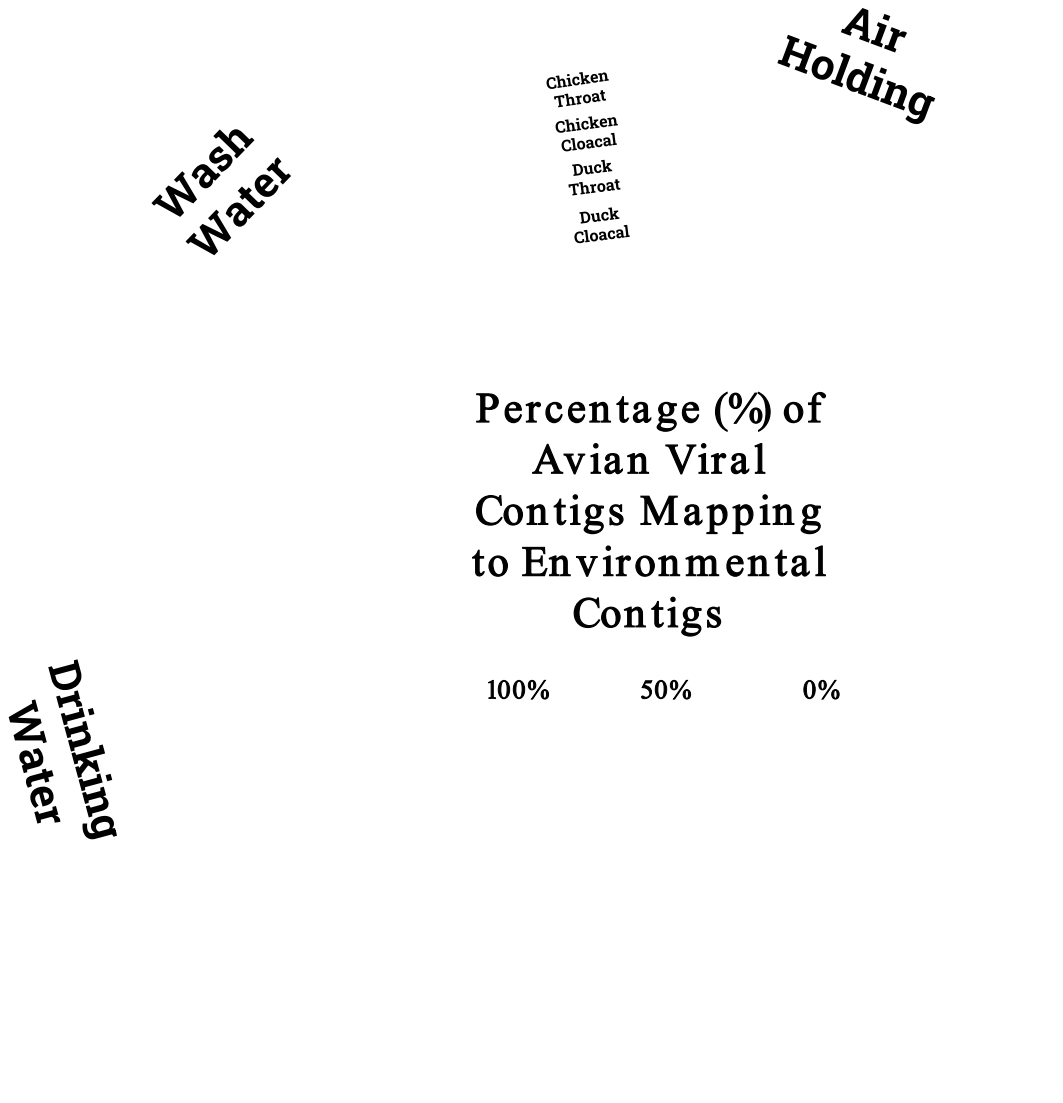
# Are ES samples able to effectively replicate what is detected in poultry in the live bird market?

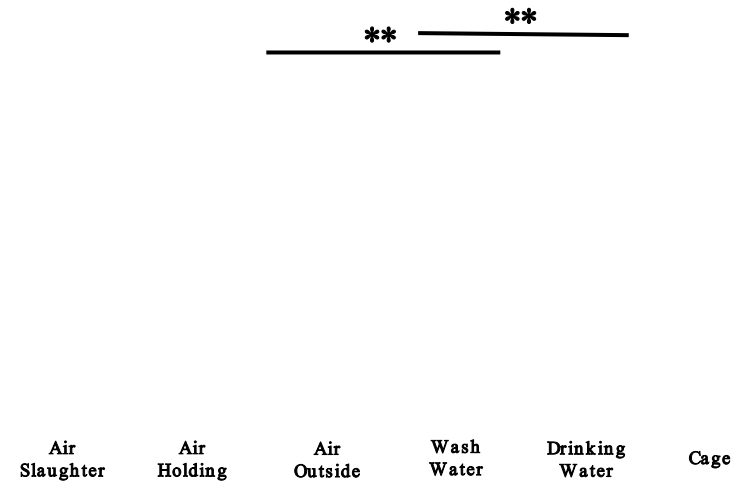
## Majority of Taxa in Avian Samples Also Detected in Environment at Each Visit

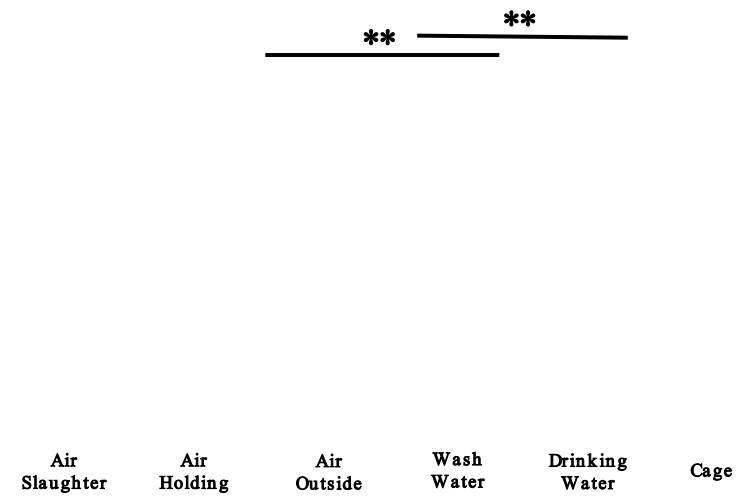


# Are ES samples able to effectively replicate what is detected in poultry in the live bird market?

## Majority of Avian Viral Contigs Map to Environmental Contigs

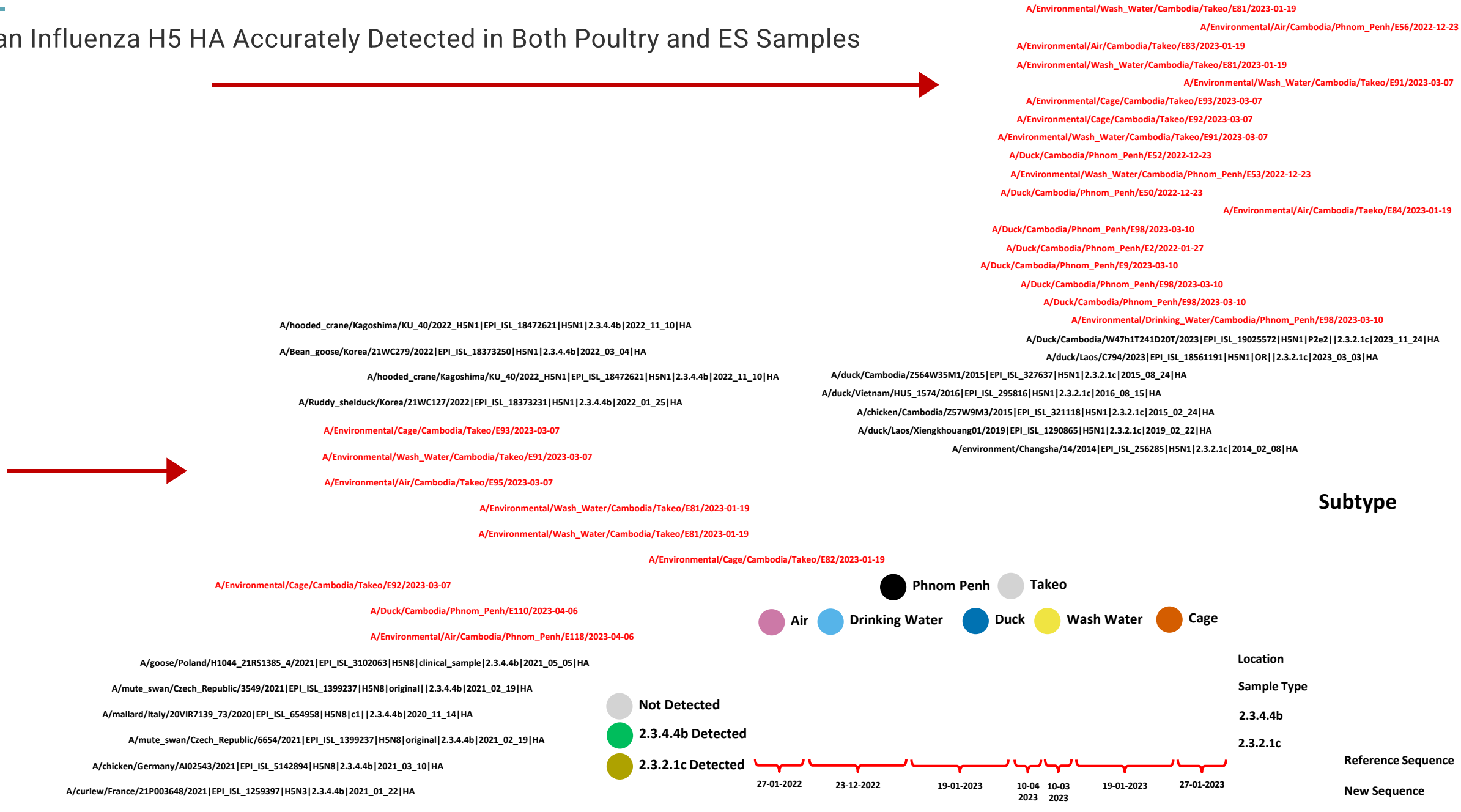


# Are ES samples able to effectively replicate what is detected in poultry in the live bird market?

## Avian Influenza H5 HA Accurately Detected in Both Poultry and ES Samples



Can we use NGS to detect viruses in ES from the live bird market?

**YES**

Is NGS comparable to qRT-PCR on ES and poultry samples from live bird markets?

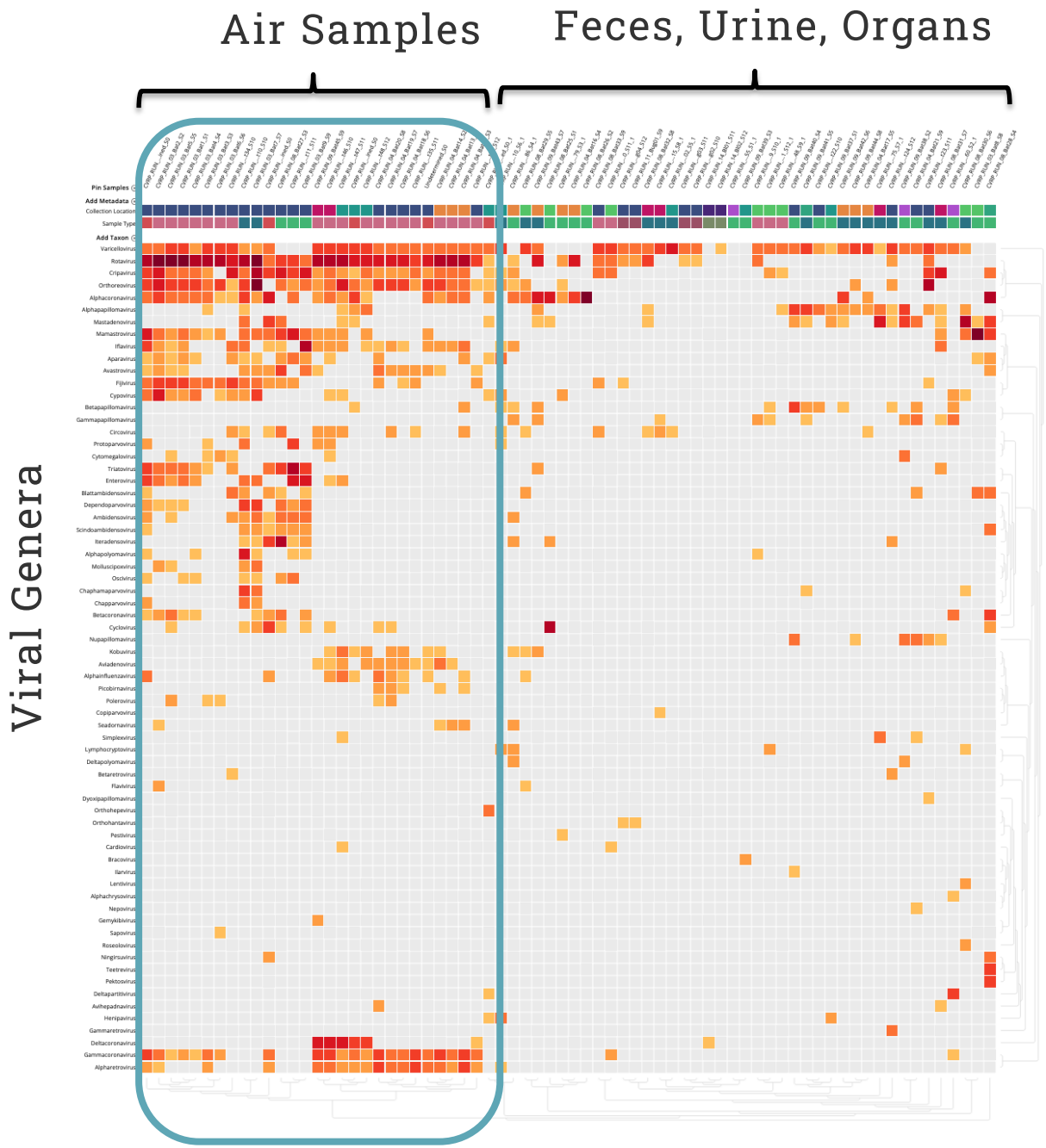
**YES** – with caveats: need to define standards, how should the data be interpreted?, segmented viruses, etc…

Are ES samples able to effectively replicate what is detected in poultry in the live bird market?

**YES** – with caveats: may need to take multiple ES sample types, etc…

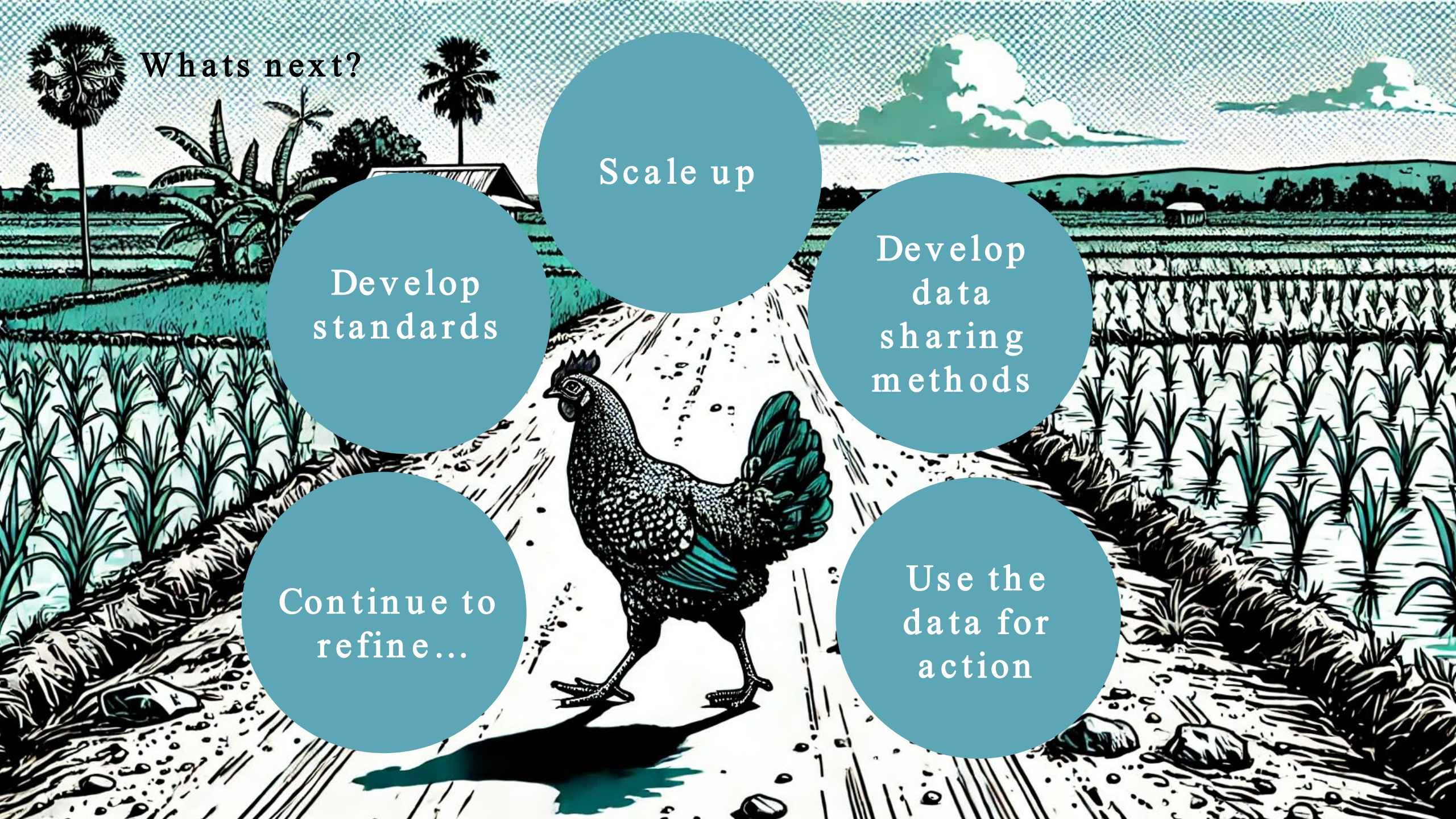
# Targeted Metagenomics in ES samples from Bat Interfaces

Comparison of air samples from bat interfaces and individual bat samples




**All virus genera can be accurately detected in air samples that are detected in feces, urine, and organs of bats...and more!!

# Whats next?


Scale up
Develop standards
Develop data sharing methods
Continue to refine...
Use the data for action

# Thank You

Institut Pasteur du Cambodge
PASTEUR NETWORK

Research and Reponse at IPC would not be possible without these funders, partners, and collaborators and many others

ekarlsson@pasteur-kh.org
@E_A_Karlsson
@eakarlsson.bsky.social
@ekarlsson@mstdn.science

# How much will it cost me?

# *Every second counts between detection and response*

..and the cost of an outbreak goes up every second after spillover

## INITIAL SETUP

Purchase of air samplers and in-field testing equipment, sequencer, and laptop, etc..

**$35,500**

## TESTING/VISIT/SITE

Cost for reagents, field mission, testing, etc…for each visit at each site

**$5,000**

## TRAINING and SUPPORT

Costs for international training, backstopping visits, etc…

**$45,000**

## PREVENTING PANDEMICS

..and potentially many, many other human and animal diseases

**PRICELESS**