Integrating Tiered Orthogonal Strategies into a Global Immune System for BioSurveillance

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COLUMBIA UNIVERSITY MEDICAL CENTER
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CENTER FOR INFECTION AND IMMUNITY
the path forward

...the only sensible defense against biologic warfare, whatever its immediate nature, is the cultivation of science, its orientation towards basic research that will be broadly applicable, and an orderly growth of well-rounded institutions where such developments are possible and are encouraged.

- Gilbert Dalldorf; New York State Department of Health, 1949

Effects Of Delayed Pathogen Identification

Deaths related to West Nile virus reach 22 across Texas

Published On: Aug 21 2012 04:46:16 AM CDT Updated On: Aug 20 2012 06:22:06 PM CDT
FORT WORTH, Texas -
The deaths of two more North Texans have been blamed on the West Nile virus.

Tarrant County officials Monday reported the deaths of a Fort Worth man and a North Richland Hills woman, both of whom were in their 80s and had underlying medical conditions.

That brings to four the number of dead in Tarrant County, to 16 the number of West Nile-related fatalities in the Dallas-Fort Worth area, and to 22 the number of dead in Texas.

Tarrant County officials are performing ground spraying to kill mosquitoes that carry the virus, while Dallas County is performing aerial spraying. Dallas County has reported 10 West Nile deaths.

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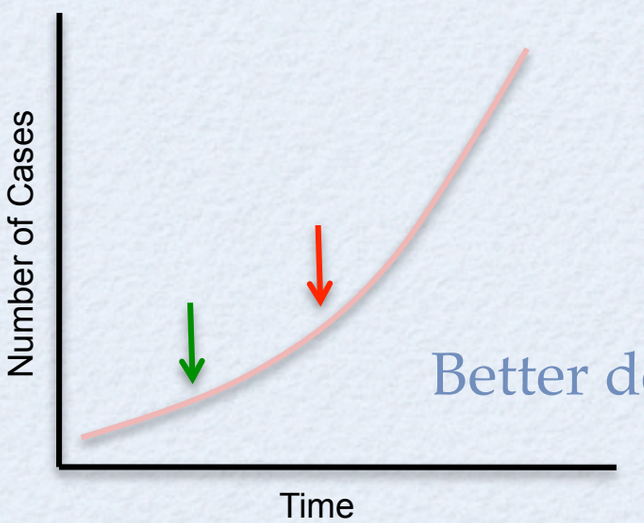
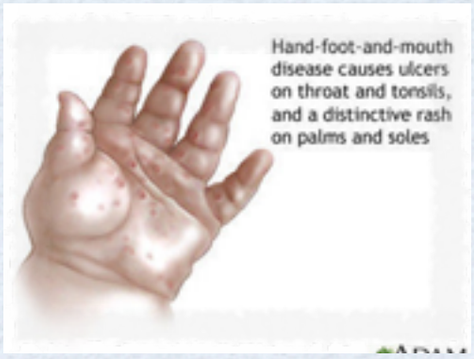




July 5, 2012 5:51 AM

Cambodia mystery illness kills 61 kids

(AP) PHNOM PENH, Cambodia - Health officials in Cambodia are searching for the cause of a mystery disease that has killed more than 60 children over the past three months, the World Health Organization said Thursday.

The "undiagnosed syndrome" has killed 61 of the 62 children admitted to hospitals since April, but there's no indication that is it spreading from person to person, said WHO spokeswoman Aphaluck Bhatiasevi.

She said health workers are trying to determine whether the cases were all the same disease or a collection of various illnesses.

The children were less than 10 years old and first fell ill with a high fever, followed by neurological symptoms and severe respiratory problems that quickly progressed. The cases have been reported in hospitals in 14 provinces, with most occurring in southern Cambodia.

The statement quoted Health Minister Man Bung Heng as saying identification of the cause may take some time. It said neighboring countries have been alerted.

Better detection methods limit the spread of disease & saves lives

Current gaps in the Federal Government's strategy

- Incomplete & fractured biosurveillance efforts have led to delayed outbreak recognition & prolonged response times.
- Current infrastructure is unfocused & inadequate to respond to emerging & re-emerging public health threats.
- Incomplete understanding of innate & adaptive immunity and host/pathogen interactions is a barrier to broad-based solutions.
- Existing strategies do not take advantage of modern science base & its full potential.

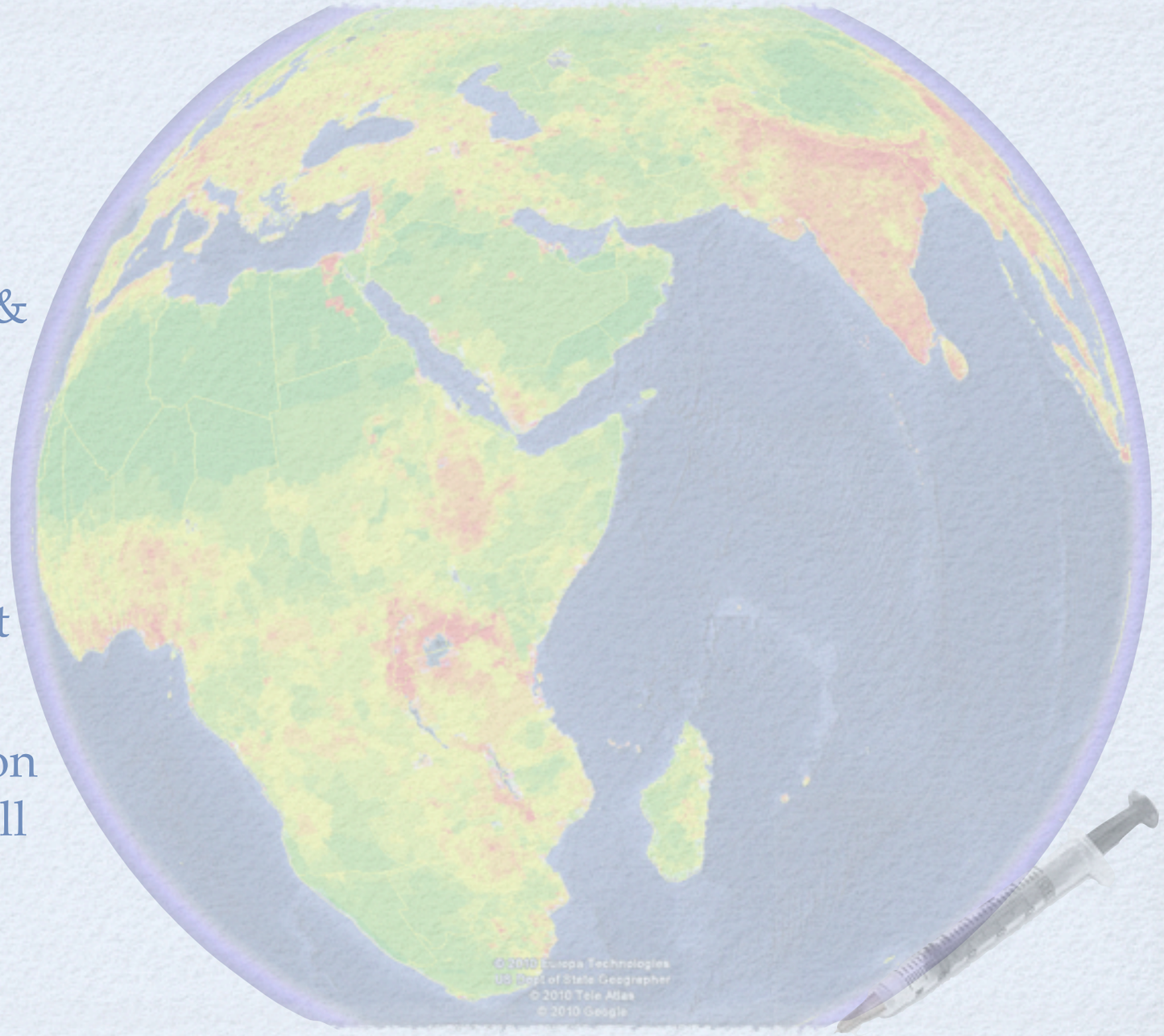
Result: Vulnerability to bioterrorism & emerging health threats

Solutions and Objectives

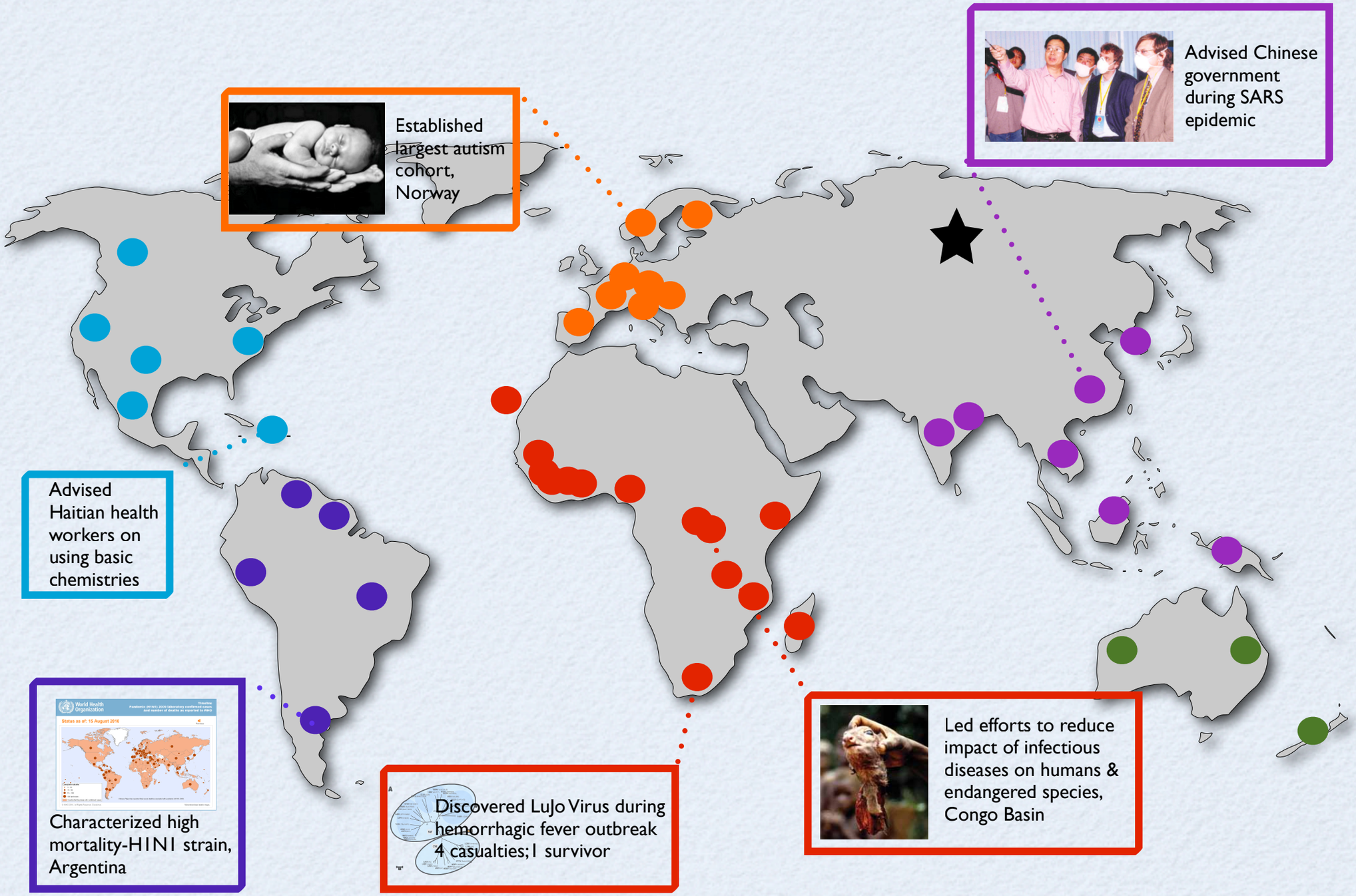
- Establish global awareness, surveillance & collaborations in regions considered highest risk for emerging diseases.
- Create a sustainable, comprehensive global biosurveillance program through development of outbreak response infrastructure & protocols.
- Develop pipelines for biomarker discovery & host/pathogen interactions that can be harnessed quickly to develop diagnostic assays, vaccines & therapeutics
- Use cutting-edge, advanced technologies to rapidly develop global solutions.

Deliverables

- Rapid, global outbreak detection, characterization & diagnostics
- Predictive global risk models for emerging & re-emerging disease hotspots
- Community access to global sample & sequence repository
- Training program for domestic & international collaborators & government agencies in disease diagnostics, bioinformatics & hotspot modeling
- Rapid development & implementation of immunotherapies, vaccines & small molecule therapeutics



CII's Global Network

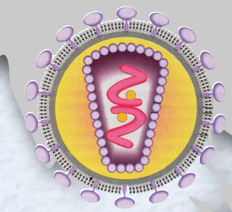


Open network architecture allows engagement with other ongoing efforts, such as the Cooperative Threat Reduction Program

Assembling Global Health

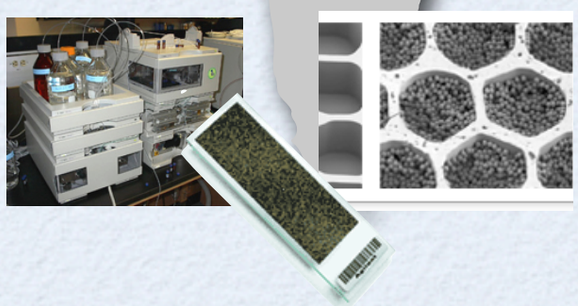
SURVEILLANCE

OUTBREAK RESPONSE



HOST DISCOVERY

DISCOVERY DIAGNOSTICS



Influenza Research Activities Emerging Infections Department NAMRU-6

Handshake Phase

- Central research/clinical lab identified
- 200 samples selected and sent to Columbia
- Samples processed through our staged strategy (MassTag, GreeneChip and sequencing)
- Phase 1 training at Columbia (personnel and more samples)
- MassTag platform and other technologies installed at remote site
- Phase II training- Columbia personnel travel to remote site and run samples on site
- Survey of site and satellite research stations
- Site personnel begin to run process samples on MassTag independently
- Samples sent to CII for confirmation
- Remote site can independently run several hundred samples a month
- End handshake phase

Iterative Phase

- Columbia preps samples that are negative by MassTag and arrays and send out to a trusted sequencing lab
- Data is shared with bioinformatic and interface collaborators in conjunction with remote site to develop streamlined analysis pipelines that will enable remote interactions
- MassTag probes are developed to identify new pathogens. MassTag panels are updated along with GreenChip array
- Assays are validated and transferred to remote site

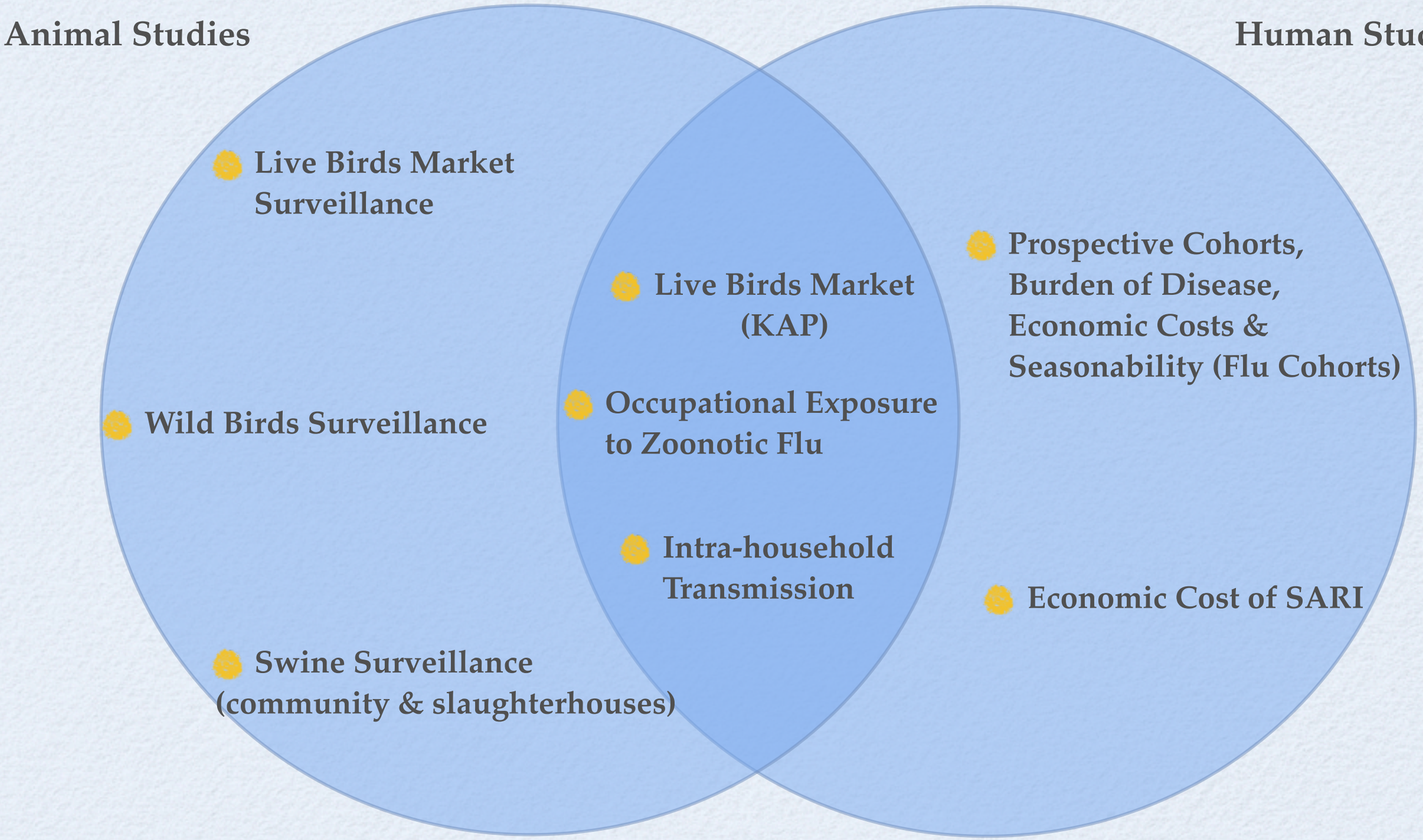
Tuning Phase

- CII begins assessing technologies that would have applications in remote lab and satellite labs
    - robotics
    - lateral flow pathogen detection assays
    - advanced *in situ* hybridization assay for pathogen diagnosis
    - sample prep methodology
    - sequencing platforms
- Informatics and sequencing laboratories begin to directly interact with the remote lab
    - Samples sent directly for sequencing
    - Direct access and training on sequencing pipeline
    - trouble shooting and advanced analysis for complex samples
    - optimized informatics and analysis

Collaborative Phase

- Assistance for technical issues
- Outbreaks
- Technology fit assessment

NAMRU-6 Program Overview

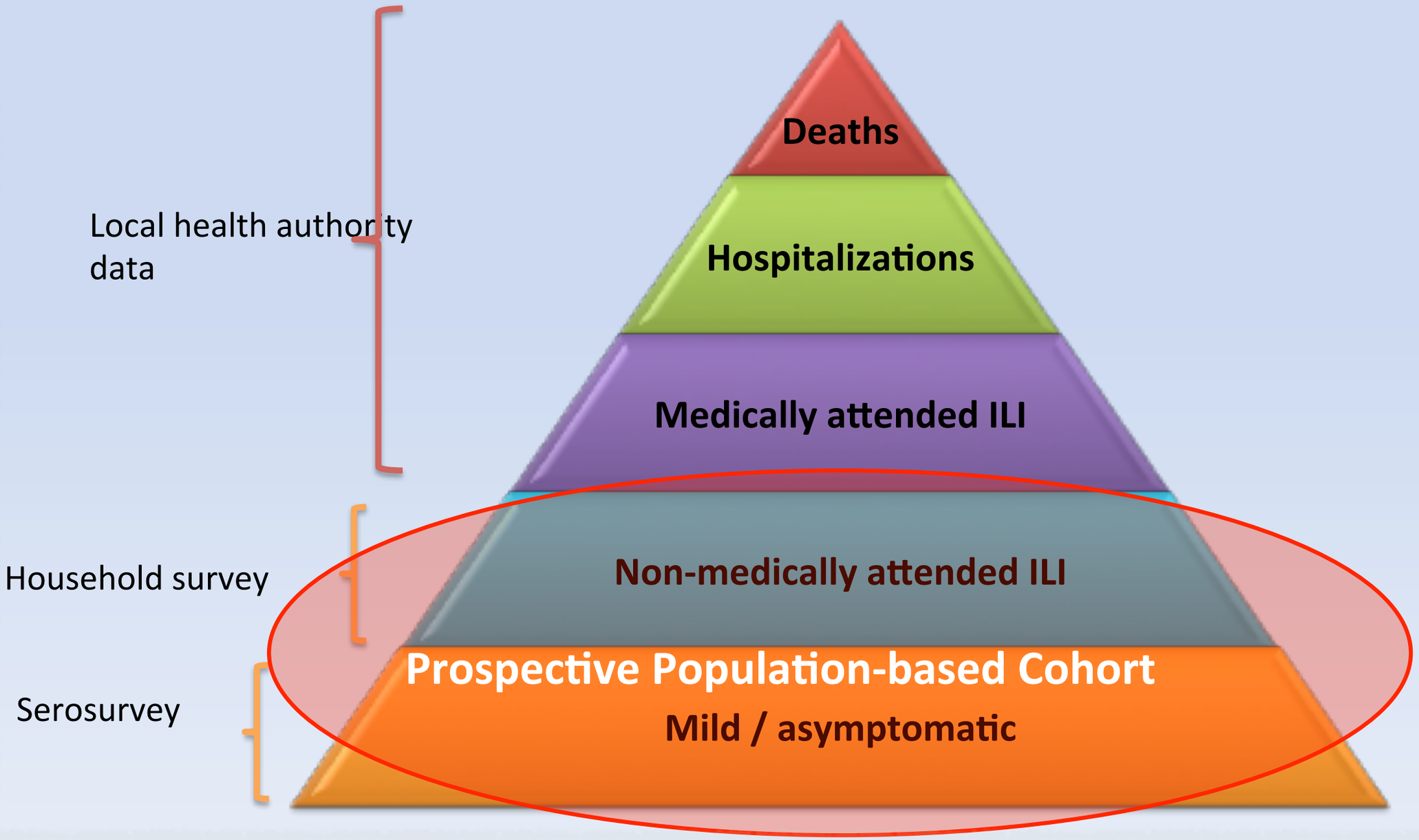


Demographics & Geography of Peru

- Population ~29 million
- Area ~1.3 million km^2 total land coverage
- 2414 Km of coastline
- Climate
  - tropical in east to dry desert in west
  - temperate to frigid in Andes
- Terrain
  - Costal Plains
  - Highlands in center
  - Eastern lowland jungle of the Amazon Basin

Disease Pyramids

Sources to estimate disease prevalence and burden



Methods

Multi-site, population-based prospective cohort - active ILI surveillance (3 years)

Location

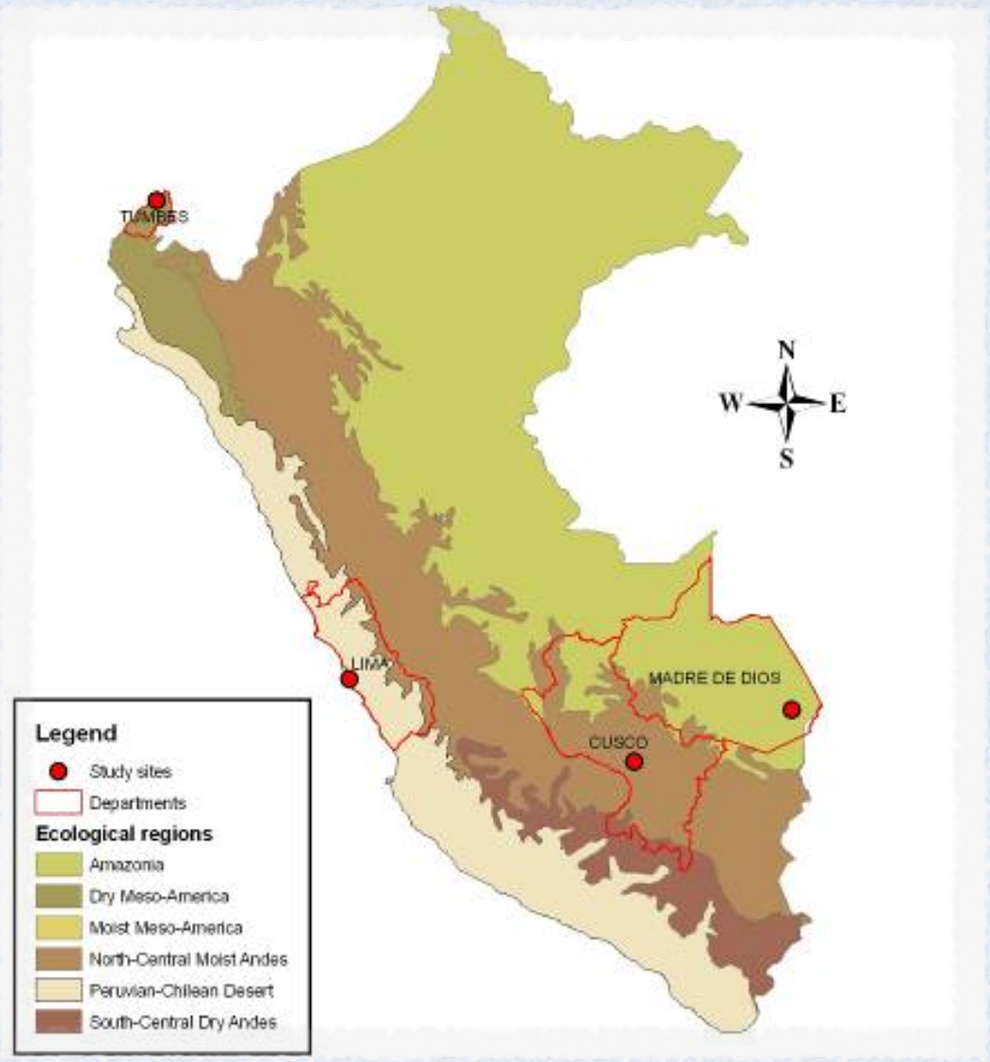
- 4 sites (375 households per site)
- Randomly selected households - using a census list
- At least 60% of household members required for participation
- All households are geo referenced

Population

- 6000 individuals (~1800/ site)
- all ages

Peru Influenza ILI Surveillance Cohorts

- Prospective Population Based Cohort:
  - Objective: To better define epidemiology of influenza virus & other respiratory viruses
- Methods
  - Four geographically distinct regions of Peru
    - Tumbles
    - Lima
    - Cuzco
    - Madre de Dios
  - Active surveillance (3d w household visit) for ILIs
  - Oropharyngeal swabs for qPCR & serum for Luminex testing
  - Follow up of ILI cases for 2 weeks



PROYECTO INFLUENZA PERU

Other Etiologies by Luminex Antibody Serology

ILI samples (N=201)			
Adenovirus	4	Influenza B, Bocavirus	2
Bocavirus	1	Para 1	4
Corona 229E	1	Para 2	1
Corona HKU1	8	Para 3	3
Corona NL63	2	Para 3, Adenovirus	1
Enterovirus/Rhinovirus	59	Para 3, Bocavirus	1
Enterovirus/Rhinovirus, Adenovirus	2	Para 3, Enterovirus/Rhinovirus	2
Enterovirus/Rhinovirus, hMPV	1	Para 4	2
hMPV	10	RSV	3
Influenza A (H3)	6	RSV, Para 2, Enterovirus/rhinovirus	1
Influenza A Matrix	8	RSV, Para 4, Enterovirus/Rhinovirus	1
Influenza B	7	No result	71

Other Etiologies by Cell Culture

ILI samples (N=1254)			
ADENOVIRUS	32	FLU A-PARAFLU 2	1
COXSACKIE B	7	FLU A-PARAFLU 3	1
ECHOVIRUS	1	FLU B	2
ENTEROVIRUS	13	FLU B - HSV	1
ENTEROVIRUS-PARAFLU 3	1	FLU B-ADENOVIRUS	1
FLU A	394	HMPV	11
FLU A - COXSACKIE B	1	HSV	21
FLU A - PARAFLU 3	1	HSV-ADENOVIRUS	2
FLU A-ADENOVIRUS	8	HSV-ENTEROVIRUS	2
FLU A-COXSACKIE B	2	PARA FLU 1	2
FLU A-ENTEROVIRUS	3	PARA FLU 2	20
FLU A-HMPV	1	PARA FLU 3	16
FLU A-HSV	6	MUESTRA CONTAMINADA	8
		NO AISLAMIENTO	696

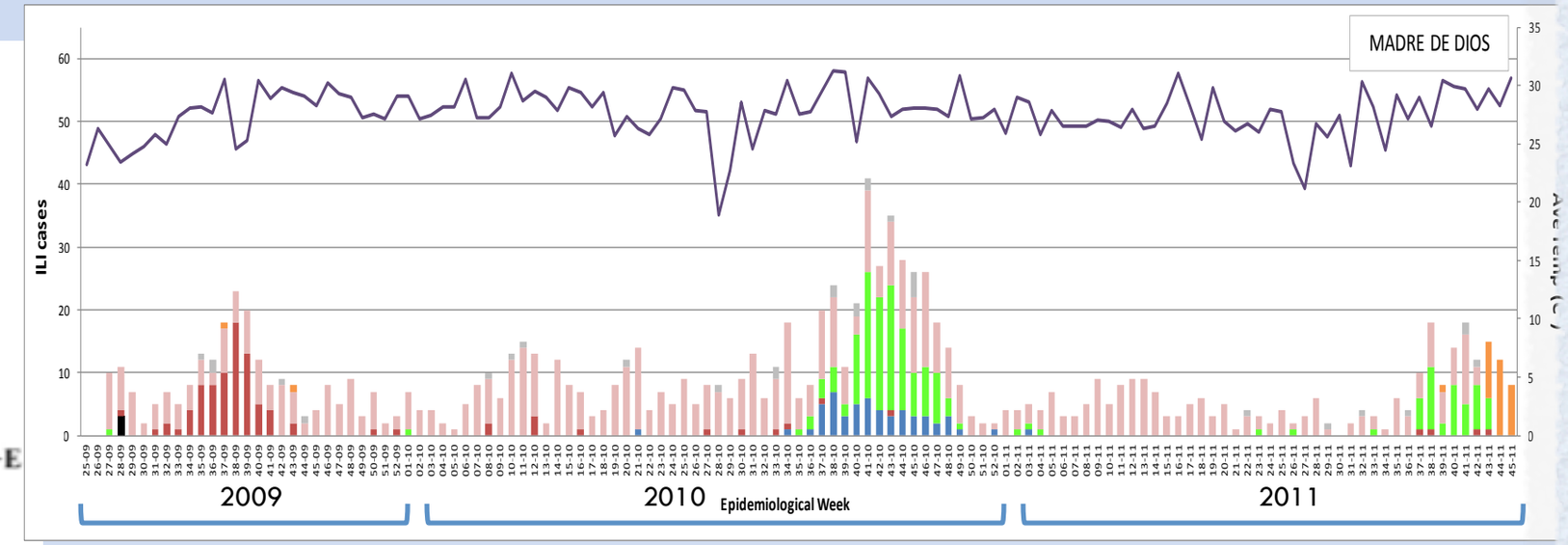
Puerto Maldonado

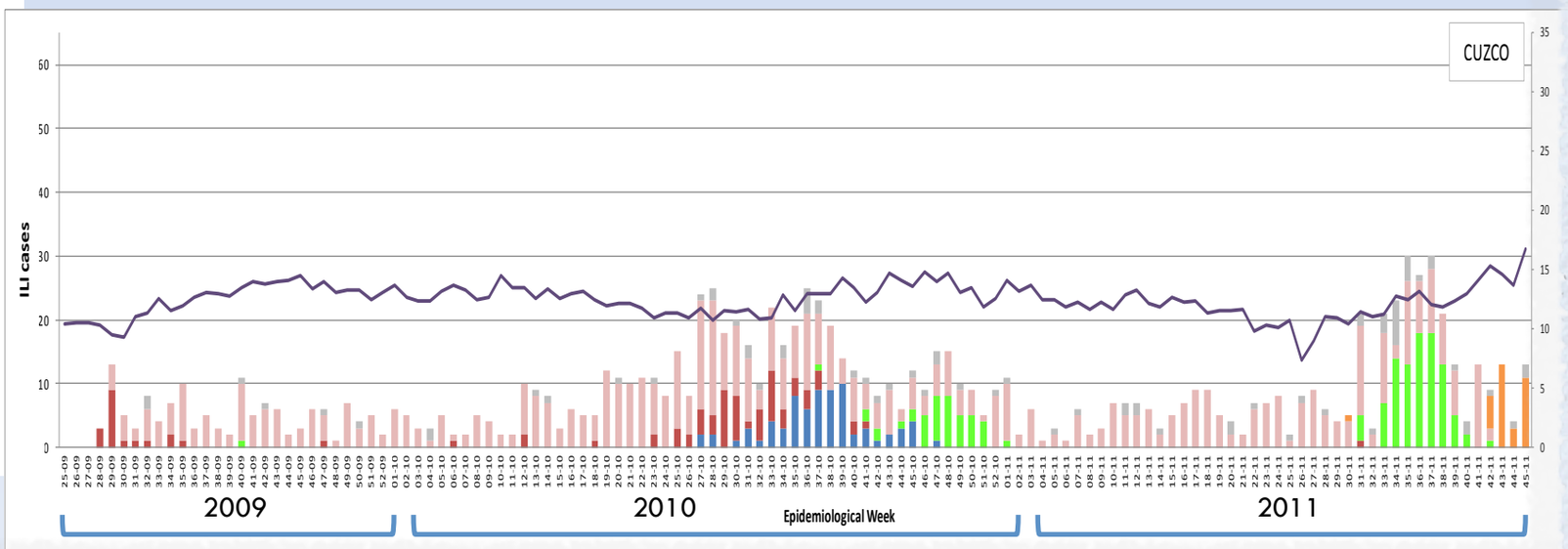
- Southern Amazon 55 km west of Bolivia
- Temperature 24-38°C
- >1000 mm of rainfall/year
- City of Puerto Maldonado has a population of 92,000 people
- The study site involves a small peri-urban community
- Poultry and small mammals are raised for consumption
- Interoceanic highway is being constructed through the center of the town
- New study site

Interoceanic Highway Will Bring Change to the Region

Flu Epidemic Curves- Amazon & Andes

Madre DE Dios & CUZCO Weeks 25 (2009)-45(2011)



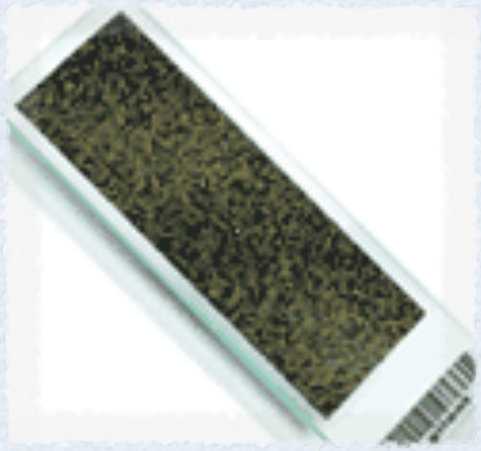


Multiplexed Approaches

A Staged Strategy for Pathogen Discovery

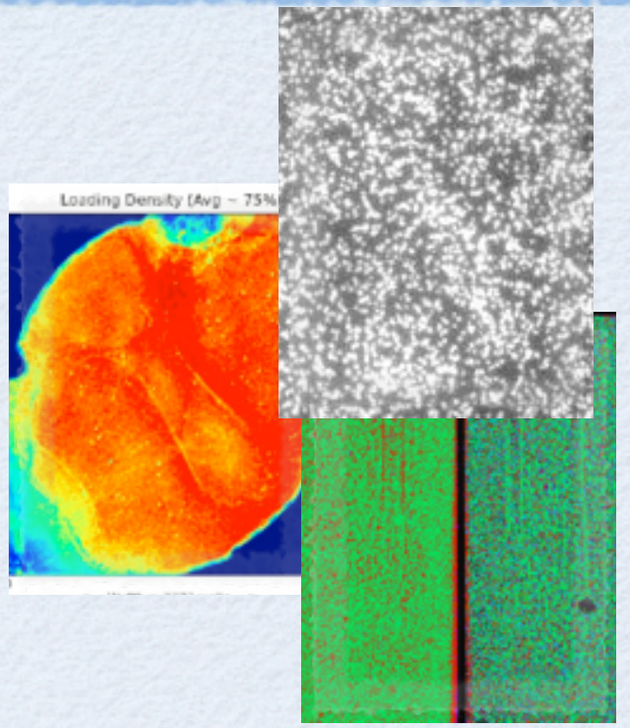
MassTag PCR Panels

- Respiratory disease
- Hemorrhagic fevers
- Meningoencephalitides
- Poxviruses
- $15/assay, 96 samples in 6 hours



GreeneChips

- Viral
- Pan-pathogen
- Respiratory
- $75/assay, 8 samples in 15 hrs

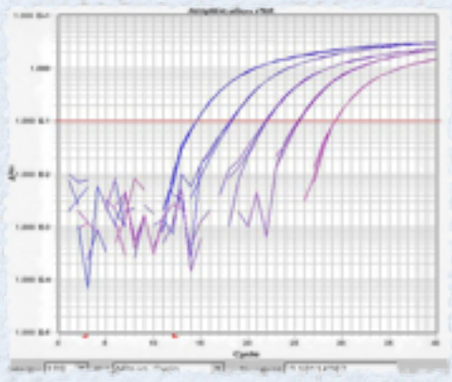


Shotgun Sequencing

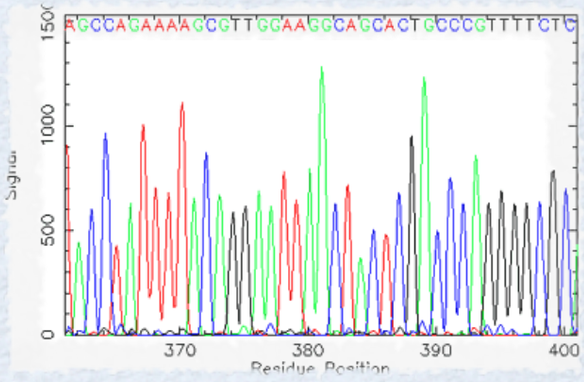
- $5,000/assay – 3 days

Serology (IgM, increase in IgG titer)
Pathology (agent distribution)
Challenge experiments (reproduce disease)

Quantitative Real-Time PCR

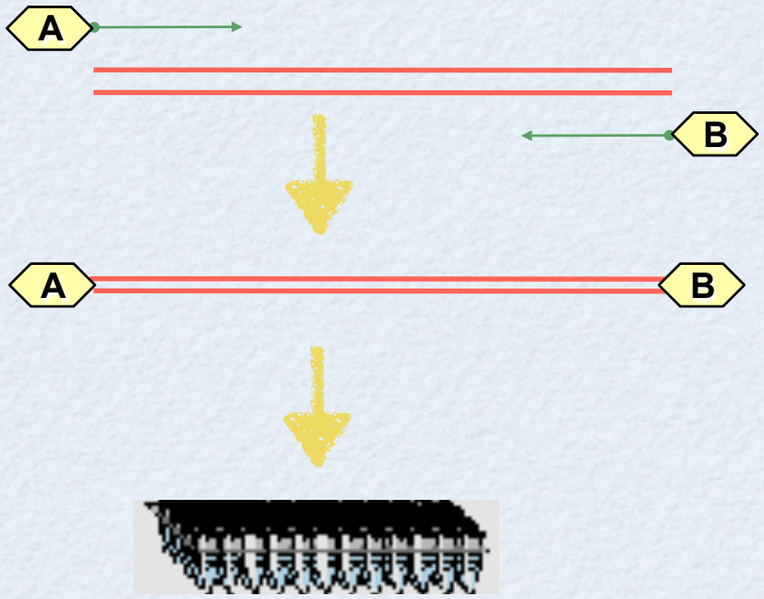


Consensus PCR Cloning & Sequencing



MassTag Pathogen Detection

1. PCR amplification with conjugated primers
120 minutes

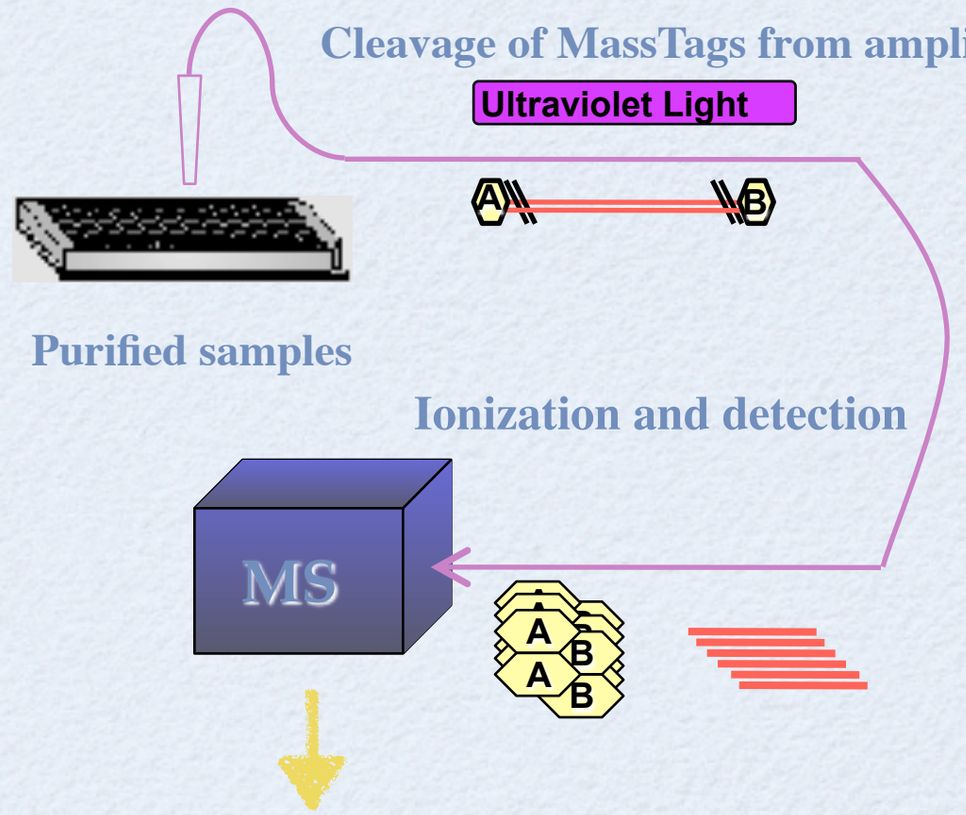


96-well thermocycler plate

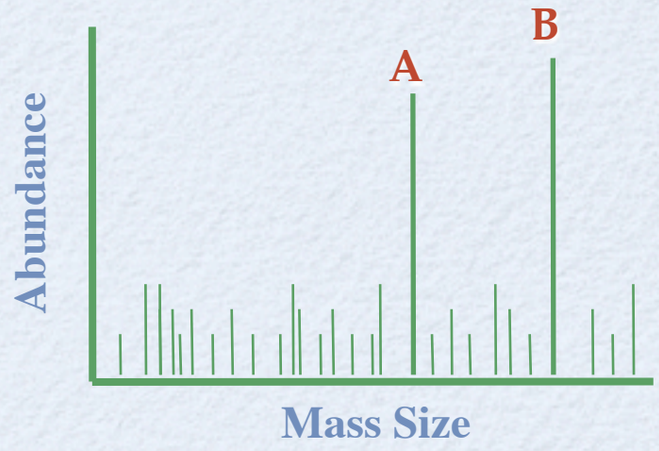
2. PCR purification on filter plate
30 minutes

3. Elution into 96-well loading plate for mass spectrometer analysis

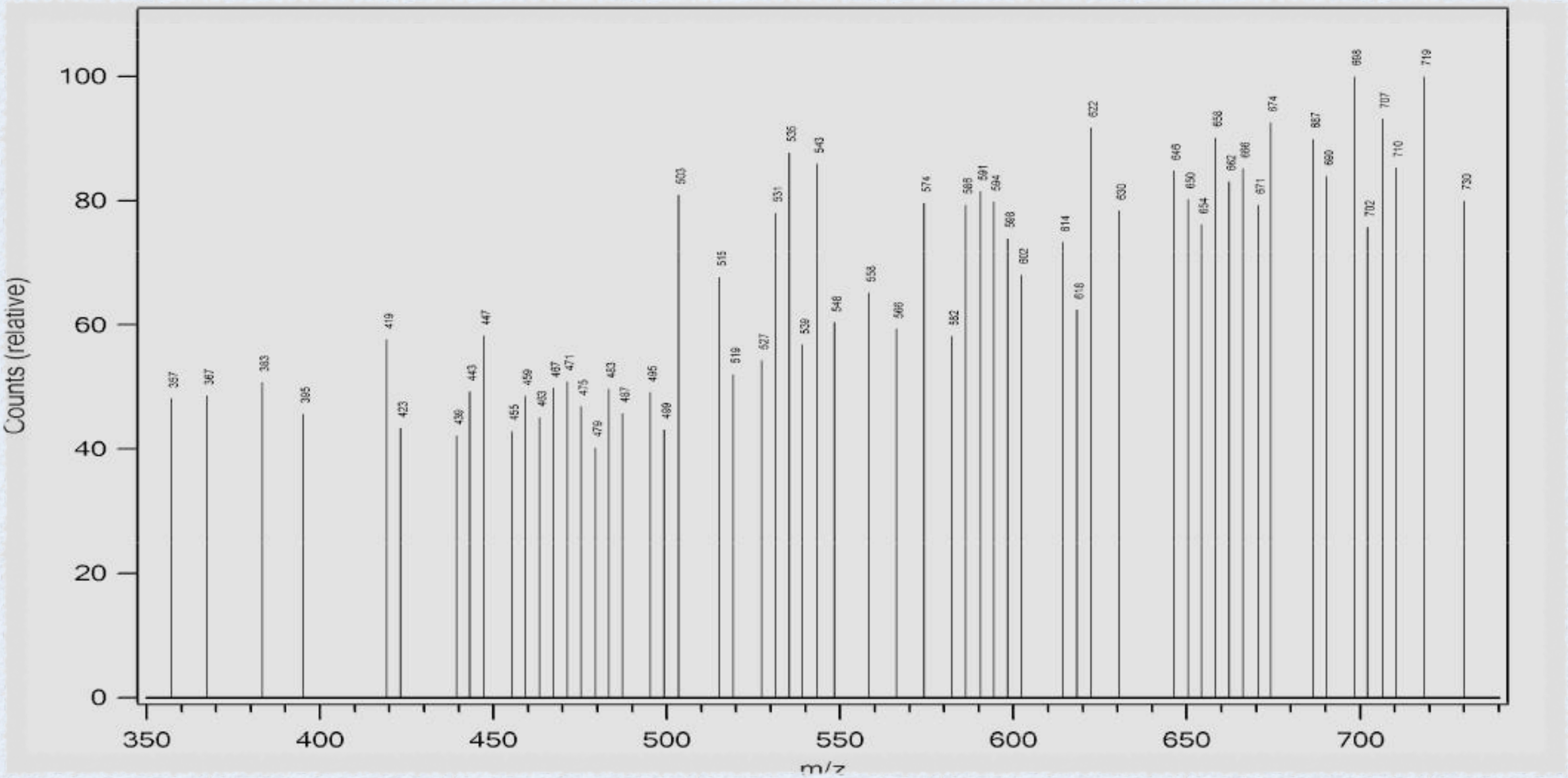
4. Automated sample injection, photocleavage & detection
1 minute/sample



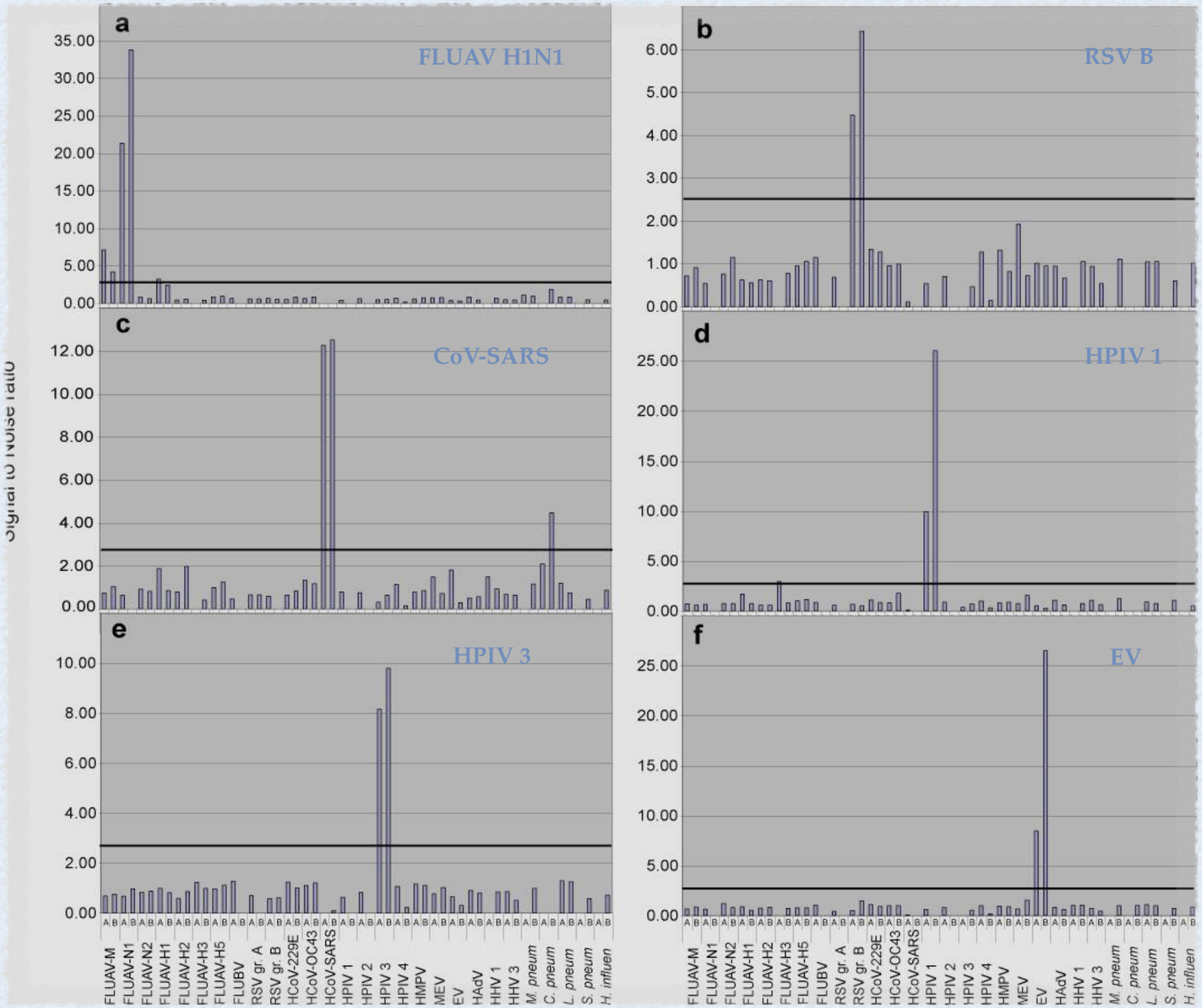
5. Identification of pathogen by signal analysis



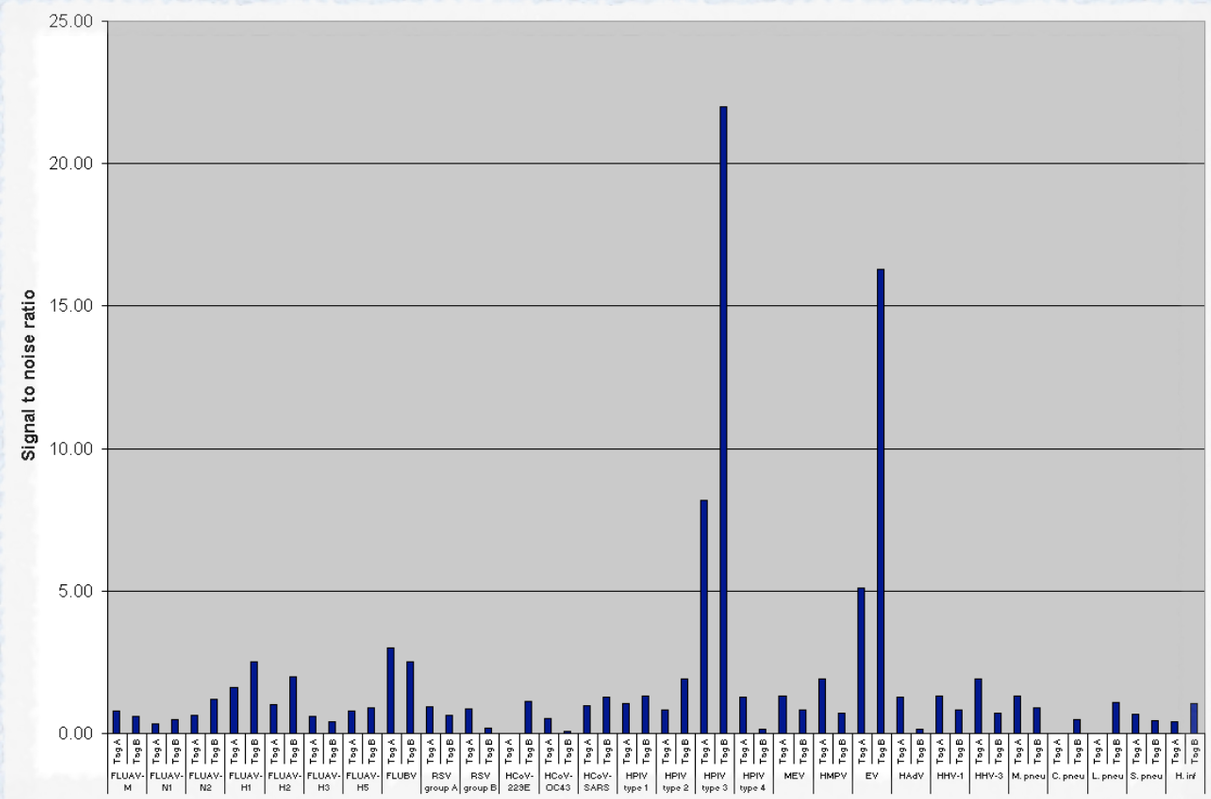
Detection of 58 Different MassTags by APCI-MS

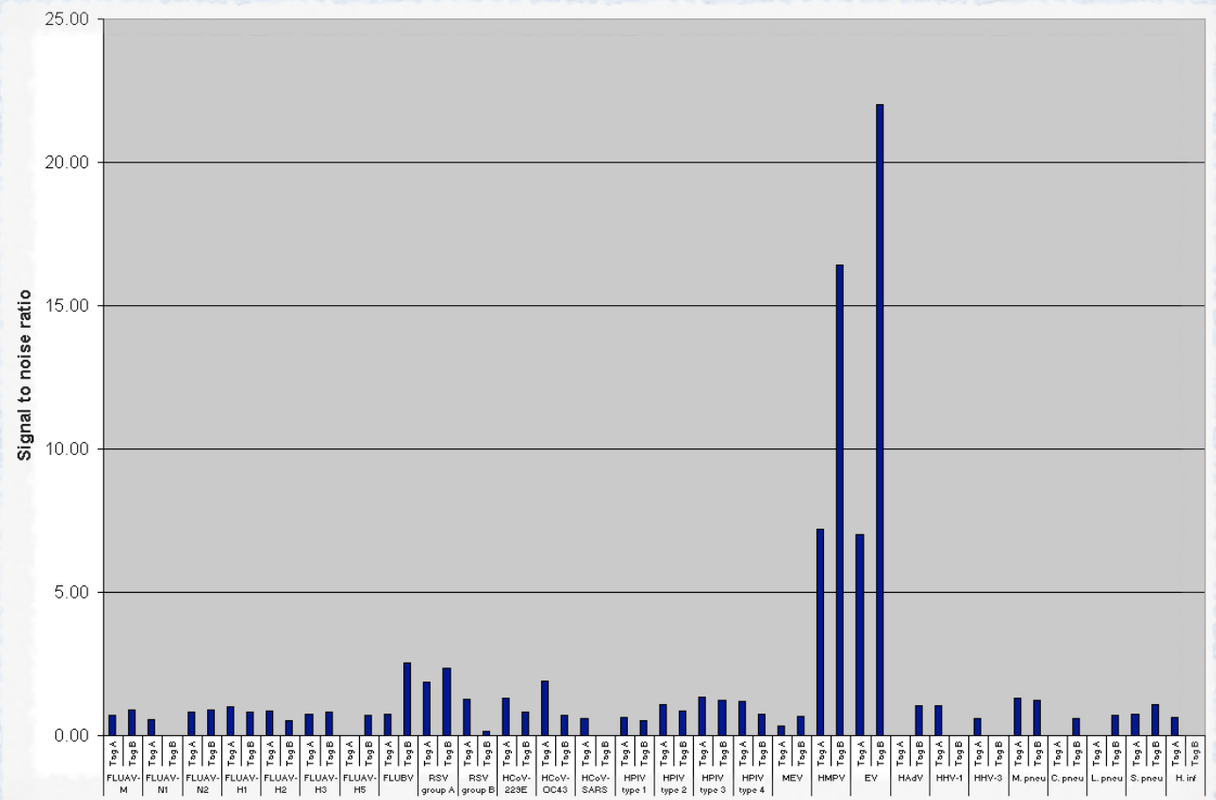


30-Plex Assay of Patient Samples



Co-Infection in Clinical Samples





- HPIV-3 / EV co-infection
  - Previously identified by multiplex PCR
  - Confirmed in duplicates

- HMPV / EV co-infection
  - Previously diagnosed as EV; not tested for HMPV
  - Confirmed by single-plex for both

Mass Tag Panels

- **West African fever panel**
  - Lassa fever virus
  - Chikungunya virus
  - Yellow fever virus
  - LCMV
  - O'nyong'nyong virus
  - Dengue fever virus
  - Bacillus anthracis
  - Yersinia pestis
  - Plasmodium spp
  - Rickettsia spp
  - Borrelia spp relapsing fever
  - Leptospira interrogans
  - Neisseria meningitides
  - Francisella tularensis
- **Hemorrhagic fever panel**
  - Lassa IV virus
  - Seoul virus
  - Yellow fever virus
  - Rift Valley fever virus
  - Crimean Congo Hemorrhagic fever virus
  - Ebola Zaire
  - Marburg virus
  - Chikungunya virus
  - Hantaan virus
  - Dobrava virus
  - Kyasanur forest virus

MassTag PCR Panels

Meningitis/Encephalitis		Respiratory Disease	
RNA agents	**DNA agents**	**RNA agents**	**DNA agents**
Eastern Equine Encephalitis virus	Adenovirus	Influenza A	Adenovirus
Nippah/Hendra virus	Cytomegalo virus	Influenza B	*Chlamydia pneumoniae*
Japanese Encephalitis virus	Epstein Barr virus	RSVA	*Legionella pneumophila*
Parecho virus	Varicella Zoster virus	RSVB	*Mycoplasma pneumoniae*
Powassan virus	Herpes Simplex virus 1	HPIV1	*Neisseria meningitidis*
Lacrosse virus	Herpes Simplex virus 2	HPIV2	*Haemophilus influenzae*
Lymphocytic Choriomeningitis virus	HHV6	HPIV3	*Streptococcus pneumoniae*
St. Louis Encephalitis virus	*Haemophilus influenzae*	HPIV4	*Mycobacteria tuberculosis*
Enterovirus	*Streptococcus pneumoniae*	MPV	*Moraxella catarrhalis*
West Nile virus	*Neisseria meningitidis*	Coronavirus OC43	CMV
Western Equine Encephalitis virus	*Cryptococcus neoformans*	Coronavirus 229E	*Bordetella pertussis*
Venezuelan Equine Encephalitis virus	*Leptospira interrogans*	Enterovirus/rhinovirus	
Rabies virus	*Mycobacterium tuberculosis*		
Influenza A virus	*Candida albicans*		
	Toxoplasma gondii		

West African Fever	Hemorrhagic Fever	Tick-borne	Biothreat
Lassa fever virus	Lassa virus	*Anaplasma spp*	*Bacillus anthracis*
Chikungunya virus	Seoul virus	*Bartonella henselae/quintana*	*Francisella tularensis*
Yellow fever virus	Yellow fever virus	*Borrelia spp Lyme Borreliosis*	*Yersinia pestis*
LCMV	Rift Valley fever virus	*Borrelia spp relapsing fever*	*Burkholderia mallei*
Onyong nyong virus	CCHF virus	*Babesia spp*	*Burkholderia pseudomallei*
Dengue virus	Ebola Zaire	*Coxiella burnetti*	*Brucella spp*
Bacillus anthracis	Marburg virus	*Ehrlichia spp*	*Rickettsia prowazekii*
Yersinia pestis	Hantaan virus	*Francisella tularensis*	*Clostridium botulinum*
Plasmodium spp	Dobrava virus	*Rickettsia spp*	*Coxiella burnetii*
Rickettsia spp	Kyasanur forest virus		Orthopox virus
Trypanosoma brucei			Ebola virus
Borrelia spp relapsing fever			Marburg virus
Leptospira interrogans			
Neisseria meningitides			
Salmonella spp.			

Bacterial Enteric	Viral/Parasitic Enteric
Listeria monocytogenes	Astrovirus
Clostridium perfringens	Rotavirus-A,B,C
Clostridium difficile	Norovirus-GI
Campylobacter jejuni	Norovirus-GII
Campylobacter coli	Sapovirus GI/II
Salmonella	Adenovirus F
Salmonella typhi/paratyphi	Giardia lamblia
Vibrio	Cryptosporidium
Vibrio vulnificus	Entamoeba histolytica
Yersinia enterocolitica	
Yersinia pseudotuberculosis	
Escherichia coli	

Oropharyngeal swabs from 162 individuals in Cuzco & Puerto Madonado with ILI symptoms tested

Results

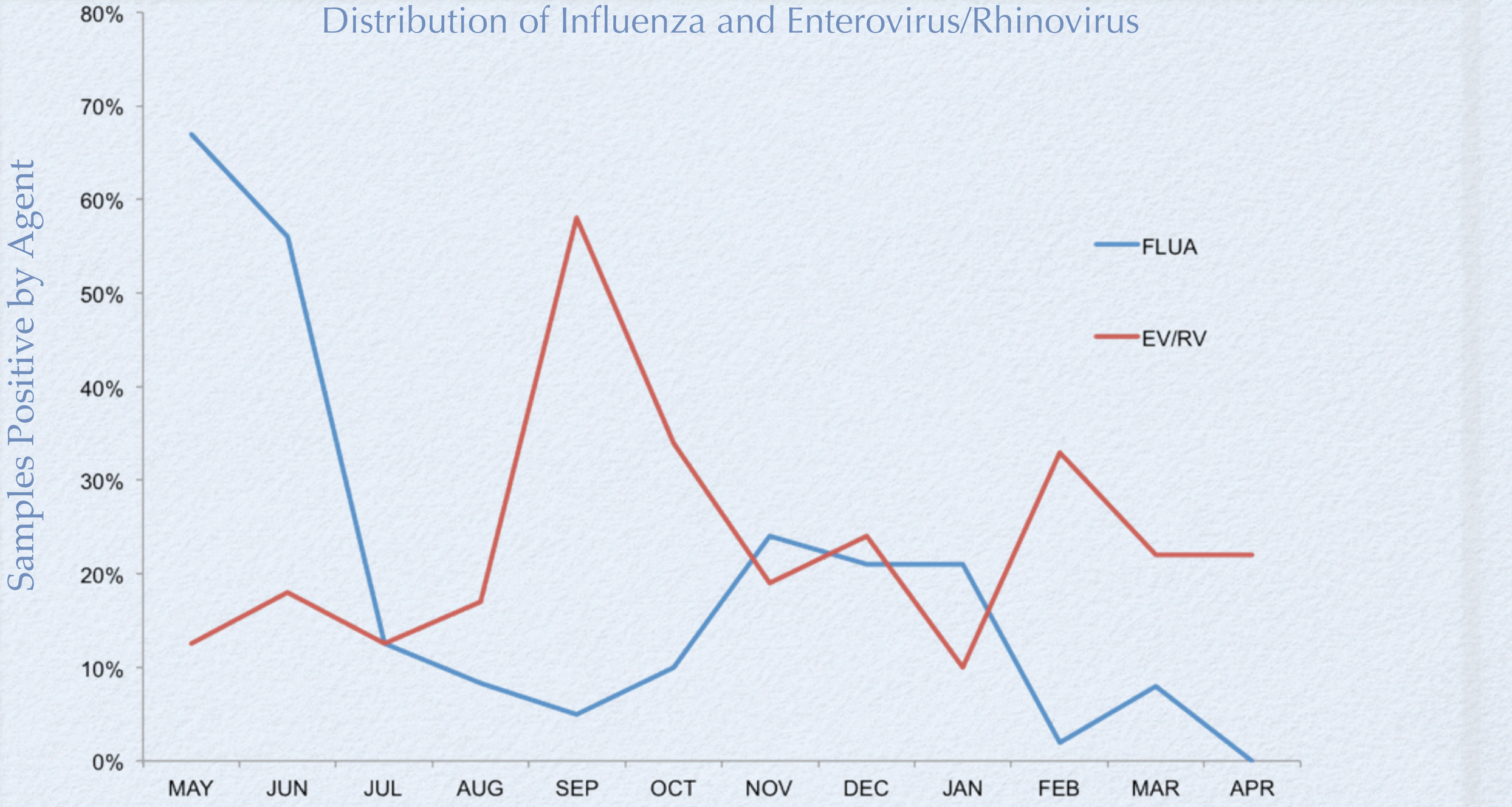
- Cases
  - 38 rhinovirus
  - 11 influenza A viruses (7 H1N1) and 4 (H3N2)
  - 11 parainfluenza viruses (3 HPV-1, 1 HPV-2, 5 HPV-3, 2 HPV-4)
  - 9 non-rhino enteroviruses (2 subsequently have been shown to be novel)
  - 7 metapneumoviruses
  - 6 influenza B viruses
  - 6 respiratory syncytial viruses
  - 3 Chlamydophila pneumoniae and 2 Legionella pneumophila
  - 14 adenovirus (10 HAdV-A, 2 HAdV-B, 2 HAdV-C, 1 HAdV-D & 2 HAdV-F)

- Many of the infections were co-infections (virus-virus or virus-bacteria)
- Etiologic agents were identified in 85 (52%) of the 162 patients with ILI including 75% of the cases where no diagnosis was previously known from monoplex assays
- Small outbreak of ILI due to enterovirus identified that was not identifiable with existing monoplex assays

  Latest results indicate that the majority of cases in July and August is largely HEV

- Unresolved and some FUO serum were prepared for Illumina and Sequenced at ECBC

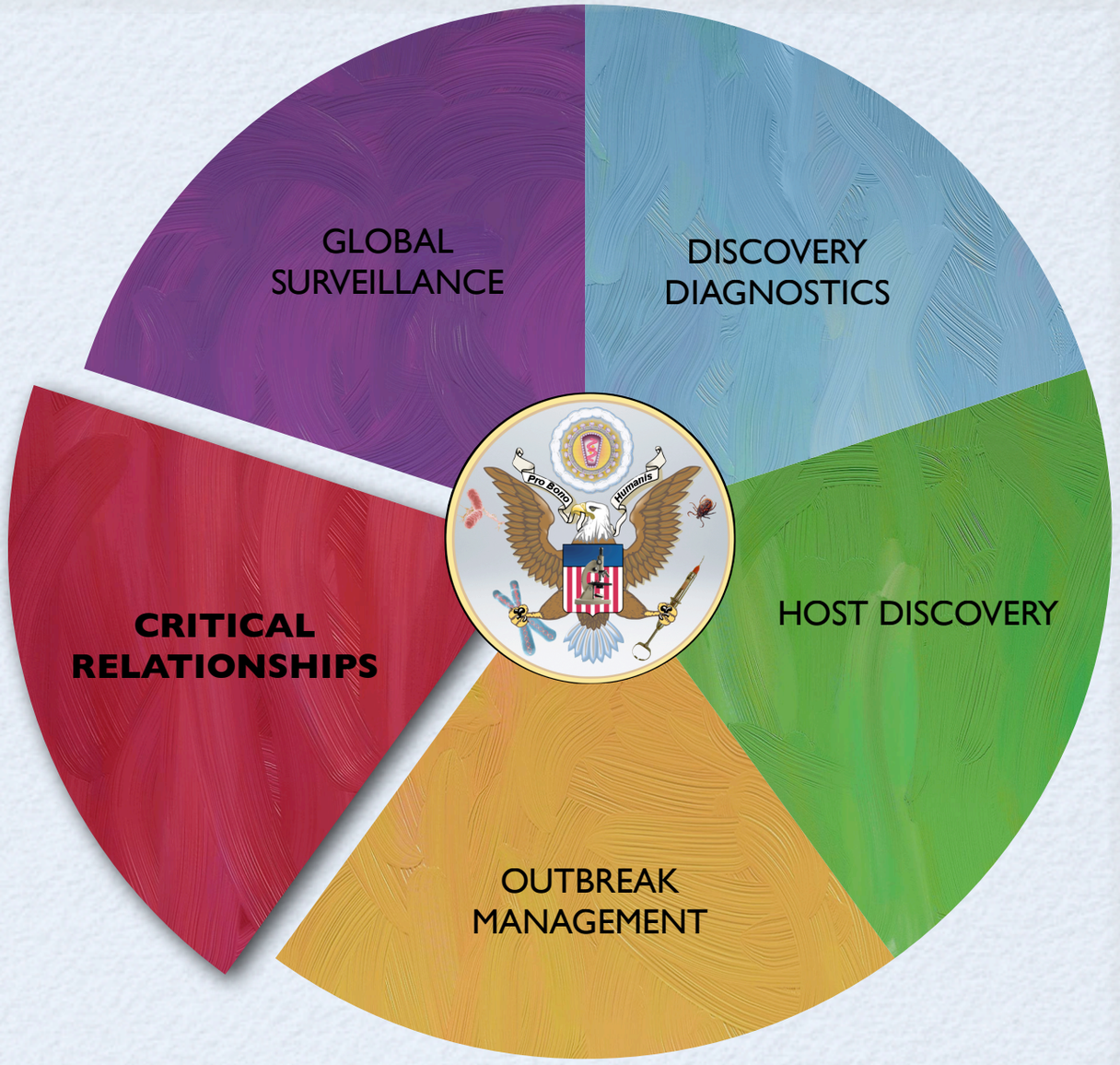
New York City Department of Health



Influenza Surveillance

Collaboration with the Federal Government

- Act as consultant to the Federal Government through regular meetings
- Liaise with existing government agencies to streamline implementation of comprehensive biosurveillance program nationally & globally
- Participate in governmental committees in order to shape the country's outbreak response strategies

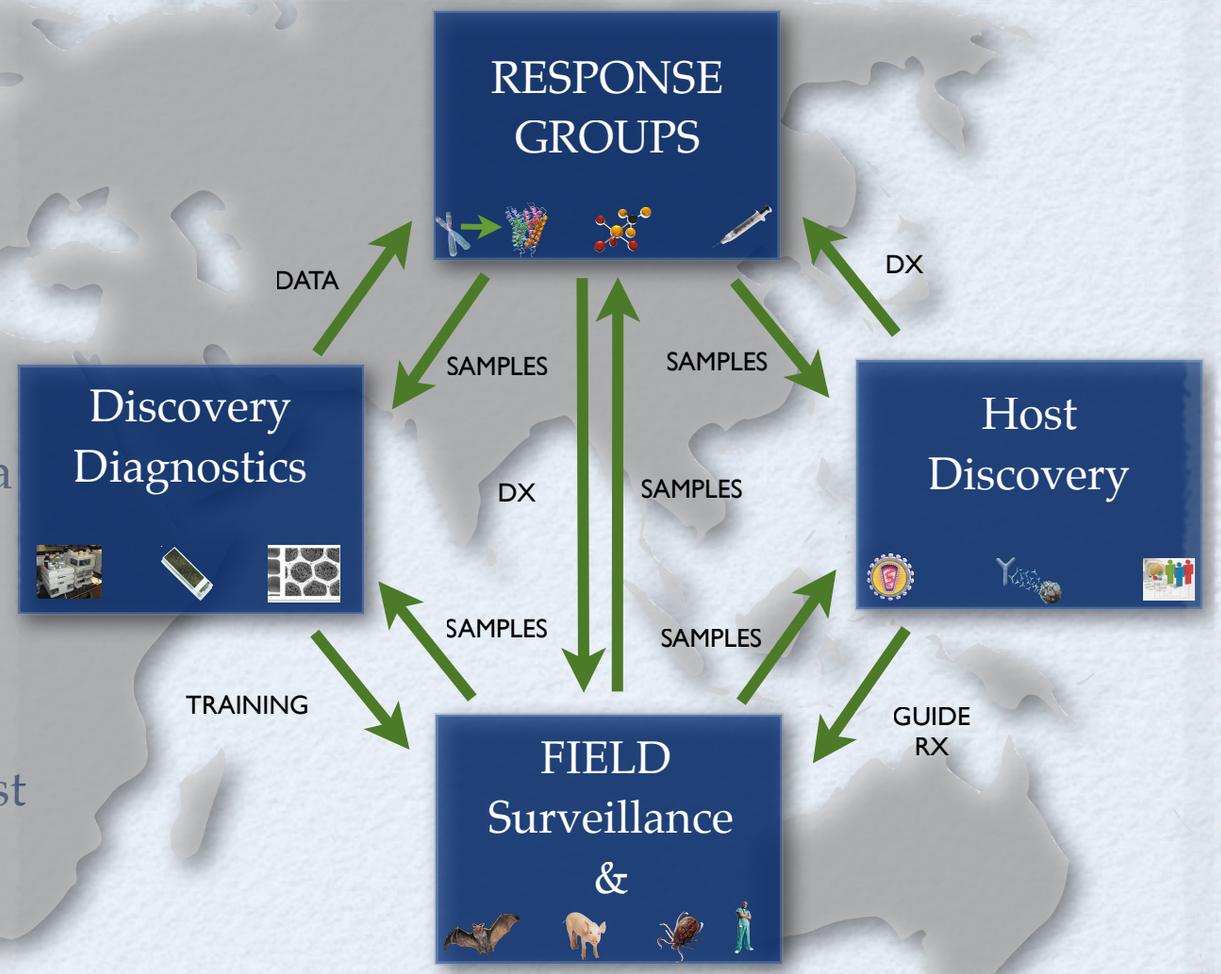


Global Legacy

- We live in a world that is increasingly interconnected, making us more susceptible to harmful pathogens. Now, more than ever, we need a unified public health strategy.

- Our international group of investigators has been instrumental in several outbreak response efforts, including the anthrax crisis, the SARS epidemic, West Nile and LuJo virus and Nipah virus encephalitis.

- By working in collaboration with the Federal Government, we can promote public health on a global scale, ensuring more efficient prevention, detection and management of potential health threats.



CII Partners

Pan American Health Organization

EcoHealth Alliance

LRN Laboratory Response Network

Lawrence Livermore National Laboratory

Caliper a PerkinElmer company

Wildlife Trust

NYC Health

Norwegian Institute of Public Health www.fhi.no

Roche

Wildlife Conservation Society

University of Houston — You are the pride

Agilent Technologies

Naval Medical Research Center U.S. Navy

Pfizer

FDA

Roche 454 Sequencing

Los Alamos National Laboratory EST. 1943

National Institutes of Health

CDC

USAMRIID United States Army Medical Research Institute of Infectious Diseases

Edgewood Chemical Biological Center A U.S. Army RDECOM Laboratory

Bill & Melinda Gates foundation

UC Davis Veterinary Medicine

NYSBC New York Structural Biology Center

Walter Reed Army Medical Center

World Health Organization

Sandia National Laboratories

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USAID From the American People

U.S. Army

BRRAT Lab

USDA

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Center for Infection & Immunity

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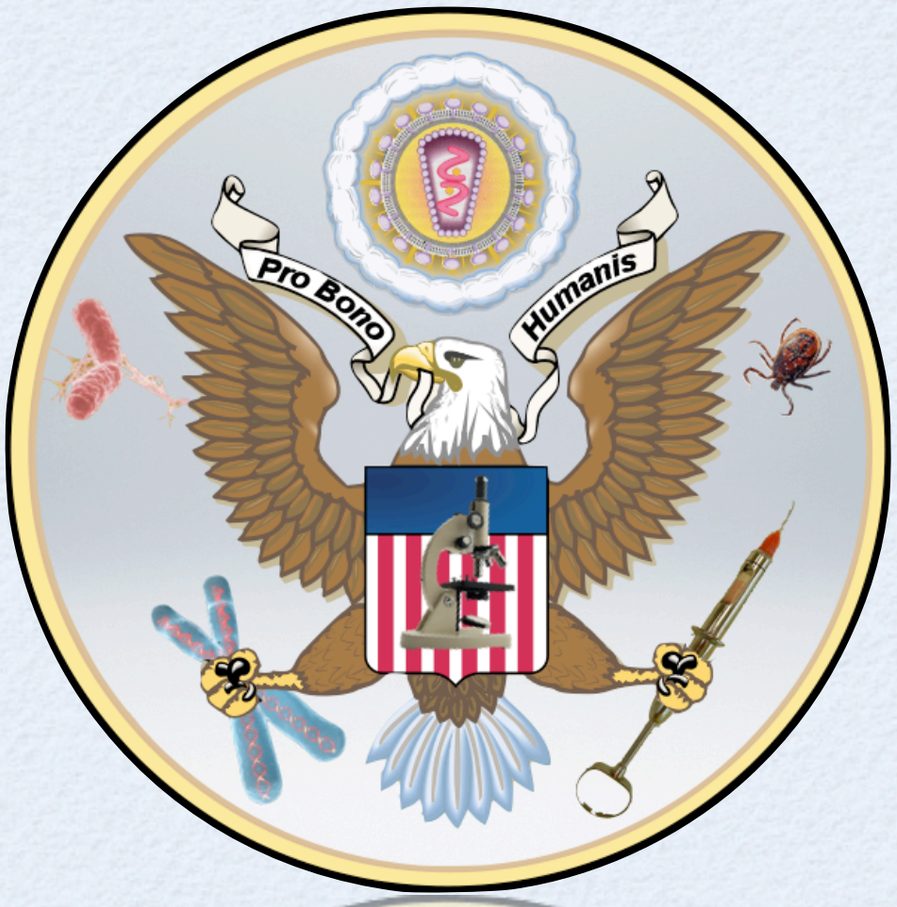
Joel Garcia
Alex Pertrosov
Alla Tashmukhamedova
Sophronia Yu
Saddef Haq
Wai Hung Wong
Ismara Navarrete
Komal Jain
Enrique Herrera

This project is funded by the DoD

Collaborators



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