

# **DNA Based Detection Technologies**

**Stephen M. Apatow**  
**President, Humanitarian Resource Institute**  
Director R & D, Humanitarian University Consortium

## **Background**

- **Humanitarian Resource Institute: Established in 1994**
- **Humanitarian University Consortium: Founded in 2002 to support the development of initiatives associated with economic, social, cultural and humanitarian issues worldwide. Academic focus areas include medicine, veterinary medicine and law.**

## Communication Networks

- **United States Networks:**
  - Grassroots Networks (Household Level)
  - Corporate
  - Municipal, State and Federal Government
  - Community Action and Interfaith Organizations – approx. 3,100 Counties.
- **International Networks:**
  - Newspaper, Radio and Television
  - Corporate
  - Intergovernmental, Non Governmental, United Nations
  - Community Action and Interfaith Organizations- approx. 195 countries.
- **Humanitarian University Consortium: UNESCO membership, college/university level, WHO, OIE, FAO.**

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**New and reemerging infectious diseases will pose a rising global health threat and will complicate US and global security over the next 20 years. These diseases will endanger US citizens at home and abroad, threaten US armed forces deployed overseas, and exacerbate social and political instability in key countries and regions in which the United States has significant interests.**

*The Global Infectious Disease Threat and Its Implications for the United States, National Intelligence Council, January 2000.*

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## Globalization

- **Geoeconomic prioritization is the reference point for the global communicable disease architecture.**

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## The Threats: Bioweapons

- **Bacterial weapons**
- **Viral weapons**
- **Rickettsial weapons**
- **Fungal weapons**
- **Toxin weapons**
- **Bio-regulators**

Alibek: Biological Weapons Threat and Defense

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## **LD<sub>50</sub> for Some Infections' Causative Agents:**

- **Anthrax: 10000-20000 spores**
- **Brucellosis: 200-400 bacterial cells (?)**
- **Coccidioidomycosis: 10-100 arthrospores**
- **Ebola: 3-10 viral particles**
- **Glanders: 100-200 bacterial cells (?)**
- **Marburg: 3-10 viral particles**
- **Plague: 500-1500 bacterial cells**
- **Smallpox: 5-10 viral particles**
- **Tularemia: 10-100 bacterial cells**
- **Q fever: 1-3 cells**

Alibek: Biological Weapons Threat and Defense

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## **Infectious Diseases of Concern**

- **West Nile Virus**
- **Foot and Mouth**
- **Severe Acute Respiratory Syndrome (SARS)**
- **Avian Influenza**

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## West Nile Virus (WNV)

- **West Nile virus was first isolated in the West Nile District of Uganda in 1937. The ecology was characterized in Egypt in the 1950s.**
- **The virus became recognized as a cause of severe human meningoencephalitis (inflammation of the spinal cord and brain) in elderly patients during an outbreak in Israel in 1957.**
- **Equine disease was first noted in Egypt and France in the early 1960s.**

CDC: West Nile Virus, National Center for Infectious Diseases, Division of Vector-Borne Infectious Diseases.

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## West Nile Virus – Equestrian Cases

- **In August 1999, 22 horses in the Riverhead area of Long Island began showing signs of an encephalitic infection: lethargy, weakness in the hindquarters, and convulsions.**
- **On Oct 19, after 13 of the horses had died or were euthanized, the USDA-APHIS announced they all tested positive for the West Nile virus.**

Veterinarians were key to discovering outbreak of exotic encephalitis., AVMA, 10.21.99

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## West Nile Virus – Bird Migration

- **USGS - National Wildlife Health Center in Madison, Wis., -- Bird species, migration, spread discussion.**



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## Arboviral Surveillance

- **During the 2000 arbovirus surveillance season, we collected 317,676 mosquitoes, submitted 9,952 pools for virus testing, and detected 363 WNV-positive pools by polymerase chain reaction (PCR).**

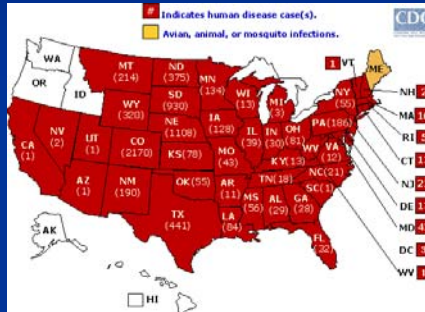
Mosquito Surveillance and Polymerase Chain Reaction Detection of West Nile Virus, New York State, CDC Emerging Infectious Diseases, Vol. 7, No. 4, Jul-Aug 2001.

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## West Nile Virus: 2002 Statistics

- 4,156 Human Cases
- 284 Fatalities
- 44 States (2289 Counties)
- 111 bird species - CDC's West Nile Virus avian mortality database.
- 14,358 equine cases in 40 states were reported to USDA APHIS - nearly 20-fold the case load reported by 20 states last year.

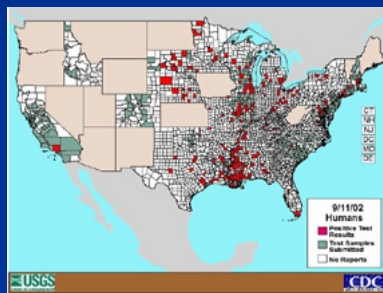
Source: Cornell Environmental Risk Analysis Program



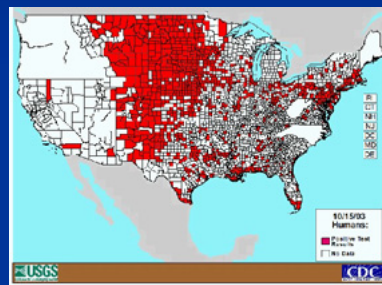
Source: CDC DVBD2

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## West Nile Virus: Epidemiological Maps



Human 2002

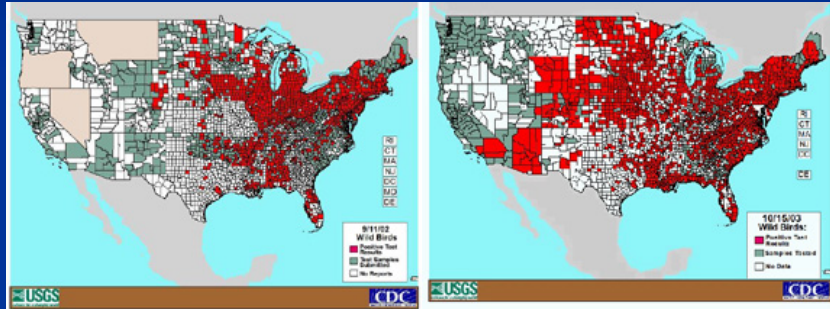


Human 2003

[www.westnilemaps.usgs.gov](http://www.westnilemaps.usgs.gov)

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## West Nile Virus: Epidemiological Maps



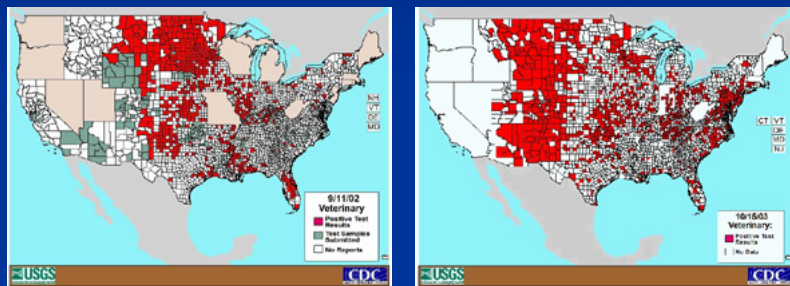
Avian 2002

Avian 2003

[www.westnilemaps.usgs.gov](http://www.westnilemaps.usgs.gov)

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## West Nile Virus: Epidemiological Maps



Veterinary 2002

Veterinary 2003

[www.westnilemaps.usgs.gov](http://www.westnilemaps.usgs.gov)

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## West Nile Virus: 2006

### Back in the News:

- **Forty-one states and the District have reported 2,171 illnesses from West Nile virus so far this year, 74 of which have been fatal.**
- **"There were 1,512 reported cases in 38 states -- including 41 deaths -- at that same point last year, and a total of 3,000 cases, including 119 deaths, by the close of 2005," said Dr. Lyle Petersen, director of the Centers for Disease Control and Prevention's Division of Vector-Borne Infectious Diseases.**

West Nile cases nearing record, The Washington Times, 22 September 2006.

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## Foot and Mouth Disease (FMD)



- **Foot-and-mouth disease (FMD) is a severe, highly communicable viral disease of cattle and swine. It also affects sheep, goats, deer, and other cloven-hooved ruminants.**
- **This country has been free of FMD since 1929, when the last of nine U.S. outbreaks was eradicated.**

USDA, APHIS, Veterinary Services, Emergency Programs

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## UK FMD Outbreak – Index Case



- Index case found 20 days after estimated start of the outbreak.
- One month after the index case, the number of determined infected farms was 707, with 342 infected premises that had not yet been detected.

Martin Hugh-Jones, Foot & Mouth Disease: The UK Lessons, 2002 Ontario Veterinary Medical Association Annual Meeting.

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## FMD: Biodetection

- If an outbreak is detected, the time required to diagnose FMD and initiate the appropriate measures will be crucial to determining the outbreak's ultimate effect.
- Research has suggested that a one week delay could increase the proportion of infected premises from 18% to more than 90%.

Ekboir: Potential Impact of Foot-and-Mouth Disease in California: The role and contribution of animal health surveillance and monitoring services.

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## UK FMD Outbreak: Overview



Photo: Owen Humphreys, PA

- The United Kingdom Ministry of Agriculture, Fisheries and Food (MAFF) contingency plan envisaged up to ten outbreaks (premises).
- To the extent that it was a conscious decision, the cull was used because it was realized that actual tracing of the disease had become impossible.

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## UK FMD Outbreak: Statistics



(AP PHOTO/ FRANCK PREVEL)

- 10,791,000 animals culled (Meat and Livestock Commission)
- 10,472: Number of premises recorded on which animals have been or were due to be slaughtered ( DEFRA, June 2003.)
- Economic cost: \$20 billion (US) (Vannieuwenhoven , 2001 Convention News, AVMA. 18 July 2001)

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## Severe Acute Respiratory Syndrome (SARS)



(AP Photo/Str)

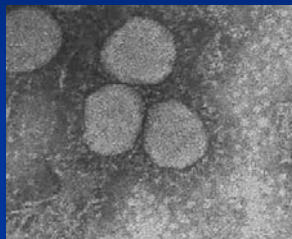
**Severe Acute Respiratory Syndrome (SARS) has crossed international boundaries within a matter of weeks.**

**In contrast to the spread of West Nile Virus from the initial index case in New York City throughout the North American Continent during the period from 1999-2003**



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## SARS - Overview



Department of Microbiology, The University of Hong Kong and the Government Virus Unit, Department of Health, Hong Kong SAR China

- **The World Health Organization formally announced on 4/16/03 that a new pathogen, a member of the coronavirus family never before seen in humans, is the cause of Severe Acute Respiratory Syndrome (SARS).**

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## **SARS: Genomic Challenges**

- **Each gene of SARS-CoV has only 70% or less identity with the corresponding gene of the known coronaviruses.**
- **Thus, SARS-CoV was only distantly related to the known coronaviruses of humans and animals.**
- **Phylogenetic analysis suggested that SARS-CoV did not fit within any of the three groups that contain all other known coronaviruses.**

Holmes, J. Clin. Invest. 111:1605–1609 (2003). doi:10.1172/JCI200318819.

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## **SARS: Sequence Analysis**

**Comparative analysis of the SARS coronavirus genome was a result of sequence information provided by:**

- **The Institute of Microbiology and Epidemiology of the Academy of Military Medical Sciences and the Beijing Genomics Institute of Chinese Academy of Sciences**
- **CDC**
- **The National Microbiology Laboratory, Canada**
- **University of California at San Francisco**
- **Erasmus University**
- **Rotterdam and Bernhard-Nocht Institute, Hamburg**

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## Avian Influenza: H5N1

- In December 2003, Highly Pathogenic Avian Influenza (HPAI) was confirmed about 80 kilometres south-east of the capital, Seoul, South Korea. Academics noted the transmission vehicle as migratory birds.
- If the outbreak moved beyond the borders of South Korea to countries in the East Asian-Australasian Flyway (via migratory birds), could we be looking at a widespread international multi-country outbreak (as observed with West Nile Virus throughout North America).

Pandemic Influenza: Contingency Planning, International Veterinary Public Health Consortium

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## H5N1: Migratory Birds

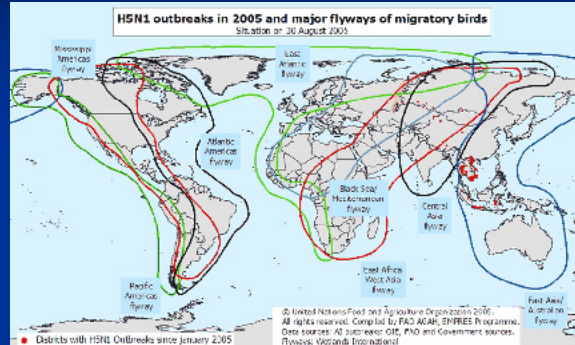


- Today, comparative genomics serves as the reference point for transboundary spread and evolution of influenza A subtype H5N1

Influenza Virus Resource: NIAID Influenza Genome Sequencing Project and GenBank.

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## H5N1: Surveillance, Containment and Control



- Discussion expands to human transmissible strains of avian influenza virus that originated from H5N1 including H3N2, H1N1, H2, H9, H7 subtypes.

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## AI: H5N1: Co-Infection

- The May issue of Lancet examines the relationship between influenza and bacterial suprainfections in past pandemics.
- Evidence available from the pandemics of 1918, 1957 and 1968 suggests that the majority of fatal cases of pneumonia in all three pandemics were associated with positive bacterial cultures, most commonly with pneumococcus, *S. aureus*, *S. pyogenes*, and *H. influenzae*.

CBN Report: Antibiotics to Treat Pneumonia: An Overlooked Essential Element of Pandemic Preparedness, Clinicians Biodefense Network, Center for Biosecurity.

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## Emerging Infectious Diseases

- 80% of "emerging" infections are animal based

*Martin Hugh Jones, Director, WHO Collaborating Center for Remote Sensing and Geographic Information Systems for Public Health, LSU School of Veterinary Medicine.*



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## DNA Based Detection Technologies

- Functional biodetection systems.
- Background discussions: OIE, FAO, WHO – published research.

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## Background Discussions: DOD/NATO

- There is no standardization of machinery or reagents.
- Just starting with the concept of definitive diagnosis using of real time PCR – 18 pathogens human & veterinary.



- The Roche LightCycler® 2.0 System has repeatedly set the standard for real-time PCR.



- The Idaho Tech R.A.P.I.D.\* (Ruggedized Advanced Pathogen Identification Device) is a specialty instrument for military field hospitals, first responders and other rough environments.

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## Functional DNA Based Detection Technologies: Reference 1

### Diagnostic System for Rapid and Sensitive Differential Detection of Pathogens

Thomas Briese,<sup>\*1</sup> Gustavo Palacios,<sup>\*1</sup> Mark Kokoris,<sup>†1</sup> Omar Jabado,<sup>\*</sup>  
Zhiqiang Liu,<sup>\*</sup> Neil Renwick,<sup>\*</sup> Vishal Kapoor,<sup>\*</sup> Inmaculada Casas,<sup>‡</sup> Francisco  
Pozo,<sup>‡</sup> Ron Limberger,<sup>§</sup> Pilar Perez-Brena,<sup>‡</sup> Jingyue Ju,<sup>\*</sup> and W. Ian Lipkin<sup>\*</sup>

Emerging Infectious Diseases, Vol. 11, No. 2, February 2005  
[www.cdc.gov/ncidod/EID/vol11no02/04-0492.htm](http://www.cdc.gov/ncidod/EID/vol11no02/04-0492.htm)

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- The paper describes a diagnostic system for rapid, sensitive, multiplex discrimination of microbial gene sequences and report its application for detecting 22 respiratory pathogens.
- Methods to directly detect nucleic acids of microbial pathogens in clinical specimens are a focus point when culturing the organism fails.
- Clinical syndromes are infrequently specific for single pathogens; thus assays are needed that allow multiple agents to be simultaneously considered.

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- The team created a polymerase chain reaction (PCR) platform in which microbial gene targets are coded by a library of 64 distinct Masscode tags (Qiagen Masscode Technology, Qiagen, Hilden Germany).
- Microbial nucleic acids (RNA, DNA, or both) are amplified by multiplex reverse transcription PCR using primers labeled by a photocleavable link to molecular tags of different molecular weight.
- Multiplex primer sets were designed to identify up to 22 respiratory pathogens in a single Mass Tag PCR reaction; sensitivity was established by using synthetic DNA and RNA standards as well as titrated viral stocks; the utility of Mass Tag PCR was determined in blinded analysis of previously diagnosed clinical specimens.

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- RNA extracted from banked sputum, nasal swabs, and pulmonary washes of persons with respiratory infections was tested by using an assay panel comprising 30 gene targets that represented 22 respiratory pathogens. Infections in each of these persons was previously diagnosed through virus isolation and conventional nested PCR.
- Reverse transcription was performed using random hexamers, and Mass Tag PCR results were consistent in all cases with the established diagnosis.

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- Infections with respiratory syncytial virus, human parainfluenza virus, SARS coronavirus, adenovirus, enterovirus, metapneumovirus, and influenza virus were correctly identified.

Pathogen	No. positive/no. tested†
RSV A	2/2
RSV B	3/3
HPIV-1	1/1
HPIV-3	2/2
HPIV-4	2/2
CoV-SARS	4/4
Metapneumovirus	2/3
Influenza B	1/3
Influenza A	2/6
Adenovirus	2/2
Enterovirus	2/2

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## Conclusions

- **Our results indicate that Mass Tag PCR is a sensitive and specific tool for molecular characterization of microflora.**

To submit correspondence to the authors or contact them at the following address:

W. Ian Lipkin, Mailman School of Public Health, Columbia University, 722 West 168th St, New York, NY 10032, USA; fax: 212-342-9044; email: wil2001@columbia.edu

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## Functional DNA Based Detection Technologies: Reference 2

### Use of Oligonucleotide Microarrays for Rapid Detection and Serotyping of Acute Respiratory Disease-Associated Adenoviruses

Baochuan Lin,<sup>1,2</sup> Gary J. Vora,<sup>1,2</sup> Dzung Thach,<sup>1,2</sup> Elizabeth Walter,<sup>2,3</sup> David Metzgar,<sup>2,4</sup> Clark Tibbetts,<sup>2</sup> and David A. Stenger<sup>1,2\*</sup>

Center for Bio/Molecular Science & Engineering, Naval Research Laboratory, Washington, DC 20375,<sup>1</sup> Epidemic Outbreak Surveillance Consortium, USAF/SGXFalls Church, Virginia,<sup>2</sup> Lackland Air Force Base, San Antonio, Texas 78236,<sup>3</sup> Respiratory Disease Laboratory, Naval Health Research Center, San Diego, California 92186<sup>4</sup>

<http://jcm.asm.org/cgi/content/full/42/7/3232>

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- **The cessation of the adenovirus vaccination program for military trainees has resulted in several recent acute respiratory disease (ARD) outbreaks. In the absence of vaccination, rapid detection methods are necessary for the timely implementation of measures to prevent adenovirus transmission within military training facilities.**
- **The cessation of a vaccination campaign that successfully prevented outbreaks of adenovirus-associated acute respiratory disease (ARD) in military facilities has resulted in the reemergence of adenovirus-associated ARD epidemics .**
- **Furthermore, there are no effective therapeutic or alternate prophylactic treatments for the ARD caused by adenoviruses.**

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- **To compound the problem, the crowded and stressed situations in military training facilities provide an ideal environment for the airborne transmission of adenoviruses.**
- **As a result, the rapid detection of adenoviruses is needed to aid in controlling viral transmission and adenovirus-associated respiratory disease.**
- **The human adenoviruses are a family of viruses consisting of 51 serotypes, can be further divided into six subgroups (subgroups A to F) according to their nucleic acid homologies, fiber protein characteristics, and biological properties.**

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- **Conventional methods for adenovirus detection and serotyping involve testing by viral shell culture, observation for cytopathic effects, and microneutralization assays or serotyping with virus serotype-specific antisera .**
- **These methods produce confirmatory results in 3 days to 3 weeks, depending on the specimen source and the concentration of viable virus within the specimen.**

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- **Although PCR-based methods have clearly facilitated the detection of adenoviruses, conventional gel-based amplicon detection techniques require multistep procedures and special laboratory setup and are entirely reliant upon DNA fragment size estimation and analysis for positive identification.**
- **More recently, fluorogenic real-time PCR has been developed as a type-specific diagnostic system.**
- **With the substantial progress in microarray technology, it is now possible to combine the sensitivity afforded by nucleic acid amplification with the specificity afforded by DNA-DNA hybridization for the detection of viruses pathogenic for humans**

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- Using the two-checkpoint scheme, our results demonstrate the ability of the assay to detect adenoviruses from laboratory and clinical samples in less than 60 min and to determine the serotype in less than 90 min.
- Using an alternate amplification and hybridization strategy, we also demonstrate a detection sensitivity of 1 to 100 genome copies for laboratory and clinical samples, concordance of the assay results with those of conventional adenovirus identification methods, and the ability to detect adenoviral contamination events in a single assay.

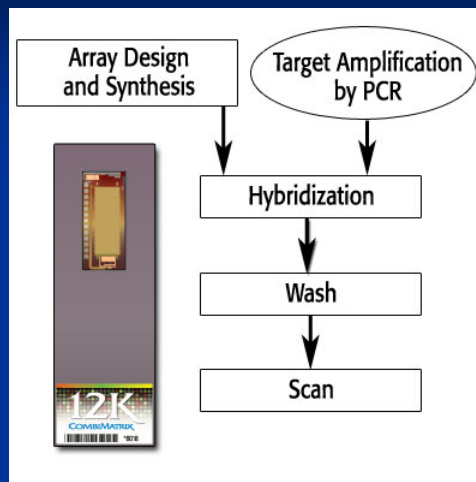
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## Logistics Discussion

- Lipkin's lab (Columbia) has a Mass Tag that discriminates 22 respiratory pathogens in a single sample for the same cost as one PCR today.
- The goal is Mass Tag PCR that identifies many pathogens at the same cost and in the same time as a single PCR today.
- This is achievable now, the question is what package of agents to cluster.

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## Microarray



CombiMatrix Corporation

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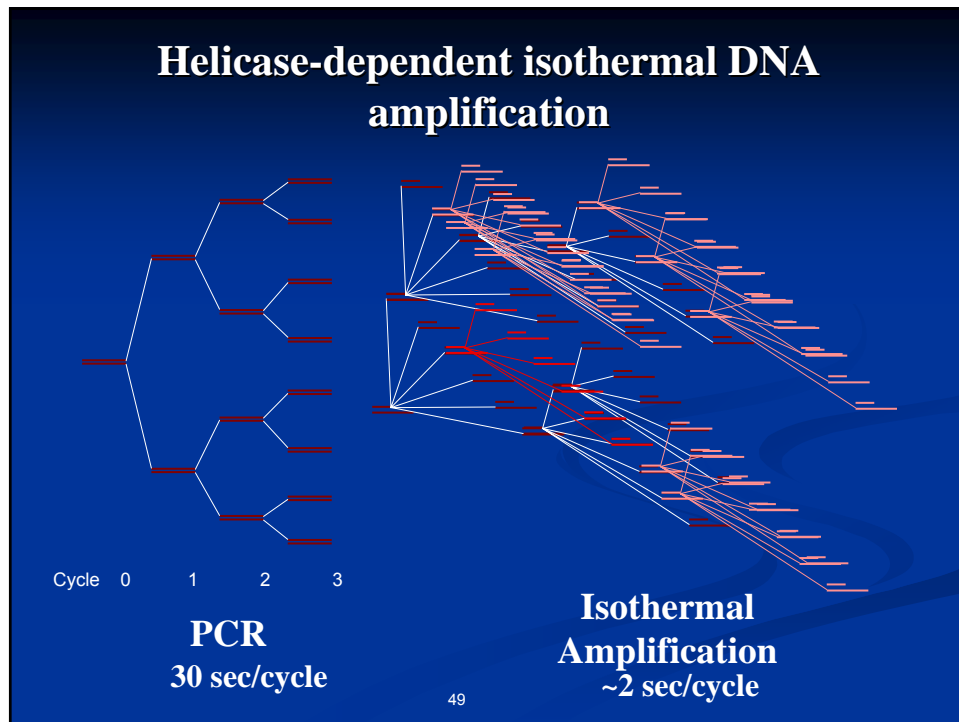
## Autonomous Pathogen Detection System



Lawrence Livermore National Laboratory (LLNL),

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## Conclusion

- The global communicable disease architecture must be strengthened by access to affordable, field-validated molecular diagnostic technologies for the medical, veterinary and lab level in every UN member country.
- A crucial need exists for concentrated research and development, rapid validation and licensing that meets a common international standard.
- The objectives set forth in the newly revised World Health Organization International Health Regulations (IHR) require a functional international epidemiological surveillance and reporting system.

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**Humanitarian Resource Institute**  
Internet: [www.humanitarian.net](http://www.humanitarian.net)

**Pathobiologics International**  
Telephone: (203) 668-0282  
Internet: [www.pathobiologics.org](http://www.pathobiologics.org)  
Email: [s.m.apatow@pathobiologics.org](mailto:s.m.apatow@pathobiologics.org)

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