



Technology Transition Workshop | *Steven A. Hofstadler, Ph.D.*

High-Throughput Analysis of Amplified Nucleic Acids with Mass Spectrometry: The Ibis™ Platform

Outline

- **The challenge of broad pathogen detection**
- **The Ibis™ approach**
 - Principle of operation
- **Bacterial detection and strain typing**
 - Group A strep – direct throat swab analysis
- **Viral detection and strain typing**
 - Influenza
 - Pandemic influenza detection and strain typing
- **Integrated platform**

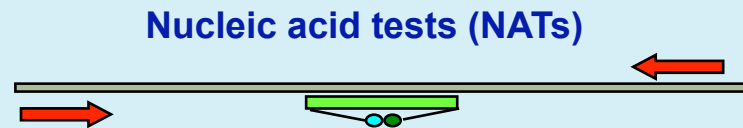
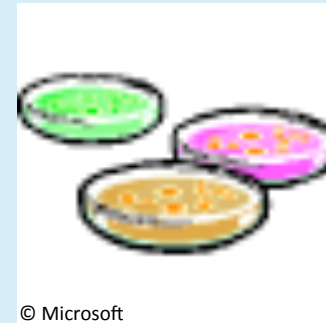
The Pathogen Detection Arena

- **Biological weapons defense is not just about anthrax**
- **Food safety is not just about E. coli 0157**
- **Hospital associated infections are not just due to Staph. aureus**
- **There are numerous naturally occurring infectious diseases**
- **Over 1000 agents known to infect humans***
 - **217 virus species**
 - **538 bacterial species**
 - **307 fungi**
 - **66 parasitic protozoa**
- **Additional plant and animal pathogens not counted**
- **Numerous strain variations**
- **Potential bio-engineered organisms**

***Taylor et al. *Phil. Trans. R. Soc. Lond. B*, (2001) 356, 983-989**

Mainstream Bioagent Detection Today

- **Culture techniques**
 - Detects a subset of all pathogens
 - Can take multiple days (weeks)
- **Single agent nucleic acid tests**
 - One test for each agent (smallpox, anthrax, plague, etc.)
 - Need too many tests
 - Fail to detect newly emergent pathogens
- **There is currently no good method to detect organisms that have never been seen before**



Bacterial Threat Symbols

Microbial Rosetta Stone Database


 NIAID Category A Priority Pathogen

 NIAID Category B Priority Pathogen

 NIAID Category C Priority Pathogen

 HHS Select Agent

 USDA High Consequence Animal Pathogen

 USDA High Consequence Plant Pathogen

 Validated Biological Weapon

 Potential Biological Weapon

 Validated Biocrime Agent

 Globally Important Human Pathogen

 Medically Important Human Pathogen

 Important Animal Pathogen

 Important Plant Pathogen

 High Potential For Bioengineering

 Zoonotic Agent

 Toxin

 CDC Notifiable Agent

 Principal Foodborne Pathogen

 Emerging Infectious Agent

Image courtesy of Christian Massire, Ph.D.

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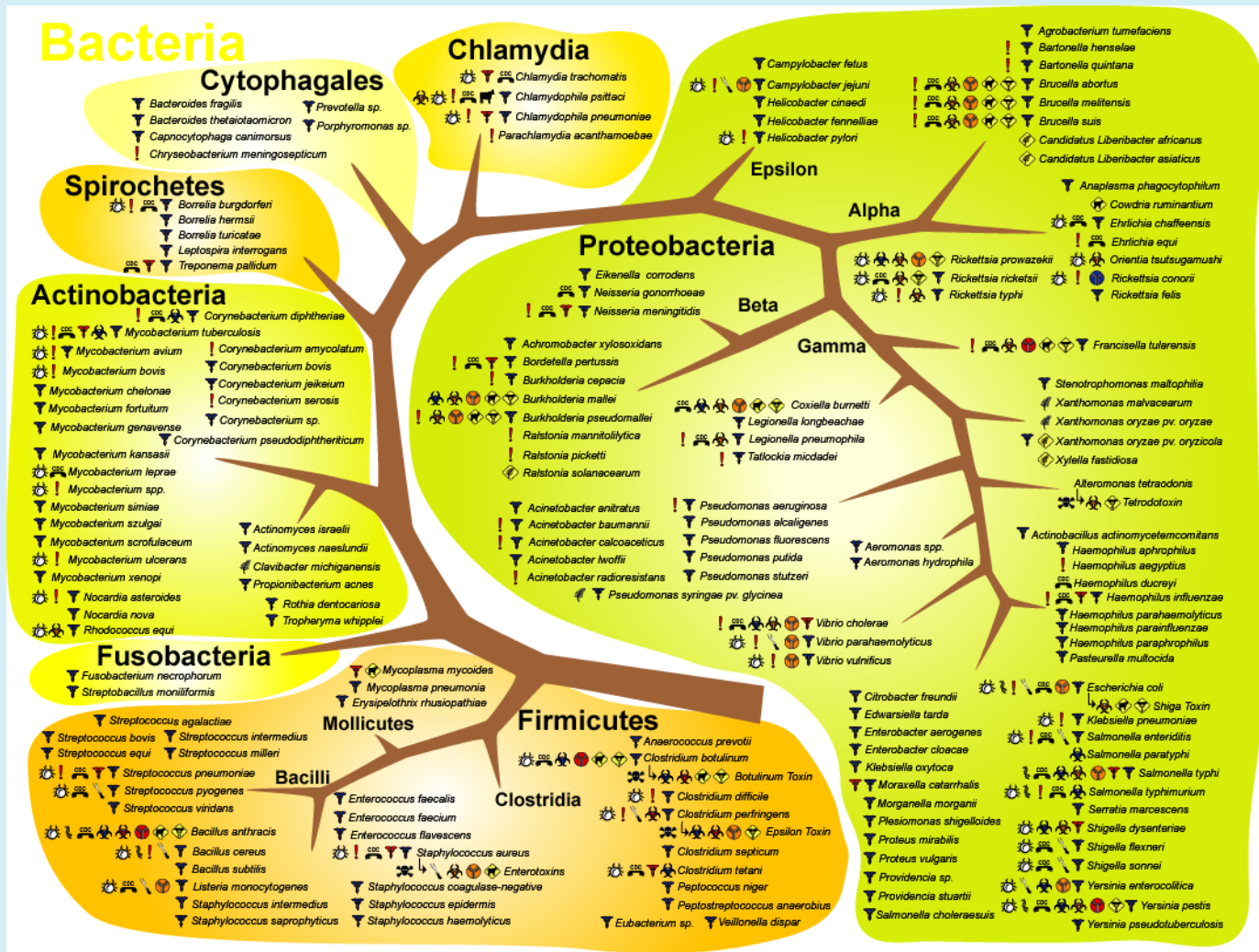


Image courtesy of Christian Massire, Ph.D.

Why Detect and/or Type Microorganisms via Nucleic Acids?

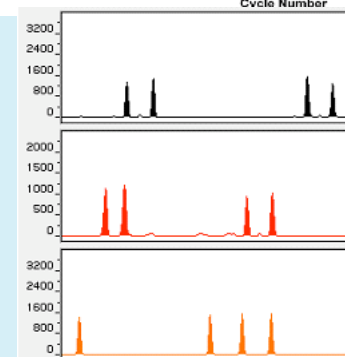
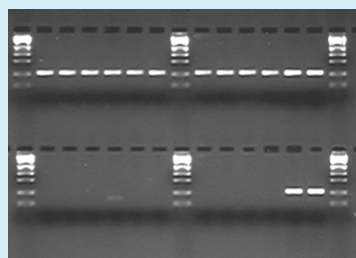
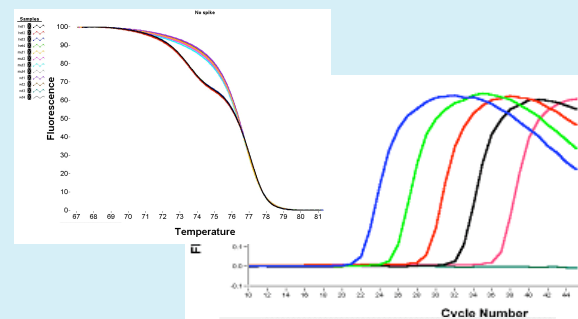
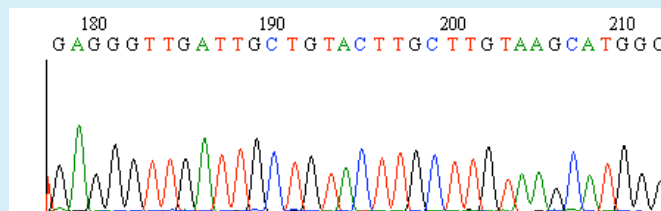
- All living things rely on DNA and/or RNA to propagate
 - All infectious agents* contain DNA and/or RNA
 - Bacteria, viruses, fungi, protozoa
 - DNA and RNA are unique among biomarkers in that they can be amplified (e.g. PCR, WGA, NASBA, etc.)
 - From trace amounts of sample
 - From highly degraded samples
 - From samples in complex backgrounds
 - **NO CULTURE REQUIRED**
 - Some genetic differences do not result in phenotypic differences
 - e.g. rRNA, VNTRs, SNPs
 - Range of specificity can be “tuned” for different applications
 - “Name that Bug”: Broad range primers
 - “Genotype/Strain-type that Bug”: species specific primers
 - “Profile that Bug”: drug resistance, virulence markers, etc.
- * Except those nasty priors!

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Interrogation of Amplified Nucleic Acids

- Sequencing
 - “Gold Standard”
- Fluorescent intercalating dye
- Hybridization
 - Specific probe with FRET pair
- DNA microarray
- Melting profiles
- Electrophoresis
 - Slab gels
 - Capillary gel electrophoresis
- **WHAT ABOUT MASS?**



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Identification and Strain Typing of Bacterial and Viral Pathogens using High Performance Mass Spectrometry: The Ibis™ Concept

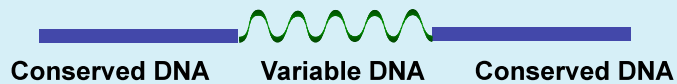


The Ibis™ Approach to Pathogen ID and Strain-Typing

STEP 1 Identify genomic regions for identification:

1

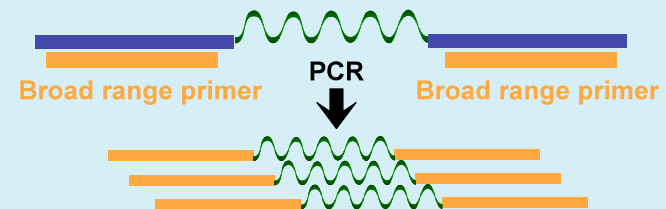
Variable DNA sequences flanked by conserved sequences



STEP 2 Amplify nucleic acids to measure:

2

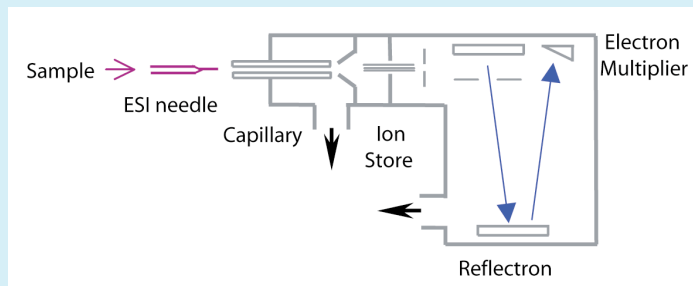
Use broad-range, unbiased PCR primers



STEP 3 Measure nucleic acid:

3

ESI-TOF

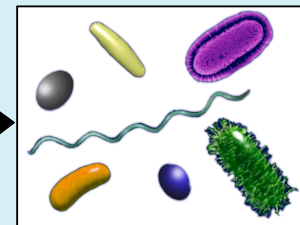


STEP 4 Identify the organisms:

4

Base-composition fingerprints

As: 17
Gs: 30
Cs: 11
Ts: 61

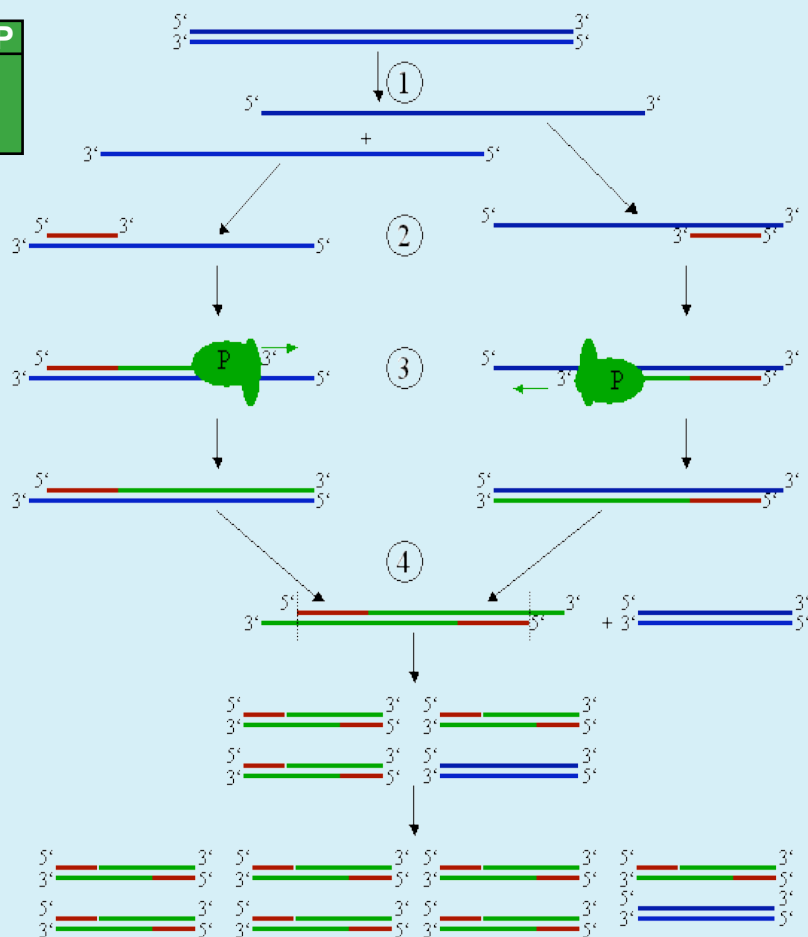


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The Polymerase Chain Reaction (PCR)

STEP
2



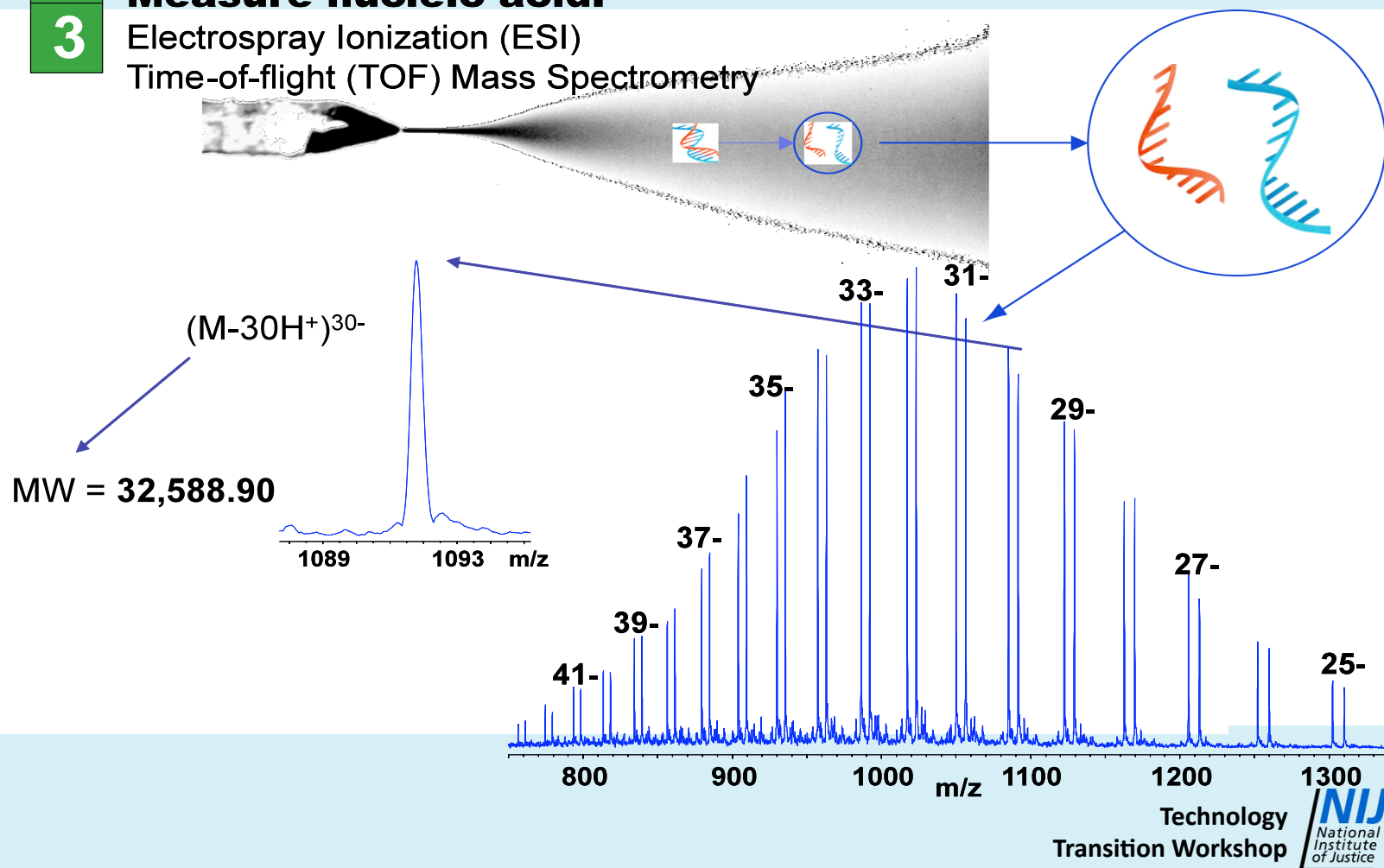
- Performed using primers designed for broad coverage
- PCR cycling conditions tolerate mismatches on initial cycles
- All primer pairs designed to work under identical PCR conditions
- Each well contains an internal calibrant
- Generally don't multiplex broad range primers (e.g. 16S and 23S rDNA)
- Multiplexing of more specific primers common (e.g. strain typing, drug resistance, virulence)

Automated ESI-TOF of PCR Amplicons on PLEX-ID™

STEP
3

Measure nucleic acid:

Electrospray Ionization (ESI)
Time-of-flight (TOF) Mass Spectrometry

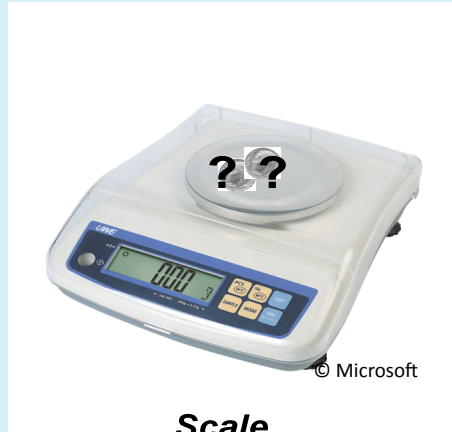


Masses to Base Composition

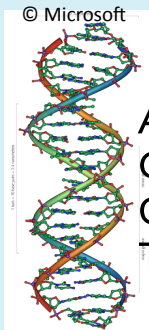
STEP
4



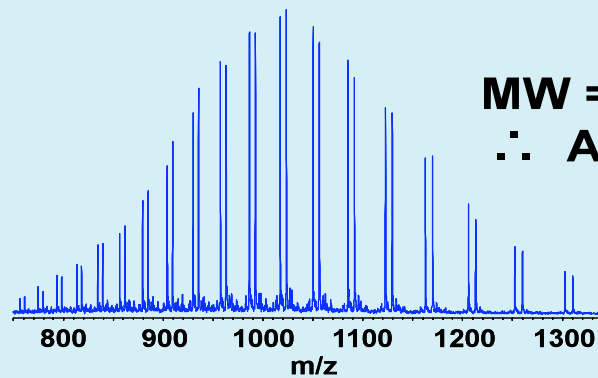
Penny = 2.500 g
Nickel = 3.950 g
Dime = 2.268 g
Quarter = 5.670 g



Weight = 4.6 grams
∴ 2 dimes



A = 313.0576 amu
G = 329.0526 amu
C = 289.0464 amu
T = 304.0461 amu



Mass spectrum

MW = 32,588.90 amu
∴ A28 G29 C25 T24

Requires 25 ppm
mass measurement error

Math takes into account
Watson-Crick base pairing

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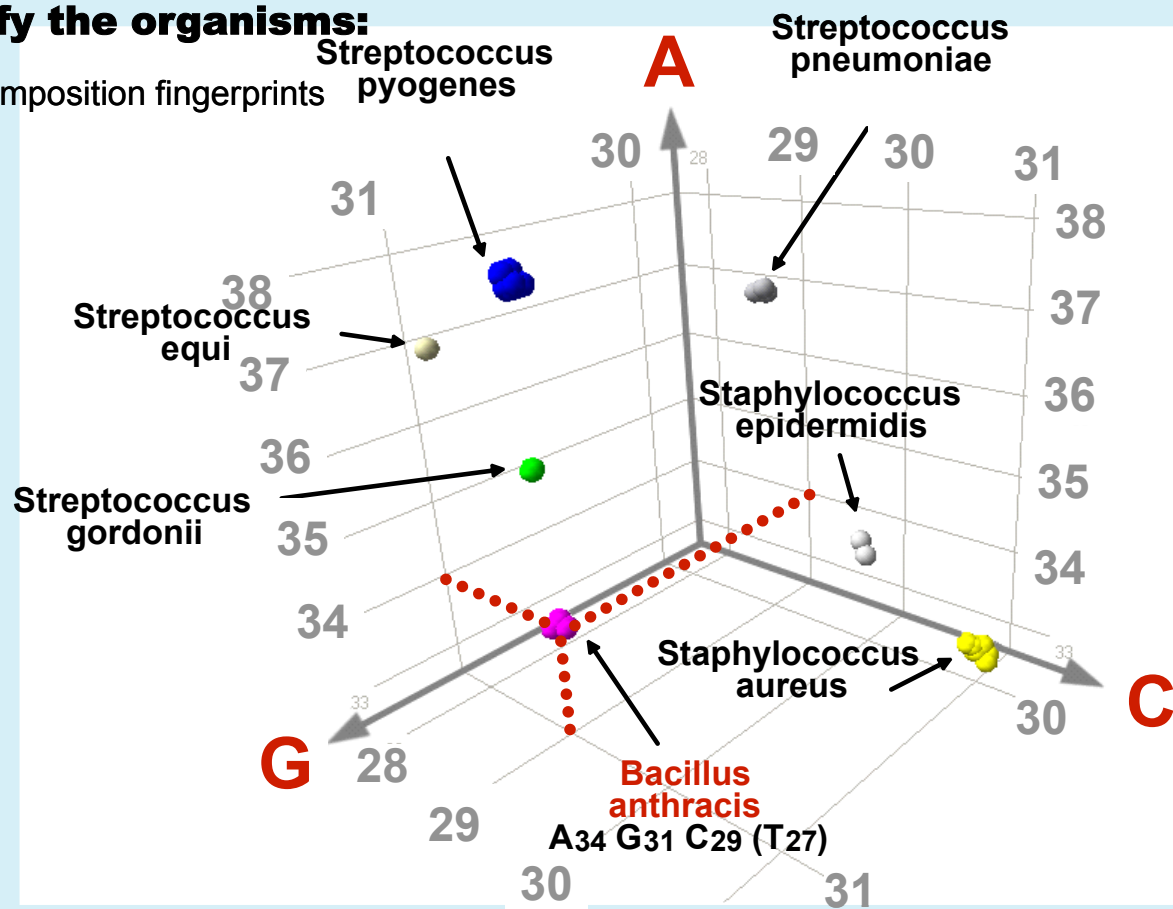
Derived and Expected Base Compositions

Primer 356 (RplB) Expected Products

STEP
4

Identify the organisms:

Base-composition fingerprints



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NIJ
National
Institute
of Justice

Broad Pathogen Detection

**Instead of asking; “Is pathogen X in my sample?”,
we ask: “Which pathogen, or pathogens, are in my
sample?”**

Group A Streptococcus (GAS)

Outbreaks in Military Settings

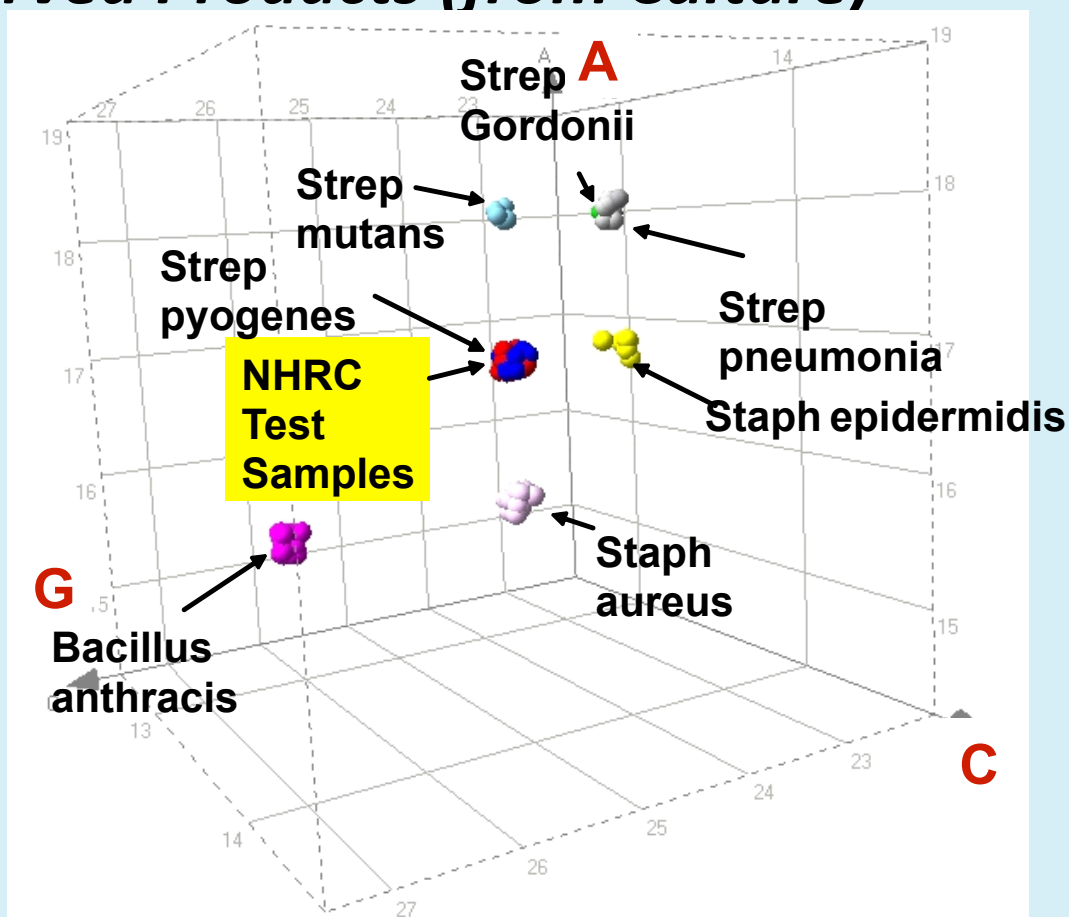
- **Outbreaks of group A strep at MCRC 2002/2003**
 - **Highly virulent strain**
 - **One death, 160 hospitalized**
 - **Training activities suspended**
- **Initial analysis of post-culture samples**
 - **80 samples sent from NHRC, Dr. Kevin Russell, December 20, 2002**
 - **“Hijacked” some BW air surveillance plates**
- **Follow up surveillance at multiple military bases**
- **Direct analysis of throat swabs without culture**



Ecker et al. *Proc. Nat. Acad. Sci. USA*, (2005) 102(22), 8012-17

Primer 17 (a 23S Primer)

Observed Products (from Culture)



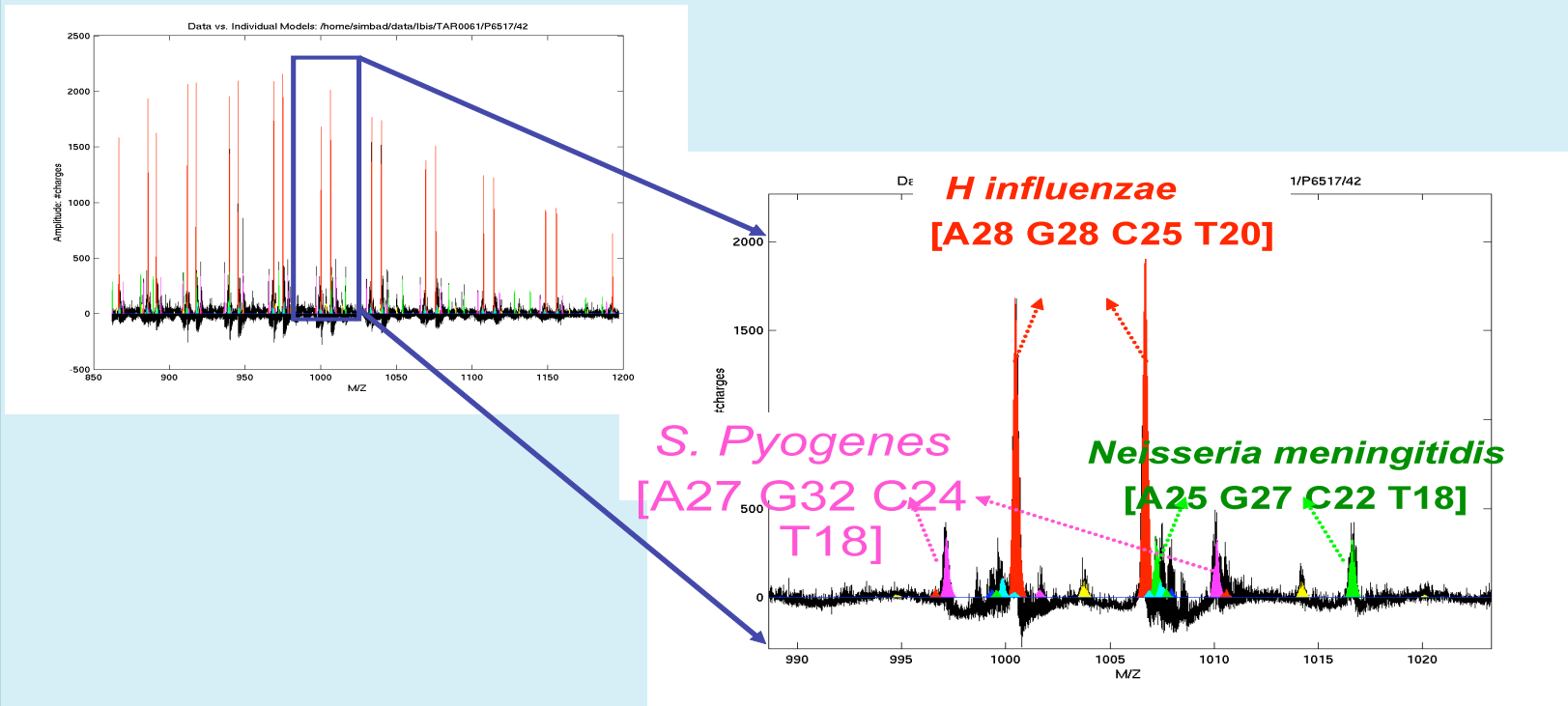
All primers of all samples consistent with *S. pyogenes*

Direct Analysis of Throat Swab*

Repeat swab positive on culture for *Streptococcus pyogenes

Primer 349: 23S rDNA 1826-1924

From an Interesting Throat Swab



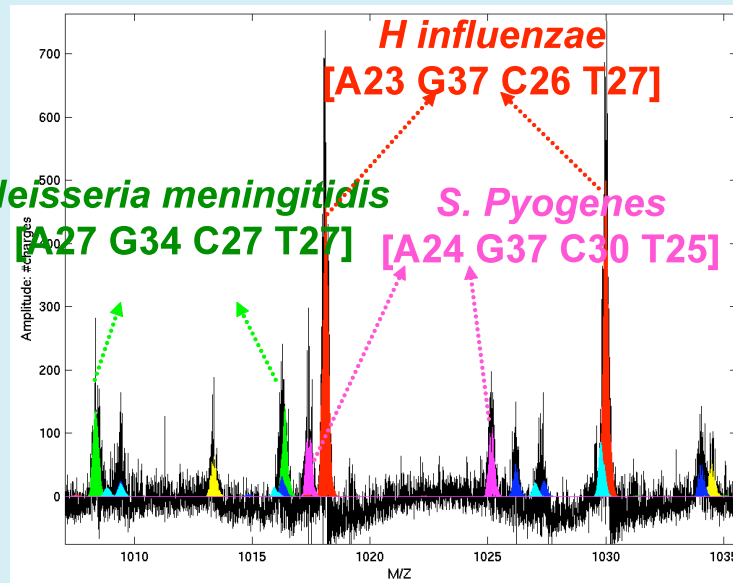
Ecker et al. *Proc. Nat. Acad. Sci. USA*, (2005) 102(22), 8012-17

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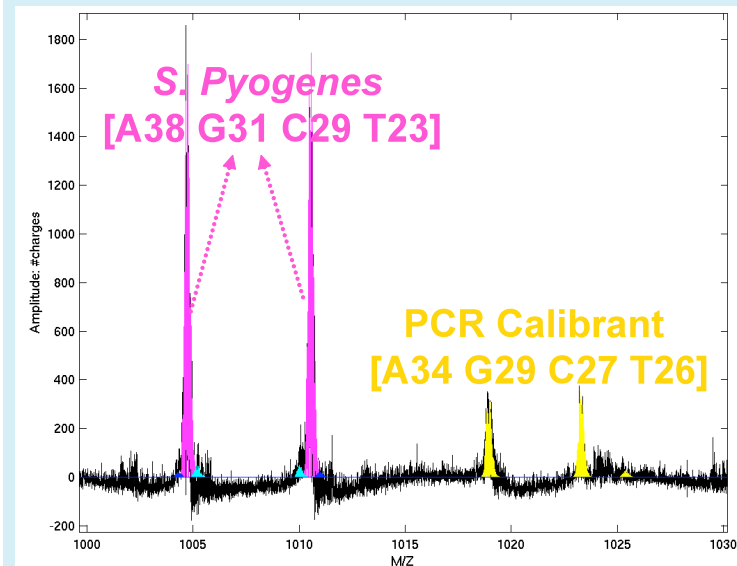


Multi-Primer Triangulation

Primer 347: 16S rDNA



Primer 356: rpIB



Organism	Cumulative Estimate of Genomes/Swab	Relative Abundances
<i>Haemophilus influenzae</i>	7.38E+05	1.00
<i>Neisseria meningitidis</i>	3.77E+05	0.51
<i>Streptococcus pyogenes</i>	1.89E+05	0.26

Ecker et al. *Proc. Nat. Acad. Sci. USA*, (2005) 102(22), 8012-17

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Haemophilus influenzae, Neisseria meningitidis, Streptococcus pyogenes (Ratio 4/2/1, 1.5 X 10⁶ genomes/swab)

Primer #1	Mass	Base Count
Blue	18234.970	A ₁₂ G ₁₇ C ₁₇ T ₁₃
Blue	17948.926	A ₁₄ G ₁₄ C ₁₂ T ₁₈
Blue	18610.017	A ₁₁ G ₁₉ C ₁₅ T ₁₅
Blue	17936.912	A ₁₁ G ₁₇ C ₁₆ T ₁₄
Blue	18877.118	A ₁₈ G ₁₅ C ₁₅ T ₁₃

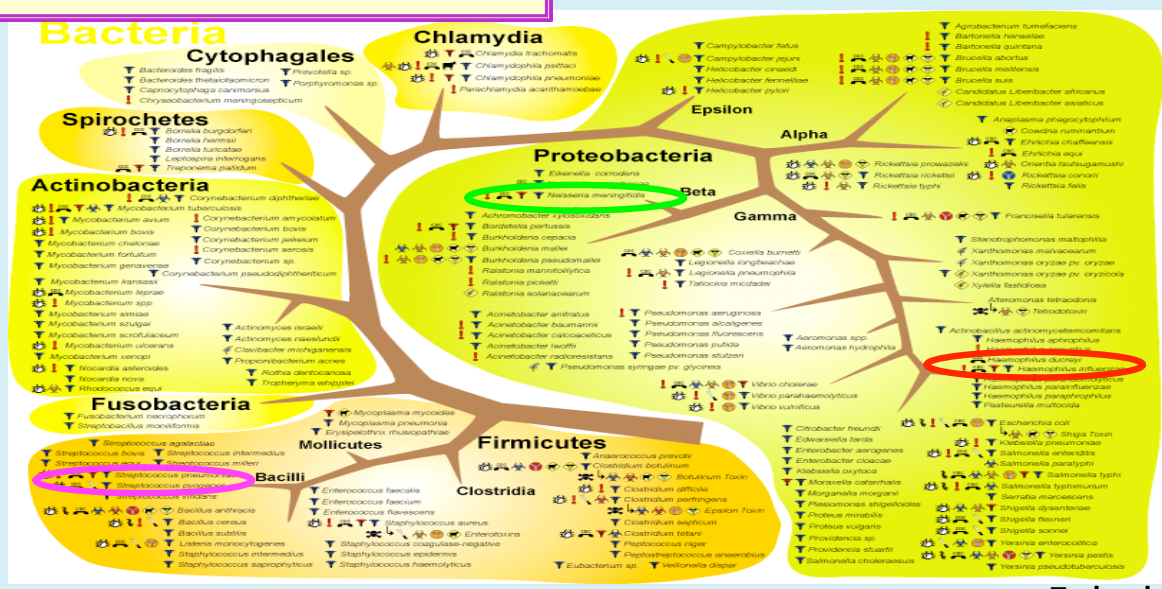
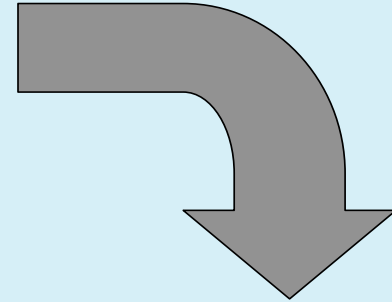
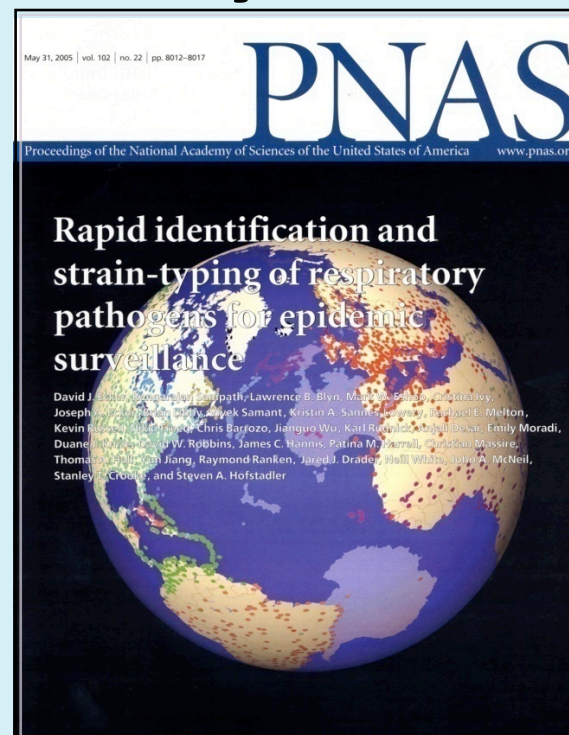


Image courtesy of Christian Massire, Ph.D.



Conclusions of Pneumonia Study*

- **Primary pathogen:**
 - Streptococcus pyogenes (GAS)
 - Known virulent strain
- **Secondary pathogens:**
 - Haemophilus influenzae
 - Neisseria meningitidis
- **Five other military facilities**
 - Determined these sites had a mixture of strain types
- **Throughput**
 - >200/samples per day



*Ecker et al. (2005) Proc. Natl. Acad. Sci. Vol. 102: p8012-8017

Virus Identification and Typing

Viral Coverage

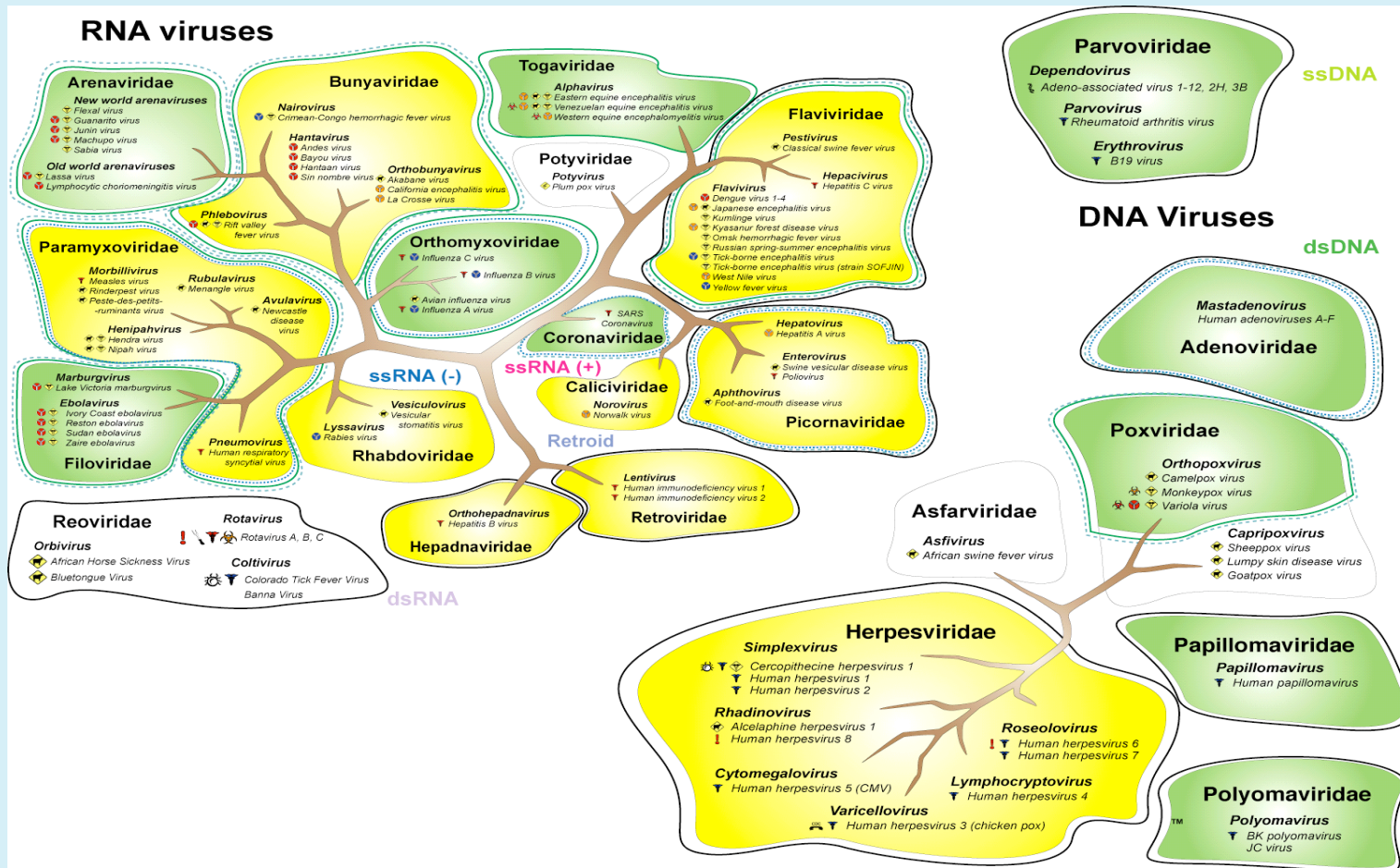


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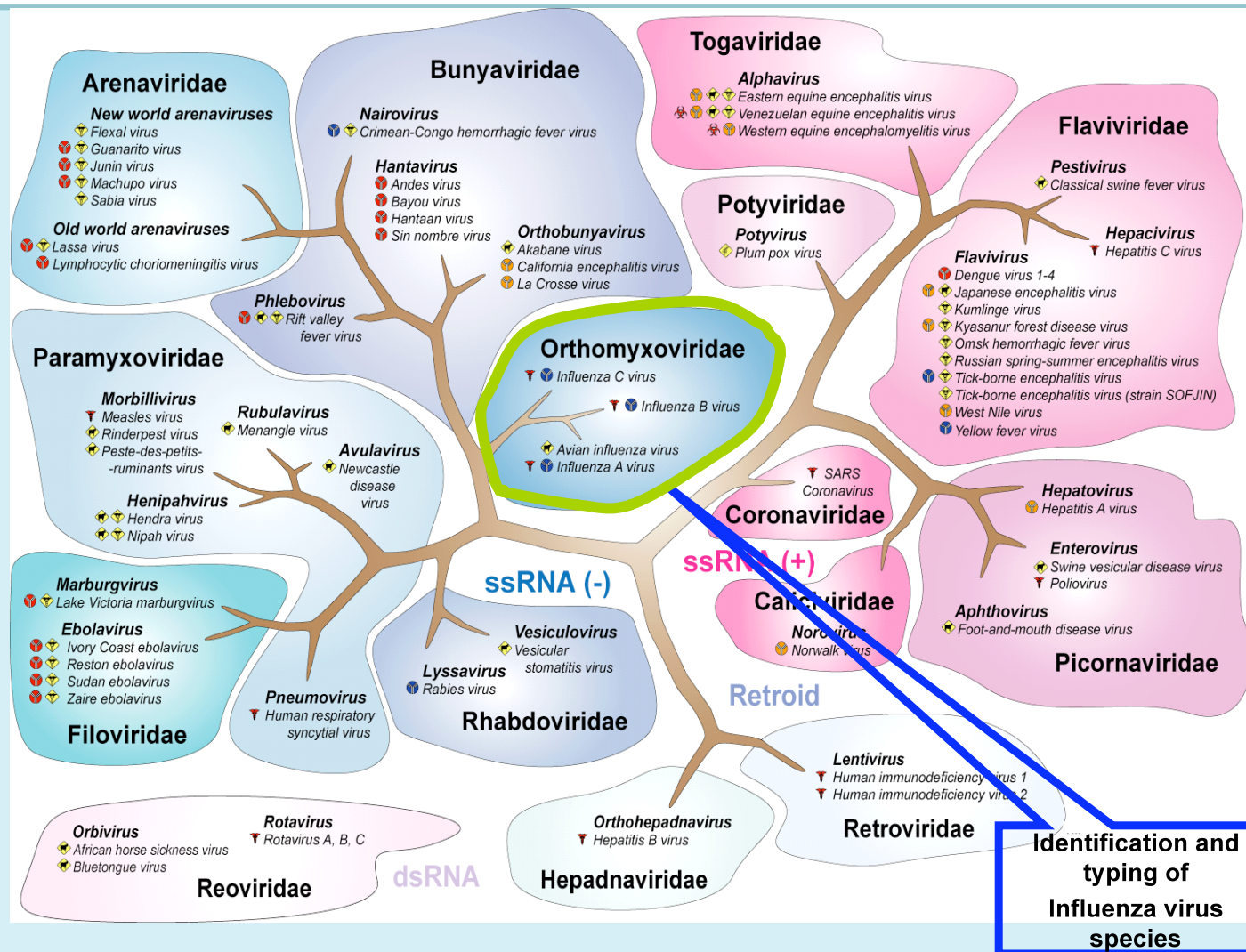


Image courtesy of Christian Massire, Ph.D.

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Influenza Virus Surveillance: Project Collaborators

CMDR Kevin Russell, M.D., Naval Health Research Center, San Diego, CA



Kirsten St. George, MAppSc, Ph.D., New York State Department of Health, Slingerlands, NY



Charlotte Gaydos, Dr.P.H. and Rich Rothman, M.D., Johns Hopkins University, Baltimore, MD



Stan Lemon, M.D., University of Texas Medical Branch, Galveston, TX



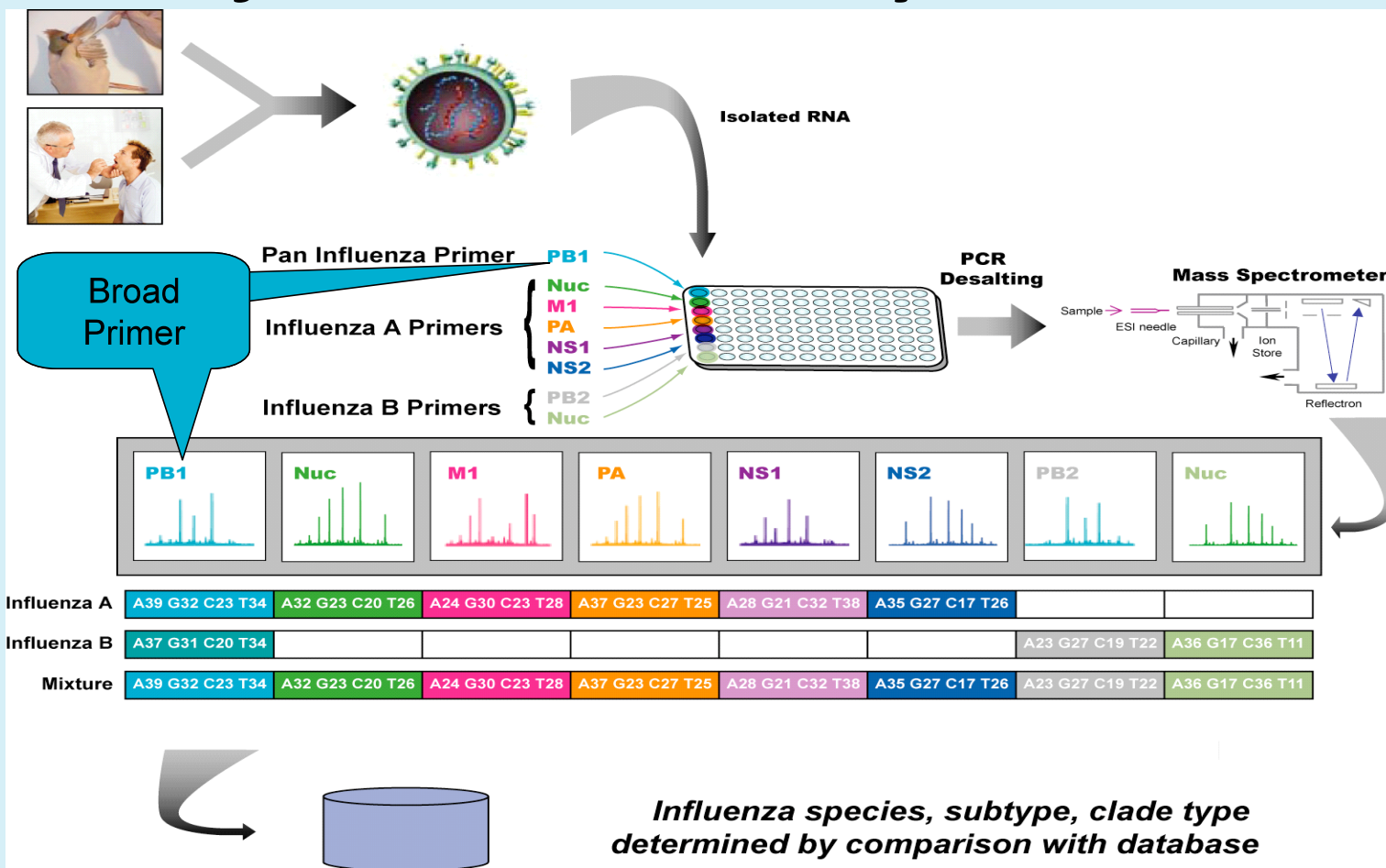
Wendy Sessions, M/SV (ASCP), Texas Department of State Health Services



Dave Stallknecht and Ginger Goekjian, College of Veterinary Medicine, University of Georgia



Ibis™ Influenza Virus Assay



Pan Influenza Primer Polymerase PB1 Primer

Human and Swine H1N1, H2N2, H3N2

Canine, Equine H3N8

Human, Avian H5N1

Other Avian, Swine

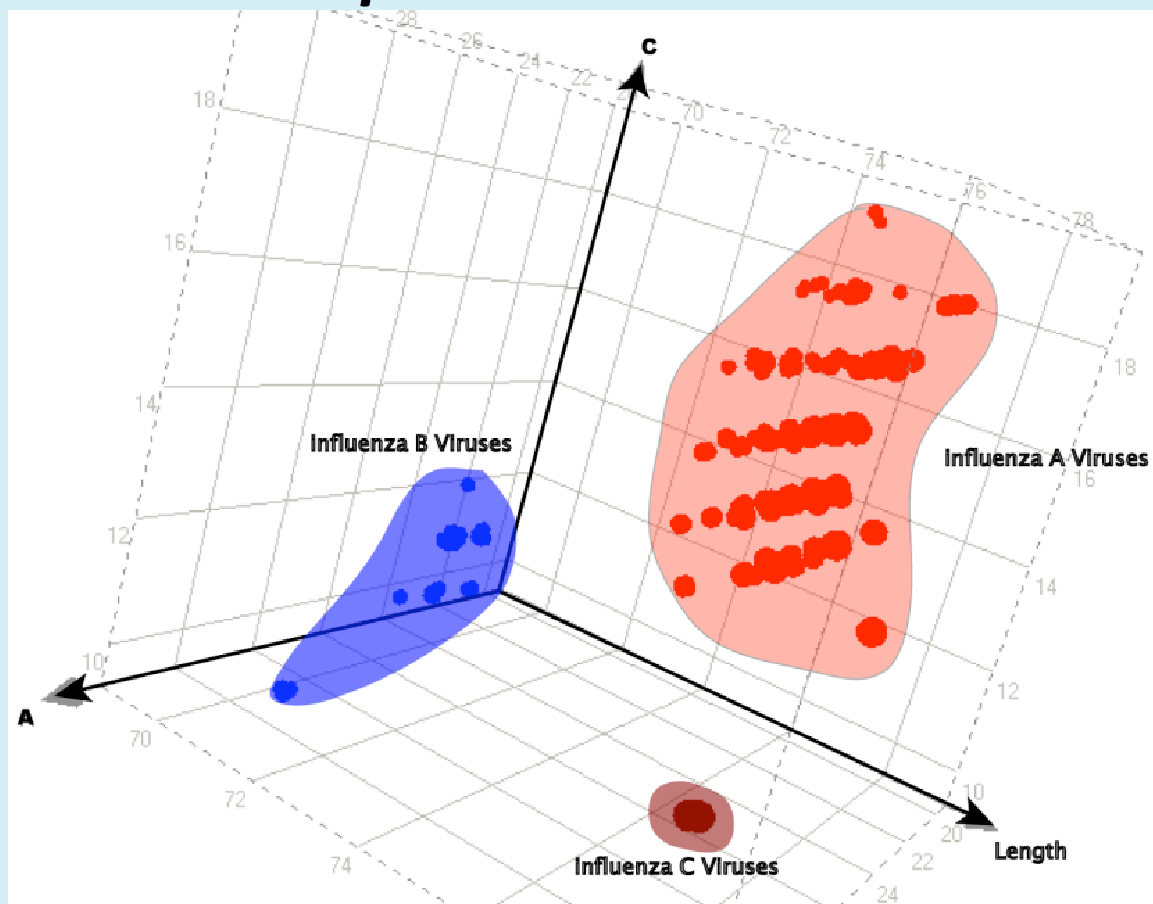
Influenza B and C

Accession	Sequence
2798 VIR2798 FLU_ALL_P	TGCTCTGGAATGATGATGGGCATGTT
REFERENCE 1918Flu_H1N1	CAATATGTTAAGCACTGATTAGGCGTCTCCATCCTGAATCTTGGCAAAAAGACATACACCAAGACTACTTACTGGT
Human H1N1 (gi 324899	CAATATGTTAAGCACTGATTAGGCGTCTCCATCCTGAATCTTGGCAAAAAGACATACACCAAGACTACTTACTGGT
Human H1N1 (gi 324897	CAATATGTTAAGCACTGATTAGGCGTCTCCATCCTGAATCTTGGCAAAAAGACATACACCAAGACTACTTACTGGT
Human H1N1 (gi 3144213	CAATATGTTAAGCACTGATTAGGCGTCTCCATCCTGAATCTTGGCAAAAAGACATACACCAAGACTACTTACTGGT
Human H1N1 (gi 1814083	CAATATGTTAAGCACTGCTTTGGGCGTCTCCATCCTGAATCTTGGCAAAAAGACATACACCAAGACTACTTACTGGT
Swine H1N1 (gi 324955 D	TAACATGTTAAGCACTGCTTTGGGCTTTCCATCTTAACTCTTGGCAAAAAGGAGTACACCAAGACTGCTTATTGGT
Swine H1N1 (gi 20068032	TAACATGCTGAGCACGCTCCTAGGAGTCTCGATCTTGAATCTTGGGCAAAAAGACATACACTAAAACCACATACTGGT
Swine H1N2 (gi 3815472	CAACATGCTAAGTACGGTTTTAGGAGTCTCGATCTTGAATCTTGGCAAAAAGAAATACACCAAGCAACATACTGGT
Swine H1N2 (gi 3815471	CAACATGCTAAGTACGGTTTTAGGAGTCTCGATCTTGAATCTTGGGCAAAAAGAAATACACCAAGCAACATACTGGT
Human H2N2 (gi 324941	CAACATGCTAAGTACAGTCTTAGGAGTCTCAATCCTGAATCTTGGCAAAAAGACTACACCAAAACAACTACTGGT
Human H2N2 (gi 324965	CAACATGCTAAGTACAGTCTTAGGAGTCTCAATCCTGAATCTTGGGCAAAAAGAAATACACCAAAACNACATACTGGT
Human H2N2 (gi 3778501	CAACATGCTAAGTACAGTCTTAGGAGTCTCAATCCTGAATCTTGGCAAAAAGAAATACACCAAAACAACTACTGGT
Human H2N2 (gi 3778503	CAACATGCTAAGTACAGTCTTAGGAGTCTCAATCCTGAATCTTGGGCAAAAAGACTACACCAAAACAACTACTGGT
Human H3N2 (gi 3778544	CAACATGCTAAGTACGGTTTTAGGAGTCTCAATCCTGAATCTTGGGCAAAAAGACATACACCAAAACAACTACTGGT
Human H3N2 (gi 3778545	CAACATGCTAAGTACGGTTTTAGGAGTCTCAATCCTGAATCTTGGGCAAAAAGACATACACCAAAACAACTACTGGT
Human H3N2 (gi 3778545	CAACATGCTAAGTACGGTTTTAGGAGTCTCAATCCTGAATCTTGGGCAAAAAGACATACACCAAAACAACTACTGGT
Swine H3N2 Influenza A	TAACATGCTGACTACAGTCTTAGGAGTCTCAATCTTGAATCTTGGCAAAAAGGATACACCAAGGACCACATACTGGT
Swine H3N3 (gi 5207815	CAACATGCTAAGTACGGTTTTAGGAGTCTCGATCTTAACTCTTAACTCTTGGGCAAAAAGGATATACCAAAACAACTACTGGT
Equine H3N8 Influenza A	CAACATGTTGAGCACTGTGCTGGGCTATCCATATTTAAACCTTGGCCAGAGGAAATACACAAAGACCACATACTGGT
Equine H3N8 Influenza A	CAACATGTTGAGCACTGTGCTGGGCTATCCATATTTAAACCTTGGCCAGAGGAAATACACAAAGACCACATACTGGT
Canine H3N8 (gi 710842	CAACATGTTGAGCACTGTGCTGGGCTATCCATATTTAAACCTTGGCCAGAGGAAATACACAAAGACCACATACTGGT
Canine H3N8 (gi 710842	CAACATGTTGAGCACTGTGCTGGGCTATCCATATTTAAACCTTGGCCAGAGGAAATACACAAAGACCACATACTGGT
Swine H4N6 Influenza A	CAACATGCTAAGTACAGTTTTAGGAGTTTTCACTTAACTCTTAACTCTTGGGCAAAAAGACATACACCAAAACAACTACTGGT
Human H5N1 (gi 1392538	TAACATGCTAAGTACGGCTTTAGGAGTCTCAATCTTAACTCTTGGGCAAAAAGGATACACCAAAACCACTACTGGT
Human H5N1 (gi 8307772	TAACATGCTAAGTACGGCTTTAGGAGTCTCAATCTTAACTCTTGGGCAAAAAGGATACACCAAAACCACTACTGGT
Avian H5N1 (gi 2884968	CAATATGCTGACTACAGTCTTAGGAGTTTTCACTCTGAATCTTGGGCAAAAAGGATACACCAAAACCACTACTGGT
Avian H5N1 (gi 2884968	CAACATGCTGACTACAGTCTTAGGAGTCTCAATCCTGAATCTTGGCAAAAAGGATACACCAAAACCACTACTGGT
Swine H5N1 (gi 5151215	CAACATGCTGACTACAGTCTTAGGAGTCTCAATCCTGAATCTTGGGCAAAAAGGATACACCAAAACCACTACTGGT
Swine H5N1 (gi 5412651	CAACATGCTGACTACAGTCTTAGGAGTCTCAATCCTGAATCTTGGGCAAAAAGGATACACCAAAACCACTACTGGT
Human H7N7 Influenza A	CAACATGTTAAGCACTGACTAGGTTATCCATATTTAAACCTAGGTCAAAAGAAATACACAAAGACCACATACTGGT
Avian H7N7 Influenza A	CAACATGCTGACTACAGTATTAGGAGTTTTCACTCTGAATCTTGGCAAAAAGGATACACCAAAACCACTACTGGT
Avian H9N1 1 (gi 313395	CAACATGCTGACTACAGTTTTAGGAGTATCAATCCTGAATCTTGGGCAAAAAGGATACACCAAAACCACTACTGGT
Avian H9N1 1 (gi 313395	CAACATGCTGACTACAGTTTTAGGAGTATCAATCCTGAATCTTGGGCAAAAAGGATACACCAAAACCACTACTGGT
Avian H9N2 (gi 5783314	CAACATGCTGACTACAGTCTTAGGAGTTTTCACTCTGAATCTTGGGCAAAAAGGATACACCAAAACCACTACTGGT
Swine H9N2 (gi 5592586	CAATATGTTAAGTACTGATTAGGCGTCTCCATCCTGAATCTTGGCAAAAAGGATACACCAAAACCACTACTGGT
Swine H9N2 (gi 5592586	CAGTATGTTAAGTACTGATTAGGCGTCTCCATCCTGAATCTTGGCAAAAAGGATACACCAAAACCACTACTGGT
Influenza B virus_(gi	TAATATGCTATCTACCGTCTTGGGAGTAGCCGCACTA
Influenza B virus_(gi	TAATATGCTATCTACCGTCTTGGGAGTAGCCGCACTA
Influenza B virus_(gi	TAATATGCTATCTACCGTCTTGGGAGTAGCCGCACTA
Influenza B virus_(gi	TAATATGCTATCTACCGTCTTGGGAGTAGCCGCACTA
Influenza B virus_(gi	TAATATGCTATCTACCGTCTTGGGAGTAGCCGCACTA
Influenza B virus_(gi	TAATATGCTATCTACCGTCTTGGGAGTAGCCGCACTA
Influenza B virus_(gi	TAATATGCTATCTACCGTCTTGGGAGTAGCCGCACTA
Influenza C virus_(gi	CAACATGCTGCTCAACAGTTCTTGGAGTAACTACATTTATGTTATATGCGATGAAGAACTAAAAGCCAAAGCATGTTTTT
Influenza C virus_(gi	CAACATGCTGCTCAACAGTTCTTGGAGTAACTACATTTATGTTATATGCGATGAAGAACTAAAAGCCAAAGCATGTTTTT

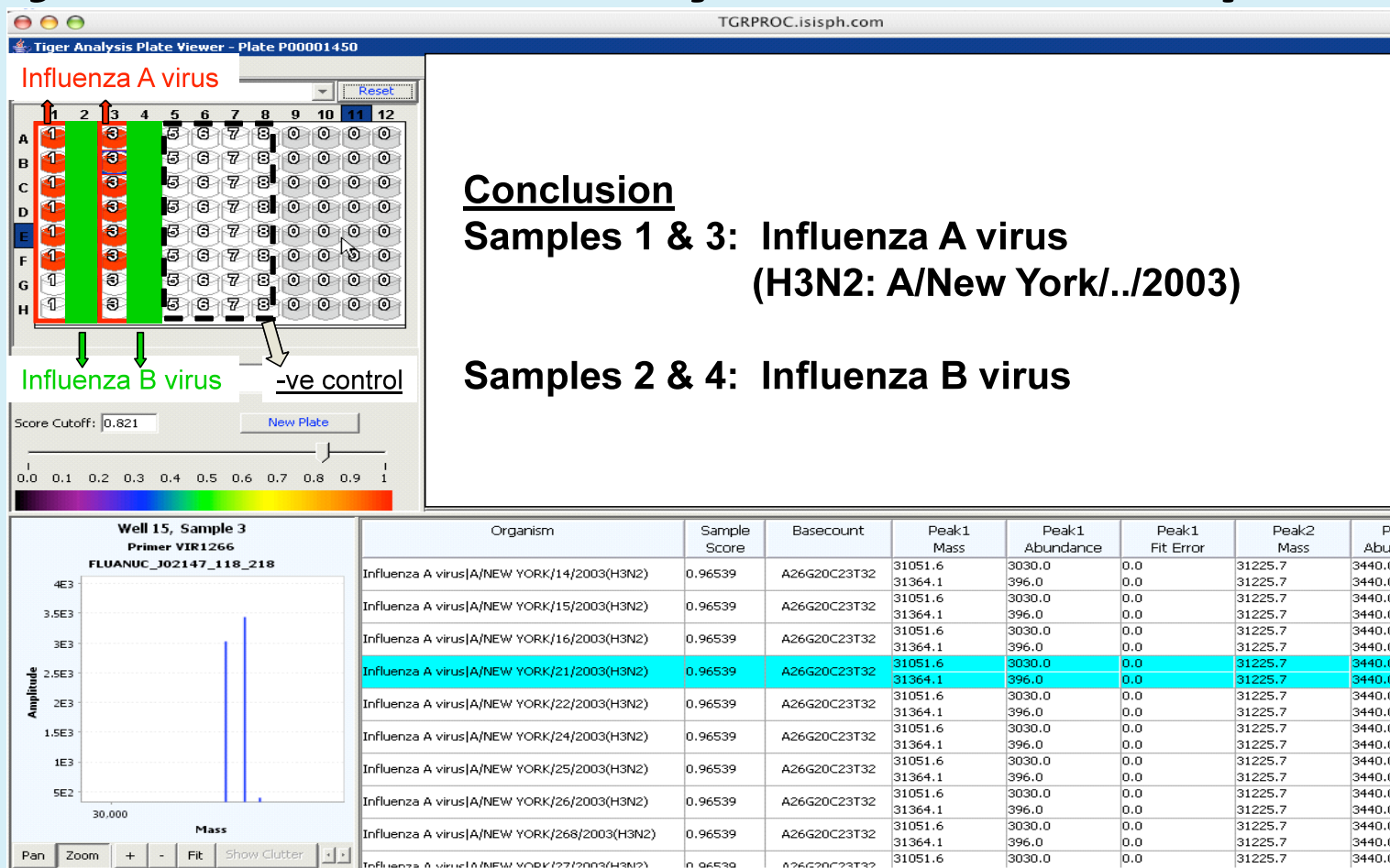
6 nucleotide deletion



Pan Influenza Primer Polymerase PB1 Primer PP2798 - Base Composition



Influenza Virus Assay Results Example



Human Influenza – Blinded Samples



Source	Location	Collection Dates	Sample type	No. of Samples
Naval Health Research Center	MCRD, San Diego Ft. Leonard Wood, Ft. Sill, Ft. Benning, Lackland AFB	1999-2005	Throat swabs, nasal swabs, nasal washes	317
Johns Hopkins University Medical Center	Baltimore, MD	2003-2005	Nasal aspirates	229
NY State Dept. of Health	Throughout NY	1999-2005	Nasal aspirates, BAL, tracheal aspirates, throat swabs	100
TX State Dept. of Health	Throughout TX	2005-2006	Throat swabs, nasal washes	10
Total				656

–Correctly identified all Influenza A types

- 149 H3N2
- 34 H1N1

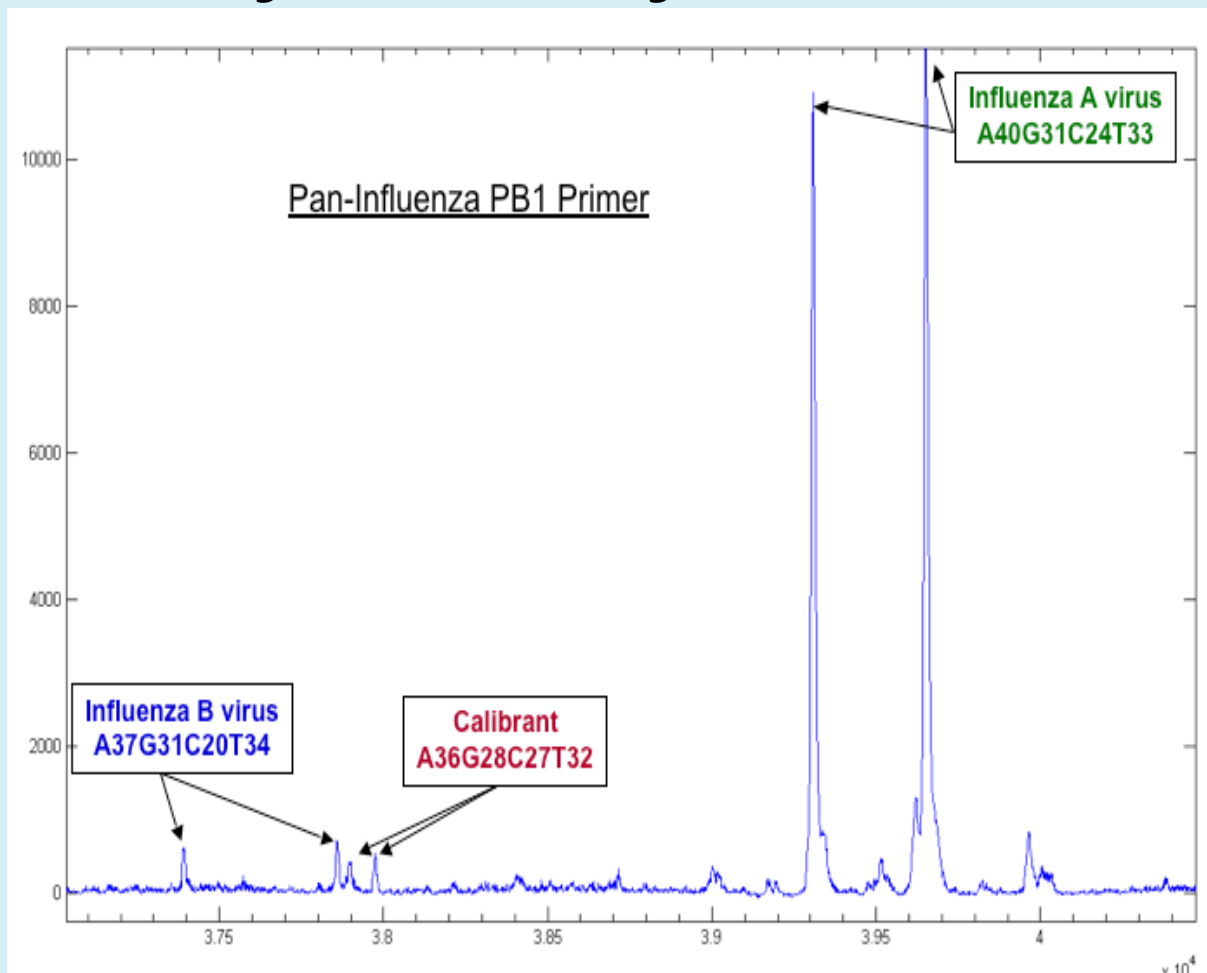
–67 Influenza B

Influenza	
Sensitivity	96.8%
Specificity	97.5%
PPV	96.0%
NPV	98.0%

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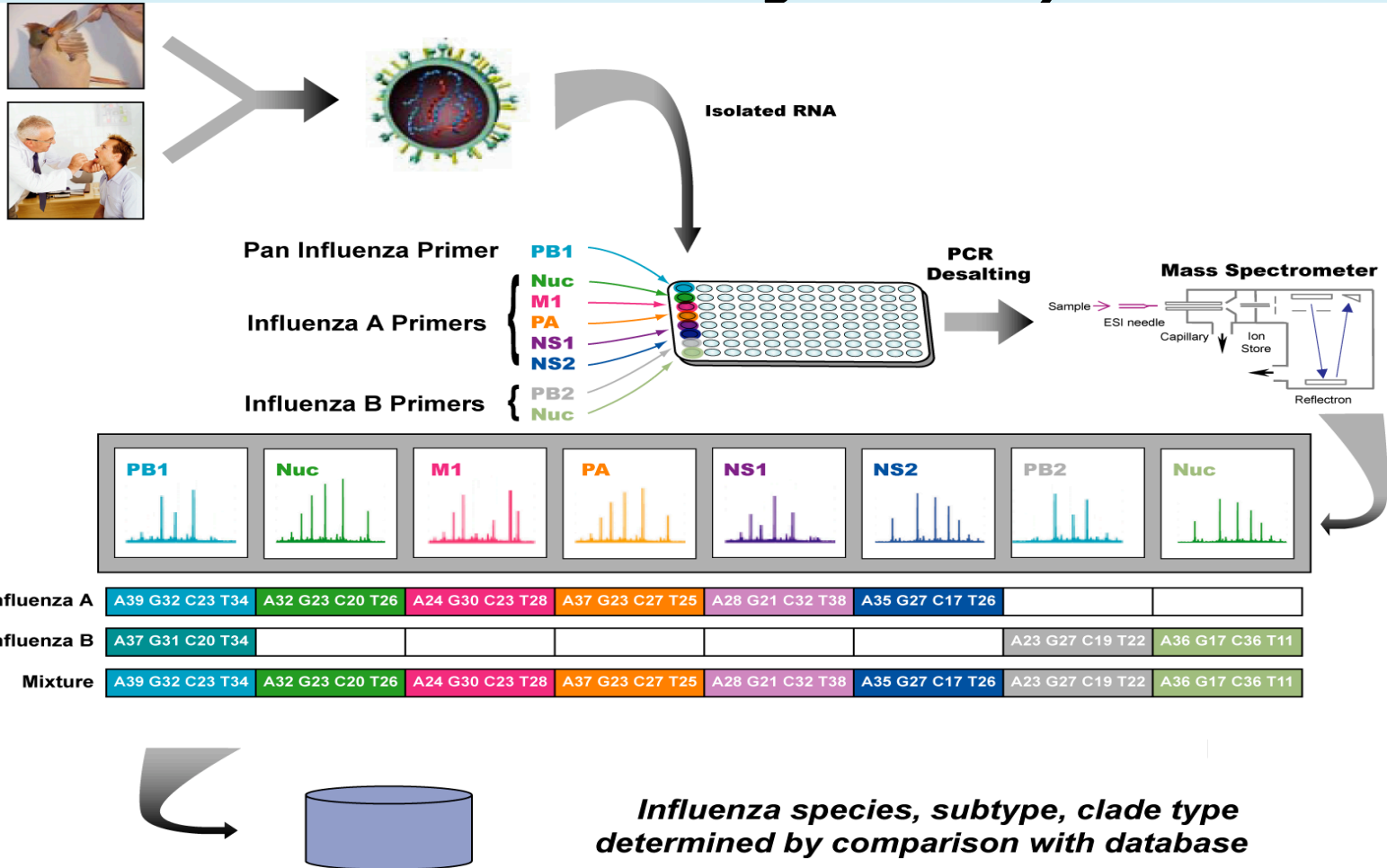
Detection of Mixed Infections



Validation Study Test Isolates from Diverse Sources

- **24 human influenza isolates**
 - 18 influenza A
 - 6 influenza B
- **63 avian influenza isolates**
 - 16 different avian species
 - Chicken, duck, goose, egret, teal,....
 - 28 distinct H/N types
 - **29 HIGHLY PATHOGENIC H5N1 isolates**
 - 8 worldwide geographic locations
 - North America, Africa, Asia
- **4 swine influenza isolates**
- **1 equine influenza isolate**

Avian Flu Detection: No Change in Assay or Primers



Avian Influenza Virus Detection: University of Georgia Samples

- **24 avian influenza virus isolates collected over a six-year period**
 - **Different host species: mallard, seagull, teal, etc.**
 - **Different combinations of H and N subtypes: H12N4, H3N8, etc.**

Avian Influenza Virus Detection: University of Georgia Samples

SCWDS ID#	Serotype	Species	Location	Date
AI00-1412	H6N1	REKN	Bower's Beach, DE	5/25/00
AI00-1794	H12N4	RUTU	Bower's Beach, DE	5/20/96
AI00-2150	H12N5	RUTU	Villas, NJ	5/15/96
AI00-629	H7N9	RUTU	Port Mahon, DE	5/19/96
AI02-262	H2N4	RUTU	Mispillion Harbor, DE	5/22/98
AI02-690	H2N9	RUTU	Reed's Beach, NJ	5/22/98
AI03-128	H9N7	RUTU	Reed's Beach, NJ	5/20/99
AI03-128	H9N7	RUTU	Reed's Beach, NJ	5/20/99
AI03-755	H9N5	RUTU	Mispillion Harbor, DE	5/20/99
AI04-127	H10N7	RUTU	Bower's Beach, DE	5/19/00
AI05-415	H3N8	RUTU	Fortescue Beach, NJ	5/21/01
AI05-415	H3N8	RUTU	Fortescue Beach, NJ	5/21/01
AI05-669	H11N8	RUTU	Reed's Beach, NJ	5/25/01
AI05-784	H11N6	RUTU	Reed's Beach, NJ	5/25/01
MN00-283	H5N2	MALL	Thief Lake, MN	9/10/96
MN00-382	H5N3	MALL	Thief Lake, MN	9/10/96
MN98-115	H4N8	MALL	Roseau Co., MN	09/ /1998
MN98-115	H4N8	MALL	Roseau Co., MN	09/ /1998
MN98-66	H6N5	MALL	Roseau Co., MN	09/ /1998
MN99-160	H4N6	MALL	Roseau Co., MN	/ /1999
MN99-17	H7N7	MALL	Roseau Co., MN	/ /1999
NC6412-009	H10N7	MALL	JM Futch, NC	12/20/00
NC675-075	H3N2	ABDU	Mattamuskeet, NC	12/21/00
TX01-32	H8N4	CITE	Brazoria Co., TX	2/11/97
TX01-7	H8N4	AGWT	Brazoria Co., TX	2/11/97
TX02-27	H1N4	BWTE	Brazoria Co., TX	2/18/98
TX02-75	H1N3	BWTE	Brazoria Co., TX	2/18/98

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Ibis™ T5000 Discrimination of Diverse Avian Influenza Virus Isolates

Serotype	Species	Location	Date	2798	1266	1279	1287	2775	2777
H1N3	BWTE	Brazoria Co., TX	2/18/98	A37G33C24T34	A35G22C22T22	A23G30C25T27	A35G27C28T22	A40G30C20T29	A35G27C16T27
H9N7	RUTU	Reed's Beach, NJ	5/20/99	A37G33C25T33	A37G21C20T23	A22G30C26T27	A35G26C28T23	A37G32C22T28	A34G27C18T26
H3N8	RUTU	Fortescue Beach, NJ	5/21/01	A37G34C23T34	A34G23C23T21	A23G30C24T28	A34G27C28T23	A37G32C22T28	A35G27C16T27
H3N8	RUTU	Fortescue Beach, NJ	5/21/01	A37G34C23T34	A34G24C20T23	A23G30C24T28	A34G27C28T23	A37G32C22T28	A34G27C18T26
H3N2	ABDU	Mattamuskeet, NC	12/21/00	A37G34C23T34	A34G24C21T22	A21G32C24T28	A35G26C29T22	A37G32C22T28	A34G27C18T26
H11N8	RUTU	Reed's Beach, NJ	5/25/01	A38G33C22T35	A35G22C22T22	A22G31C25T27	A34G27C28T23	A40G30C20T29	A35G27C16T27
H11N6	RUTU	Reed's Beach, NJ	5/25/01	A38G33C22T35	A35G22C22T22	A23G30C24T28	A34G27C28T23	A40G30C20T29	A35G27C16T27
H9N5	RUTU	Mispillion Harbor, DE	5/20/99	A38G33C23T34	A37G21C20T23	A24G28C23T30	A35G26C28T23	A37G32C22T28	A34G27C18T26
H9N7	RUTU	Reed's Beach, NJ	5/20/99	A38G33C24T33	A34G24C21T22	A22G30C26T27	A35G26C28T23	A37G32C22T28	A34G27C18T26
H6N5	MALL	Roseau Co., MN	09/ /1998	A39G32C22T35	A36G21C22T22	A21G32C25T27	A34G27C28T23	A40G30C20T29	A35G27C16T27
H4N6	MALL	Roseau Co., MN	/ /1999	A39G32C23T34	A35G22C23T21	A22G31C27T25	A34G27C28T23	A37G32C22T28	A34G27C18T26
H5N2	MALL	Thief Lake, MN	9/10/96	A39G32C23T34	A36G21C22T22	A23G31C26T25	A35G26C27T24	A37G32C22T28	A34G27C18T26
H8N4	AGWT	Brazoria Co., TX	2/11/97	A39G32C23T34	A36G22C21T22	A24G28C25T28	A34G27C28T23	A37G32C22T28	A34G27C18T26
H7N7	MALL	Roseau Co., MN	/ /1999	A39G32C23T34	A36G22C22T21	A20G32C27T26	A34G27C29T22	A37G32C22T28	A34G27C18T26
H5N3	MALL	Thief Lake, MN	9/10/96	A39G32C24T33	A34G24C21T22	A21G32C25T27	A34G27C28T23	A37G32C22T28	A34G27C18T26
H2N4	RUTU	Mispillion Harbor, DE	5/22/98	A39G32C24T33	A35G22C22T22	A22G31C25T27	A35G26C29T22	A37G32C22T28	A34G27C18T26
H12N5	RUTU	Villas, NJ	5/15/96	A39G32C24T33	A36G21C22T22	A21G32C24T28	A35G26C29T22	A37G32C22T28	A34G27C18T26
H7N9	RUTU	Port Mahon, DE	5/19/96	A39G32C24T33	A36G21C22T22	A21G32C24T28	A34G27C29T22	A37G32C22T28	A34G27C18T26
H2N9	RUTU	Reed's Beach, NJ	5/22/98	A39G32C24T33	A36G21C22T22	A23G30C26T26	A35G26C29T22	A37G32C22T28	A34G27C18T26
H4N8	MALL	Roseau Co., MN	09/ /1998	A40G31C22T35	A29G24C19T29	A25G28C25T27	A34G27C28T23	A37G32C22T28	A34G27C18T26
H4N8	MALL	Roseau Co., MN	09/ /1998	A40G31C22T35	A34G24C21T22	A25G28C25T27	A34G27C28T23	A37G32C22T28	A34G27C18T26
H12N4	RUTU	Bower's Beach, DE	5/20/96	A40G31C23T34	A35G23C22T21	A23G30C26T26	A35G26C29T22	A37G32C21T29	A34G27C17T27
H10N7	RUTU	Bower's Beach, DE	5/19/00	A40G31C24T33	A33G25C21T22	A23G30C25T27	A35G27C27T23	A38G31C22T28	A34G27C18T26
H10N7	MALL	JM Futch, NC	12/20/00	A40G31C24T33	A35G22C21T23	A23G30C26T26	A34G27C29T22	A37G32C22T28	A34G27C18T26
H8N4	CITE	Brazoria Co., TX	2/11/97	A40G31C24T33	A35G22C22T22	A22G30C27T26	A34G27C29T22	A40G30C20T29	A35G27C16T27
H1N4	BWTE	Brazoria Co., TX	2/18/98	A40G31C24T33	A35G22C22T22	A24G28C25T28	A35G26C28T23	A40G30C20T29	A35G27C16T27

*Same panel was used for human Influenza detection

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Analysis of Patient B at the NHRC

- Patient B is a 9 year old girl from Imperial County, CA
- On March 28, 2009, she had onset of cough and fever of 104.3 °F
- Diagnosed with influenza and specimen sent to NHRC as part of border surveillance program
- NHRC determined specimen was untypeable and sent to CDC on April 17, 2009
- NHRC also ran sample on the Ibis™ Influenza Surveillance plate*
 - April 23

MMWR April 24, 2009 / 58(15); 400-402

<http://blogs.sciencemag.org/scienceinsider/2009/04/behind-the-scen.html>

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Patient B Result

Find Results Analysis - Filtered on Barcode="24111, From=3/31/2009, Results=Triggered

QC	ESI	PCR	Registered	KR Type	Triggered	Analysis	Project	Experiment	Instrument	Comment
P05024111		C05009717	4/23/2009 5:46 PM	T5000 INFLUENZA SURVEILLANCE	Yes	Survey	THNHRC	NHRC INFLUENZA	TOF12	NHRC system down

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Report Type: Details Options Save Refresh Reanalyze Advanced

Detection	Organism	Strain	Host	Serotype	Amplicon	Org Corr	Score	Primer Pairs						
								Flu_ALL_PB1 (VIR2798)	FluA_Nuc (VIR1266)	1279	1287	2775	2777	1261
1-0								-	-	-	-	-	-	-
2-0								-	-	-	-	-	-	-
3-1	Influenza A virus	A/SW/MN/23124-S/01(H1N2);A/SW/MO/18777/01(H1N2)	Swine	H1N2	1	1	2/2	-	-	-	A37 G34 C19 T29	A34 G29 C16 T26	-	-
								-	-	-	-	-	2389	1354
								-	-	-	-	-	[-2 +2 +2 -2]	[-1 +1 +1 -1]
3-2	Influenza A virus	A/NEW YORK/292/2003(H1N1);A/NEW YORK/348/2003(H1N1)	Human	H1N1	1	1	2/6	-	A36 G20 C19 T26	-	A35 G26 C28 T23	-	-	-
								-	6946	-	8220	-	-	-
								-	[-2 +2 +2 -2]	-	[0 0 0 0]	-	-	-
3-3	Influenza A virus	A/MALLARD DUCK/ALB/155/1990(HN8);A/MALLARD DUCK/F	Avian	HN8	1	1	2/6	-	-	A24 G28 C24 T29	A35 G26 C28 T23	-	-	-
								-	6273	-	8220	-	-	-
								-	[-1 +1 +1 -1]	-	[0 0 0 0]	-	-	-
3-4	Influenza A virus	A/MALLARD/ALBERTA/156/2001(HN8)	Avian	HN8	1	1	2/6	-	A40 G31 C23 T34	-	A35 G26 C28 T23	-	-	-
								-	3049	-	8220	-	-	-
								-	[0 0 0 0]	-	[-1 +1 +1 -1]	-	-	-

Not cleared or approved by FDA for any clinical application. Not for use in diagnostic procedures.



2009 H1N1 New “Swine Flu” Signature

Before the new strain was added to the database, it appeared as a hybrid of several strains from different hosts – human, avian, and swine

Segment	BC	Closest match
PB1	40 31 23 34	Influenza A Virus (A/New York/473/2003(H3N2))
NP	36 20 19 26	A/SWINE/NEBRASKA/1/92(H1N1); A/SWINE/WISCONSIN/464/98(H1N1); A/SWINE/WISCONSIN/235/97(H1N1); A/SWINE/WISCONSIN/457/98(H1N1) - one SNP
M	24 28 24 29	A/SWINE/SPAIN/51915/2003(H1N1)
PA	35 26 28 23	A/DUCK/KOREA/S10/03(H3N2); A/DUCK/KOREA/S7/03(H3N2); A/DUCK/KOREA/S9/03(H3N2); A/DOVE/KOREA/S11/03(H3N2); A/CHICKEN/KOREA/S6/03(H3N2); A/MALLARD/ALBERTA/232/94(H6N8); A/MALLARD/ALBERTA/76/94(H6N8)
NS1	37 34 19 29	A/SW/MN/22860-
NS2	34 29 16 26	S/01(H1N2); A/SW/NC/45319/99(H1N2); A/SW/MN/22860-

— Human
 — Avian
 — Swine

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2009 H1N1 – Signature Added to Database

Analysis - Filtered on Project=SWINE-FLU-2009, From=3/23/2009, Results=Triggered

QC	ESI	PCR	Registered	Kit Type	Experiment	Project	Triggered	Analysis
✓	P05021907	C00012886	4/28/2009 6:19 PM	TS000 INFLUENZA SURVEILLANCE HI	CDC 20090428 SAMPLE SET	SWINE-FLU-2009	Yes	Survey

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Report Type: Details Options Save Refresh Reanalyze Advanced

Sample	QC	Organism	Strain	Serotype	Score	Primer Pairs							
						Flu_ALL_PB1 (VIR2798)	FluA_Nuc (VIR1266)	1279	1287	2775	2777	1261	1275
1	✓	Influenza A virus	A/California/04/2009-PA SNP	H1N1	6/6	A40 G31 C23 T34 49208 [-1 +1 +1 -1]	A35 G21 C20 T25 19955 [0 0 0 0]	A24 G28 C24 T29 16670 [0 0 0 0]	A35 G26 C27 T24 27810 [0 0 0 0]	A36 G35 C20 T28 16365 [0 0 0 0]	A34 G29 C16 T26 10150 [0 0 0 0]	-	-
2	✓	Influenza A virus	A/California/04/2009	H1N1	6/6	A40 G31 C23 T34 49506 [-1 +1 +1 -1]	A35 G21 C20 T25 18927 [0 0 0 0]	A24 G28 C24 T29 25412 [0 0 0 0]	A36 G25 C27 T24 20808 [0 0 0 0]	A36 G35 C20 T28 18565 [0 0 0 0]	A34 G29 C16 T26 11477 [0 0 0 0]	-	-
3	✓	Influenza A virus	A/California/04/2009	H1N1	6/6	A40 G31 C23 T34 720 [-1 +1 +1 -1]	A35 G21 C20 T25 1324 [0 0 0 0]	A24 G28 C24 T29 647 [0 0 0 0]	A36 G25 C27 T24 840 [0 0 0 0]	A36 G35 C20 T28 390 [0 0 0 0]	A34 G29 C16 T26 115 [0 0 0 0]	-	-

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Find Results-Analysis 2.7.0530 IBIS RESEARCH SIMBAD CORE

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Conclusions



- **By “weighing” DNA with mass spectrometry, unambiguous base compositions can be derived**
 - Remember coins and scale analogy!
- **Base compositions derived from broad range primers can be used to triangulate to microbial identification**

Conclusions

- **Ibis™ platform enables broad range bacterial and viral detection**
 - **Broad bacterial coverage using broad range primers**
 - **Example: Direct analysis of throat swabs**
 - **All influenza (human and avian) in same assay**
 - **Example: Human clinical specimens and avian isolates**
- **Demonstrated for bacteria and viruses without culture**
 - **Also applicable to fungi, protozoa, and humans (not shown)**

Instead of asking; “Is pathogen X in my sample?”, Ibis™

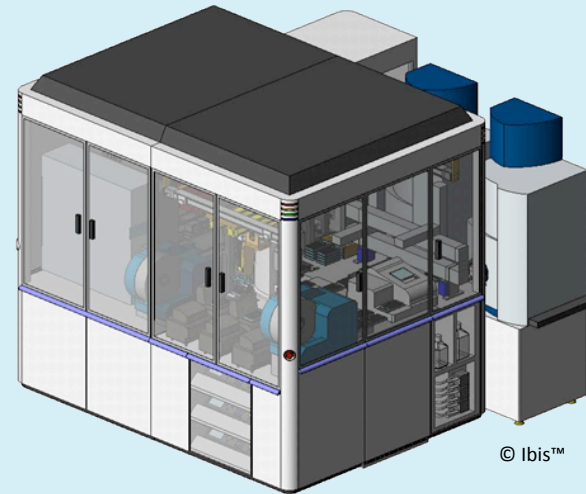
approach asks: “Which pathogen(s) are in my sample?”

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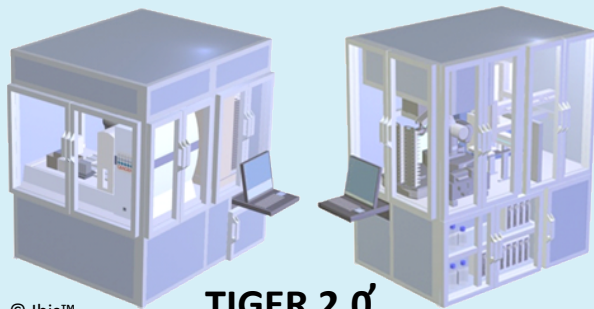
System History



TIGER 1.0
2000-2003



TIGER 2.0
2003



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TIGER 2.0'
2004-2006



IbisT5000
2006-2009



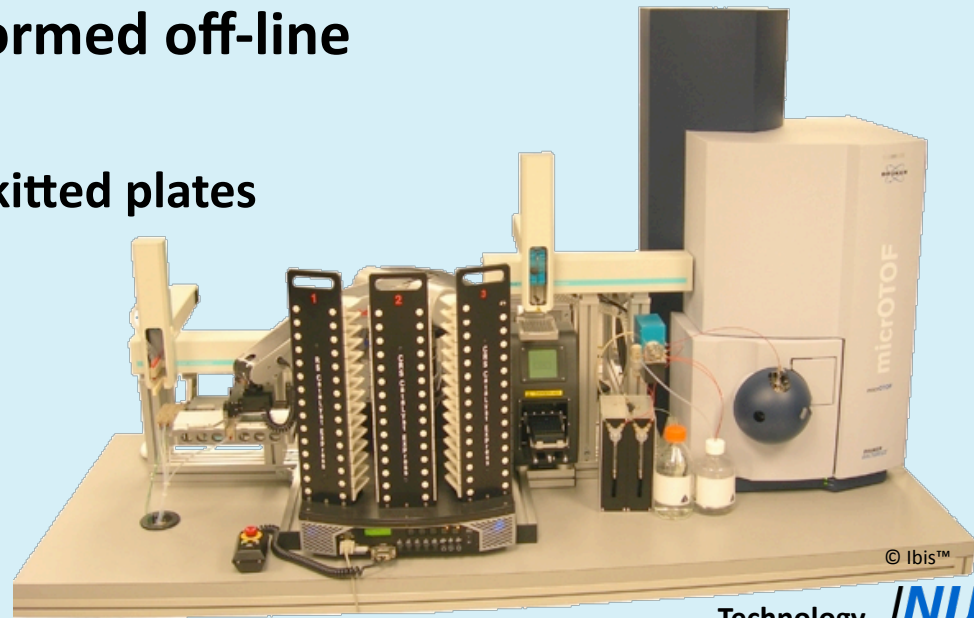
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PLEX-ID™
2010-
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Ibis™ T5000

- **Amplicon purification**
- **Automated ESI-TOF analysis**
 - Robotic arm moves plates for unattended operation
- **Data analysis**
- **Other functions performed off-line**
 - DNA/RNA extraction
 - Plate set-up into pre-kitted plates
 - PCR
- **High-throughput**
 - 1 well/minute
 - 46 sec. spray
 - 14 sec. rinse



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PLEXID² | R20

- Same magnetic bead chemistry as T5000
- Spin cuvettes (22) aligned in carousel
- Magnetic beads aliquoted from bead reservoir
 - No mag bead plate
 - No elution plate
- No robotic arms, heat sealers, LEAPS, etc.
- Accommodate “stat” sample (priority interrupt)
- Supports 30 second/well throughput
- Manufactured under FDA-compliant design/
manufacturing control



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Questions?

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Note: All images are courtesy of Dr. Steven A. Hofstadler
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