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

Steven A. Hofstadler, Ph.D.

Ibis Biosciences, Inc.

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
    - Pan-influenza detection and strain typing
- Integrated platform

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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

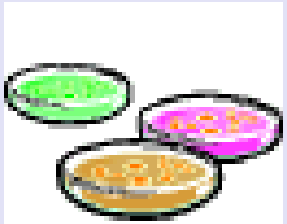
The Pathogen Detection Arena

- 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

IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

- There is currently no good method to detect organisms that have never been seen before

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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

TABLE COURTESY OF CHRISTIAN MASSIRE, PH.D.

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Bacteria

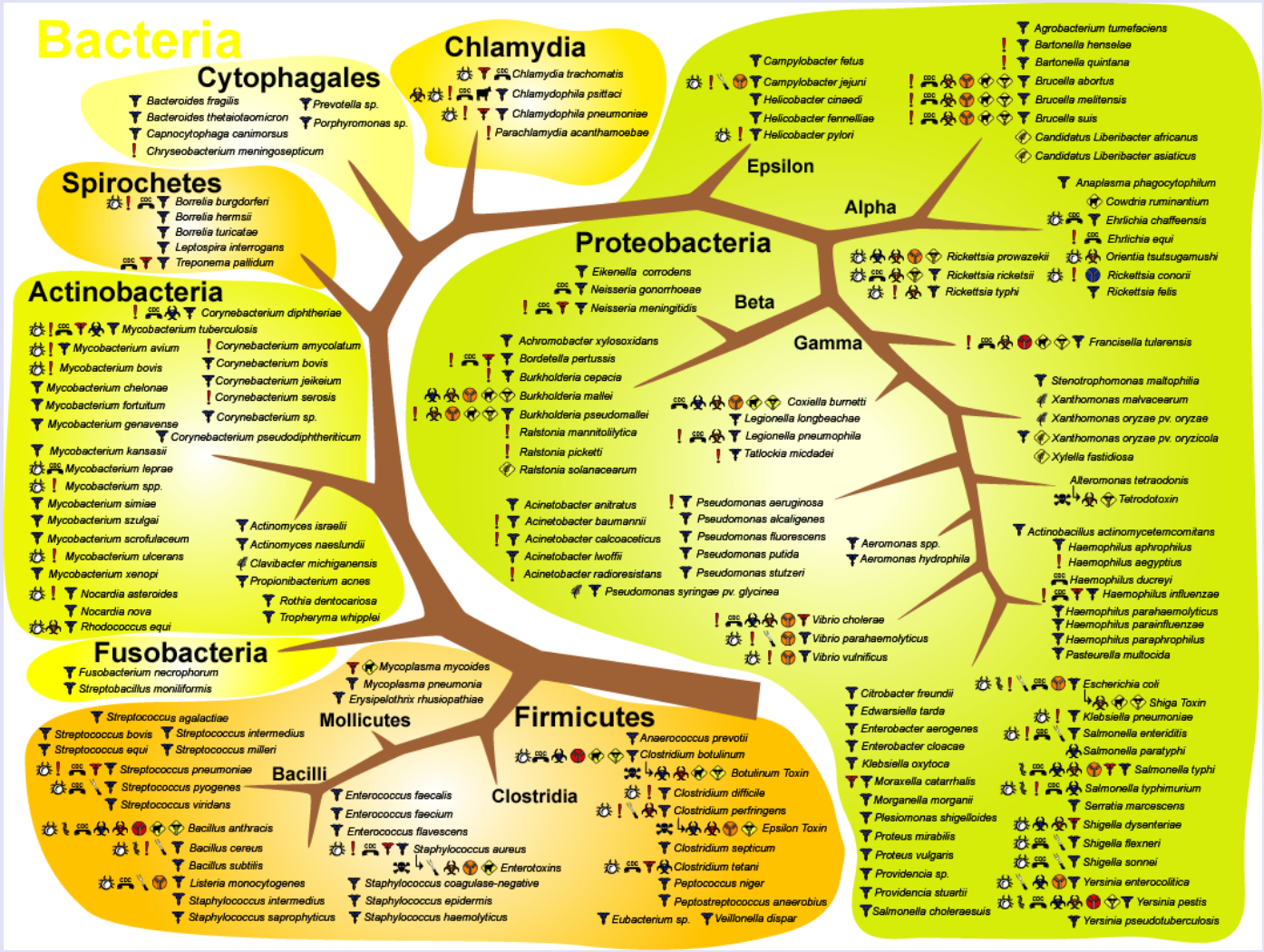

IMAGE COURTESY OF CHRISTIAN MASSIRE, PH.D.

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

**** Except those nasty prions!***

Why Detect and/or Type Microorganisms via Nucleic Acids?

- 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.

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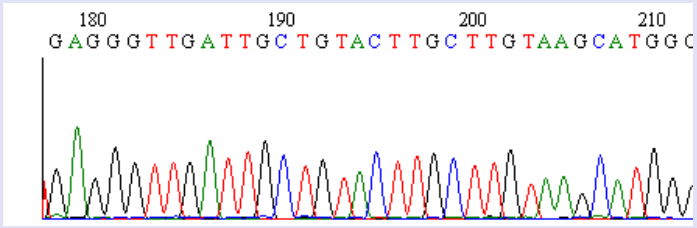
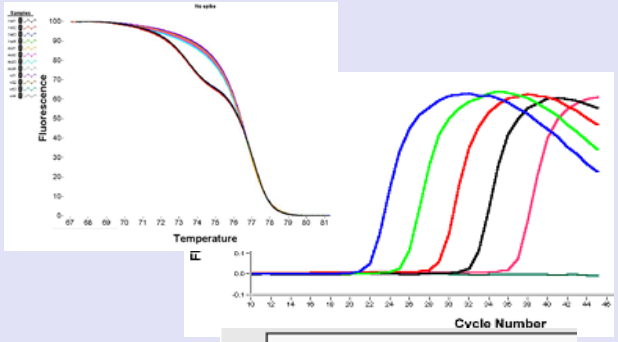
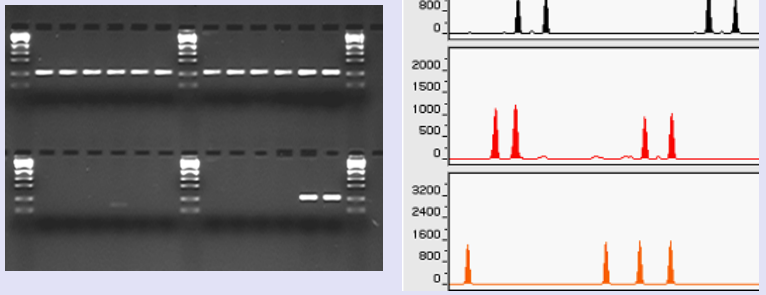
Interrogation of Amplified Nucleic Acids

- 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

Defense Advanced Research Projects (DARPA)

Centers for Disease Control (CDC)

National Institute of Allergy and Infectious Diseases (NIAID)

Department of Homeland Security (DHS)

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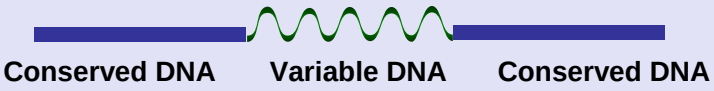
The Ibis Approach to Pathogen ID and Strain-Typing

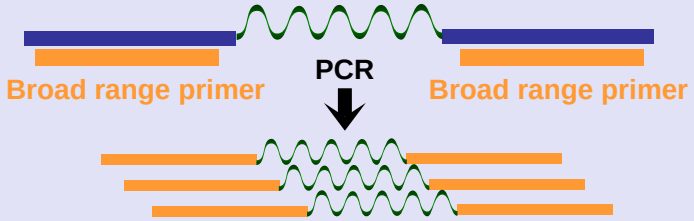
STEP 1 Identify genomic regions for identification:

Variable DNA sequences flanked by conserved sequences



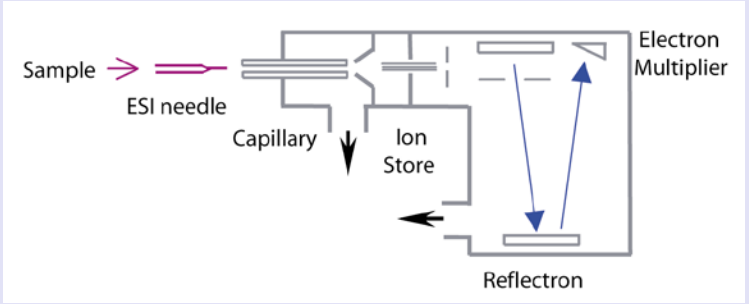
STEP 2 Amplify nucleic acids to measure:

Use broad-range, unbiased PCR primers



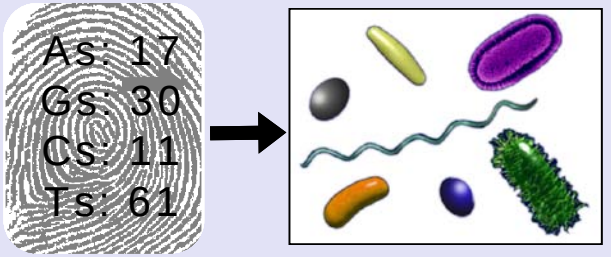
STEP 3 Measure nucleic acid:

ESI-TOF



STEP 4 Identify the organisms:

Base-composition fingerprints



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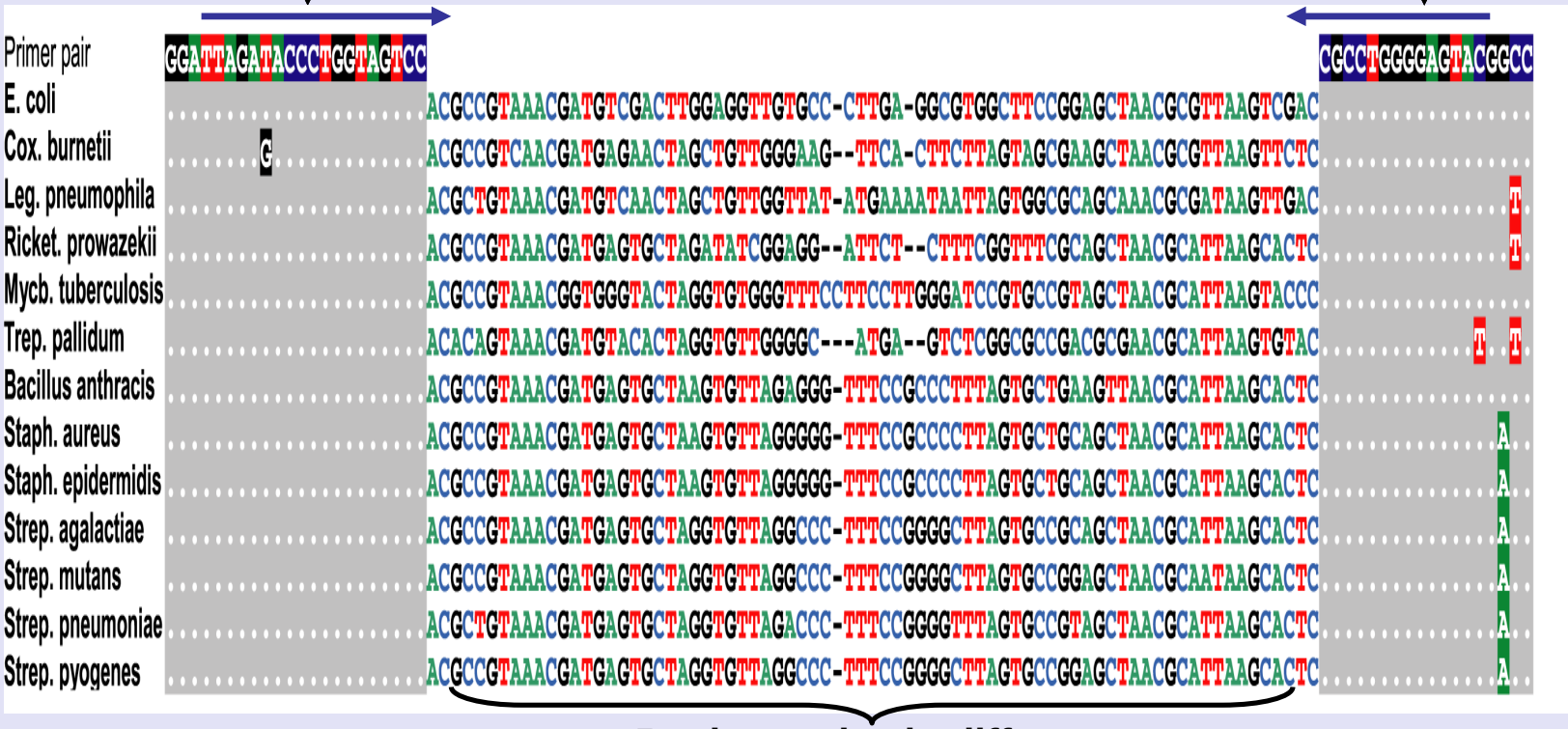
Broad Range Priming in Bacteria

STEP 1

Primers bind to conserved regions present in ALL (or groups of) bacteria



Region varies in different kinds of bacteria ⟹ $\Delta [A_w G_x C_y T_z]$

IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

The Polymerase Chain Reaction (PCR)

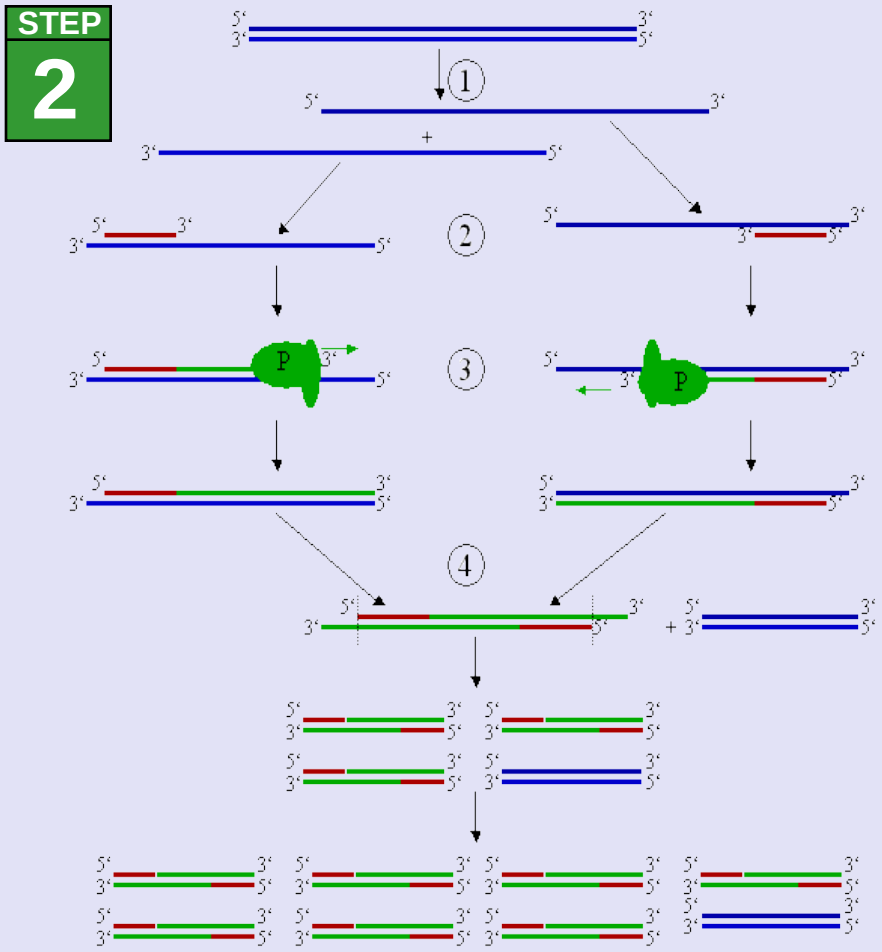


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

- **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)**

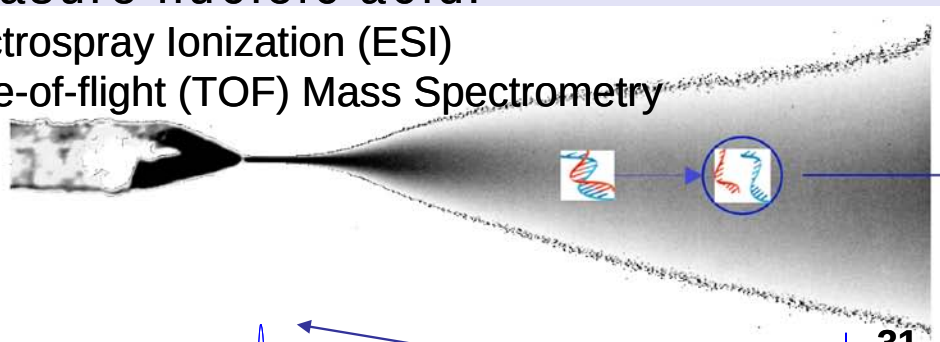
Automated ESI-TOF of PCR Amplicons on Ibis T5000™

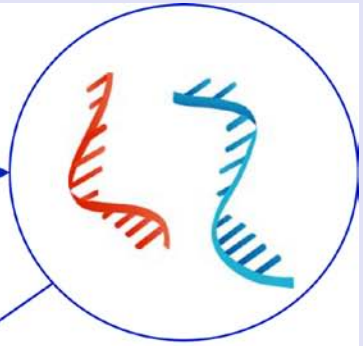
STEP 3

Measure nucleic acid:

Electrospray Ionization (ESI)

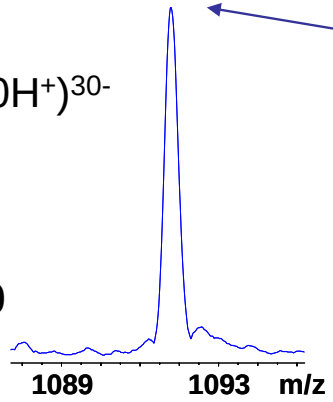
Time-of-flight (TOF) Mass Spectrometry





$(M-30H^{+})^{30-}$

MW = **32,588.90**



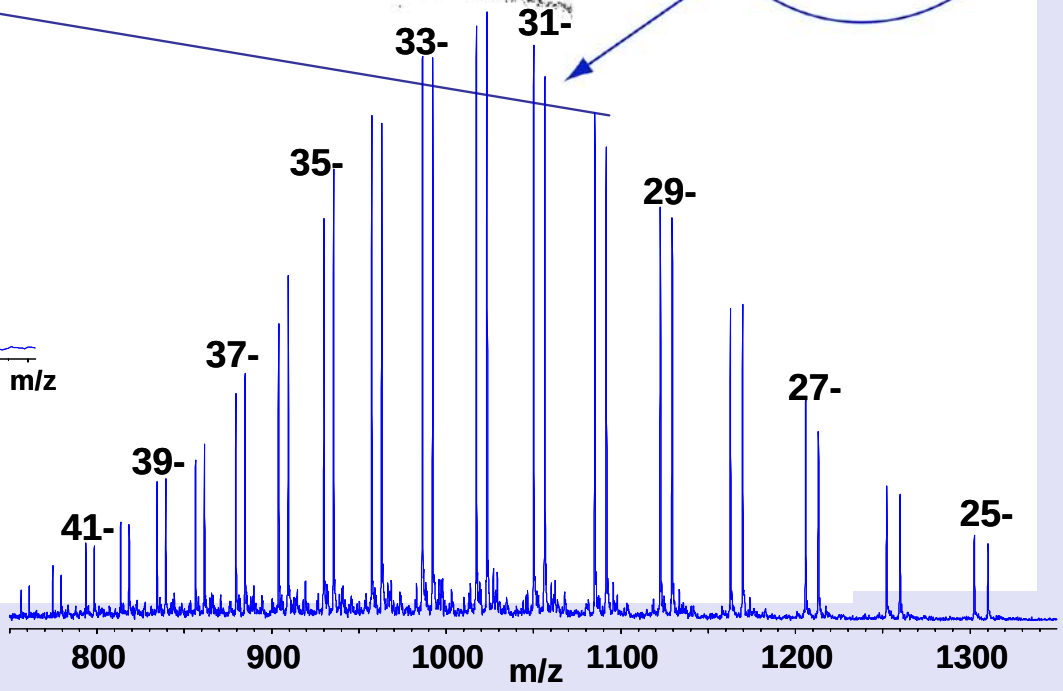


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

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Masses to Base Composition

STEP 4

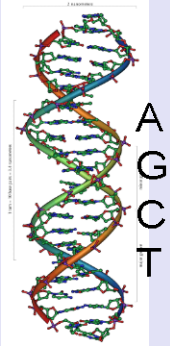
© Microsoft Media Elements

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

© Microsoft Media Elements

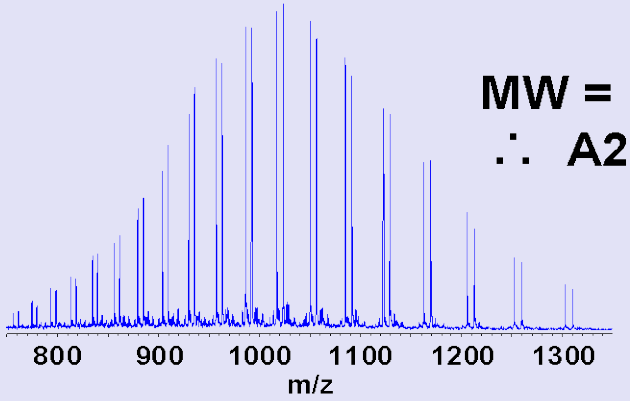
Scale

Weight = 4.6 grams
∴ 2 dimes



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

© Microsoft Media Elements



Mass spectrum

IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

MW = 32,588.90 amu
∴ A28 G29 C25 T24

Requires 25 ppm mass measurement error

Math takes into account Watson-Crick base pairing

Derived and Expected Base Compositions

Primer 356 (RplB) Expected Products

Identify the organisms:

STEP 4

Base-composition fingerprints

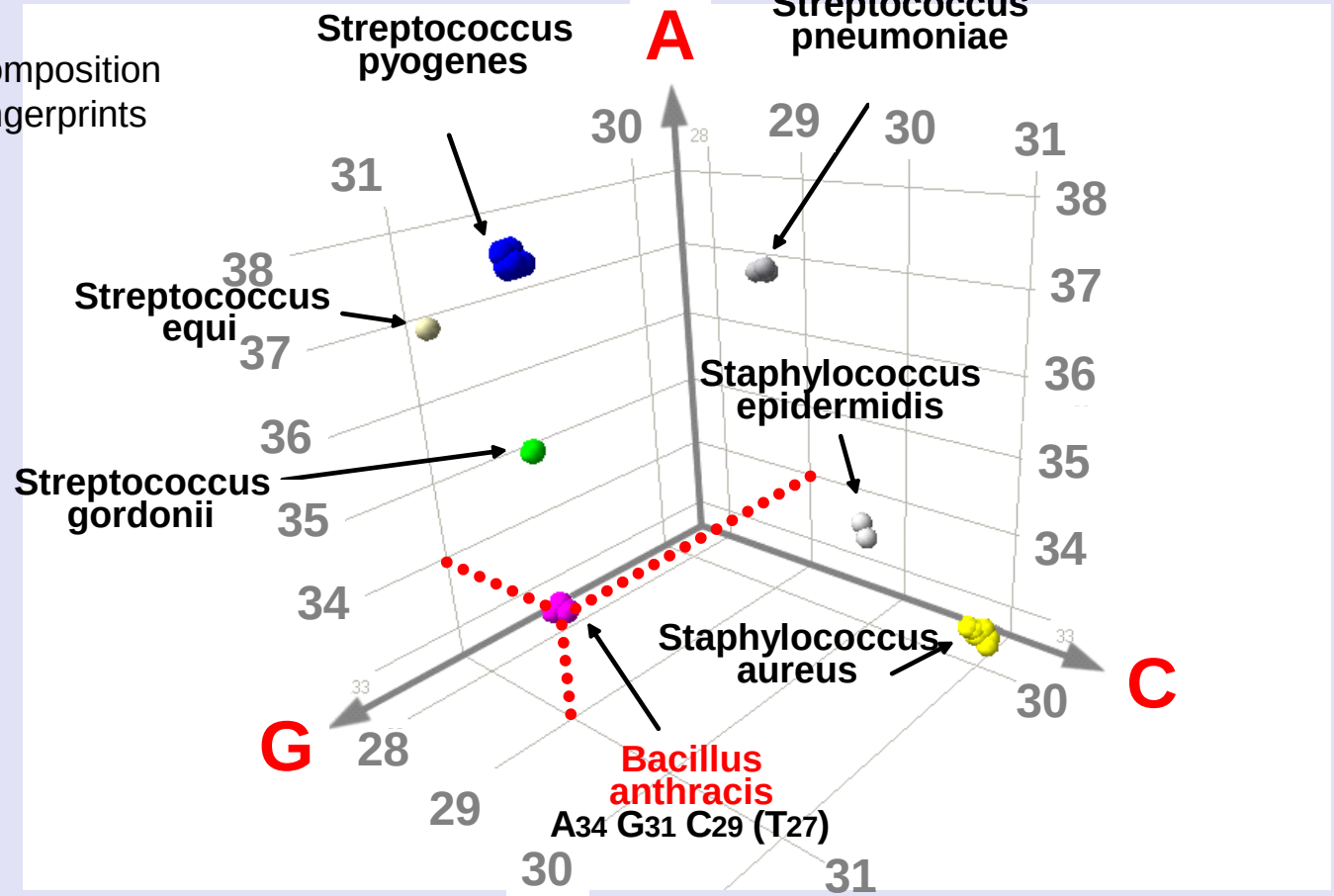


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

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)

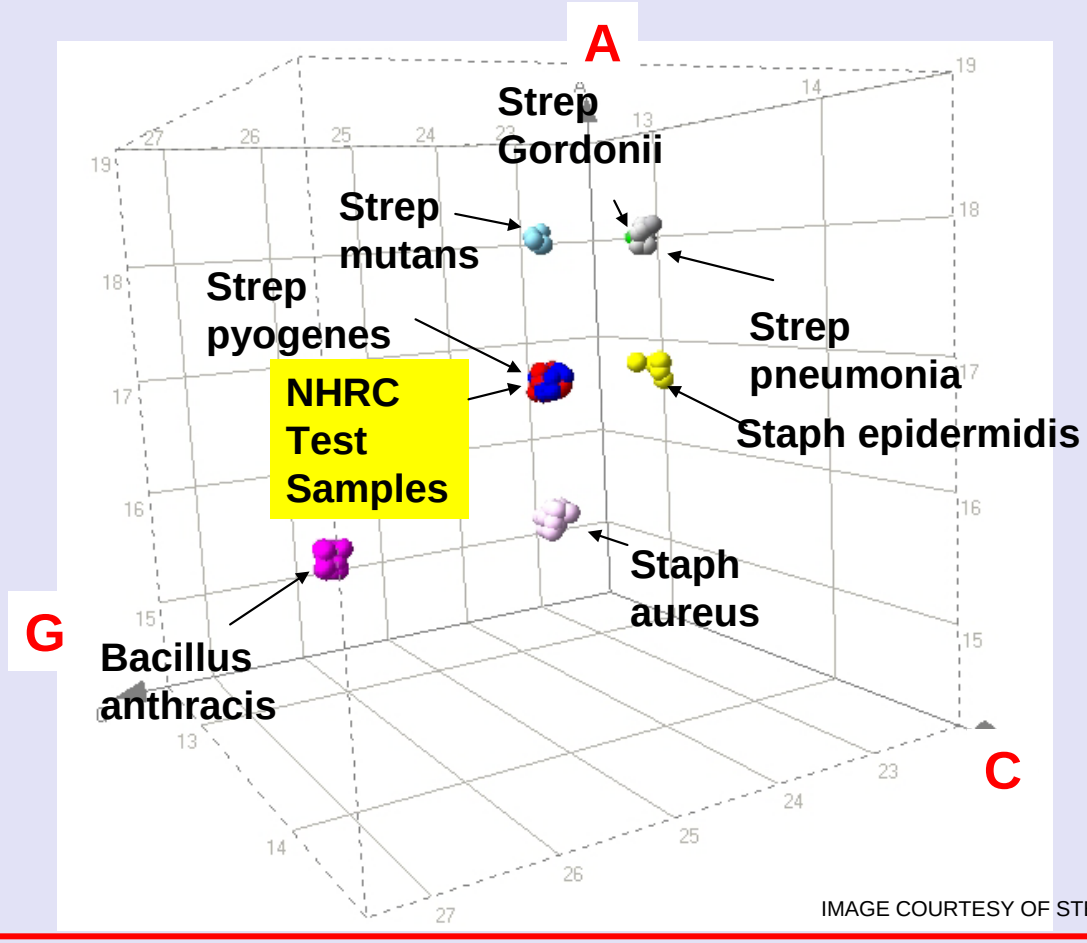


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

All primers of all samples consistent with S. pyogenes

Direct Analysis of Throat Swab*

Repeat swab positive on culture for *Streptococcus pyogenes

Multi-primer Triangulation

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

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Haemophilus influenzae, *Neisseria meningitidis*, *Streptococcus pyogenes* (Ratio 4/2/1, 1.5 X 10^6 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	$A_{18}G_{15}C_{15}T_{13}$

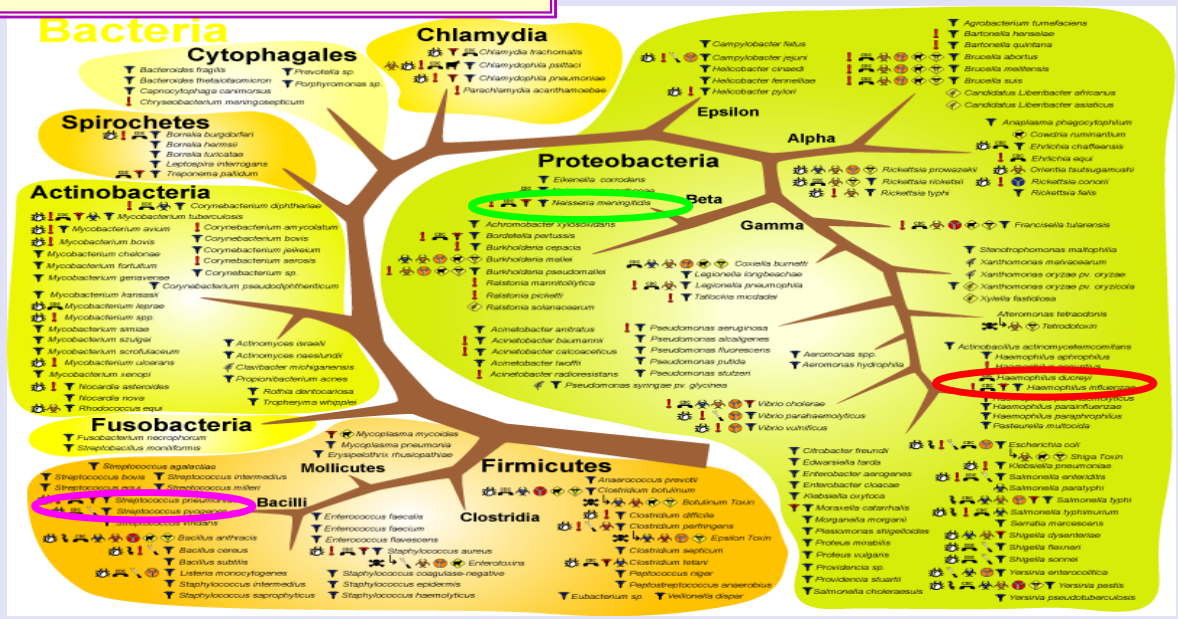

IMAGE COURTESY OF CHRISTIAN MASSIRE, PH.D.

Conclusions of Pneumonia Study*

- **Primary pathogen**
  - ***Streptococcus pyogenes* (GAS)**
  - **Known virulent strain**
- **Secondary pathogens**
  - **Haemophilus influenzae**
  - **Neisseria meningitidis**
- **5 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(22): p8012-8017**

Virus Identification and Typing

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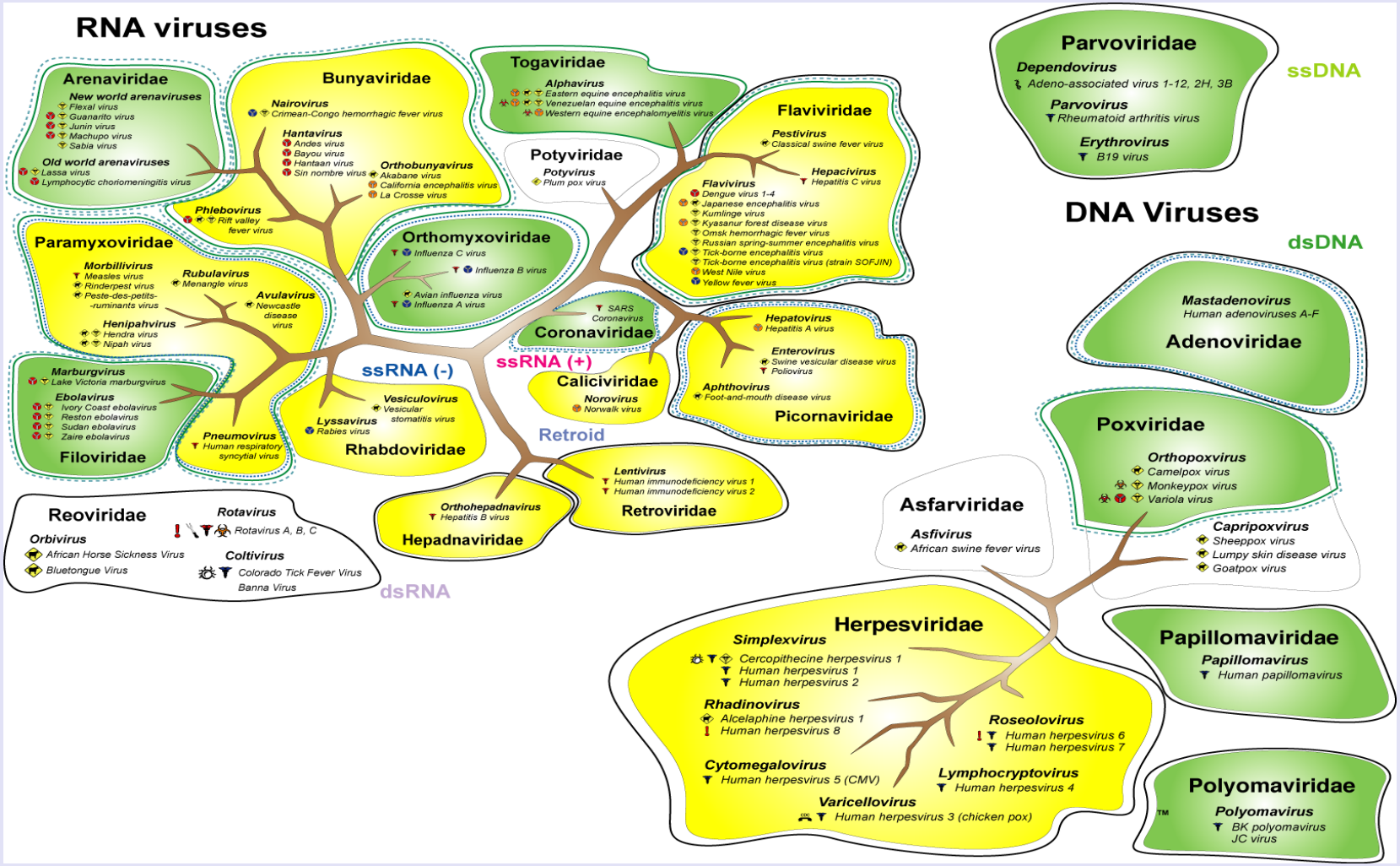
Viral Coverage



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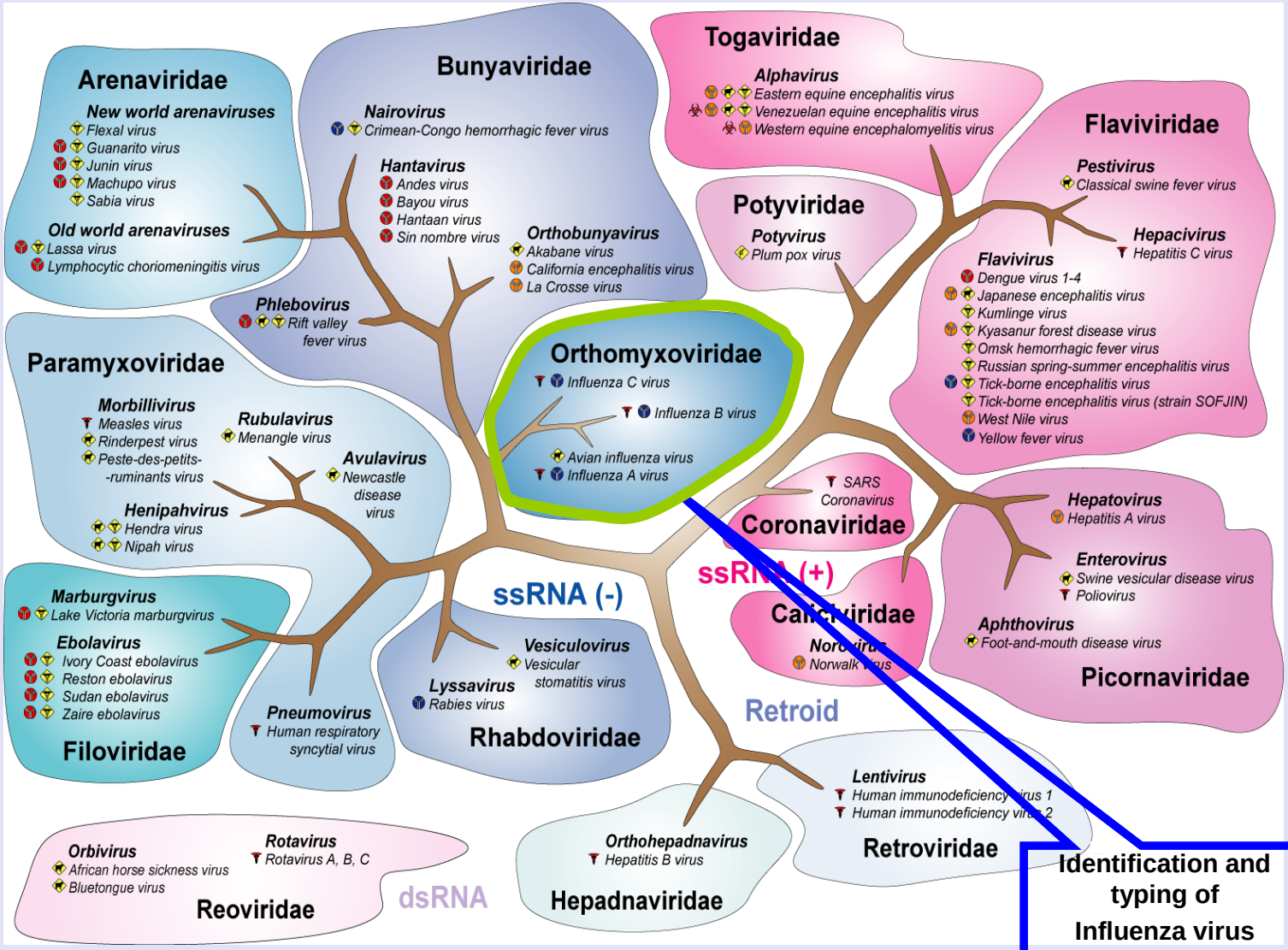

IMAGE COURTESY OF CHRISTIAN MASSIRE, PH.D.

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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, PhD, 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

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Ibis T5000™ Influenza Virus Assay

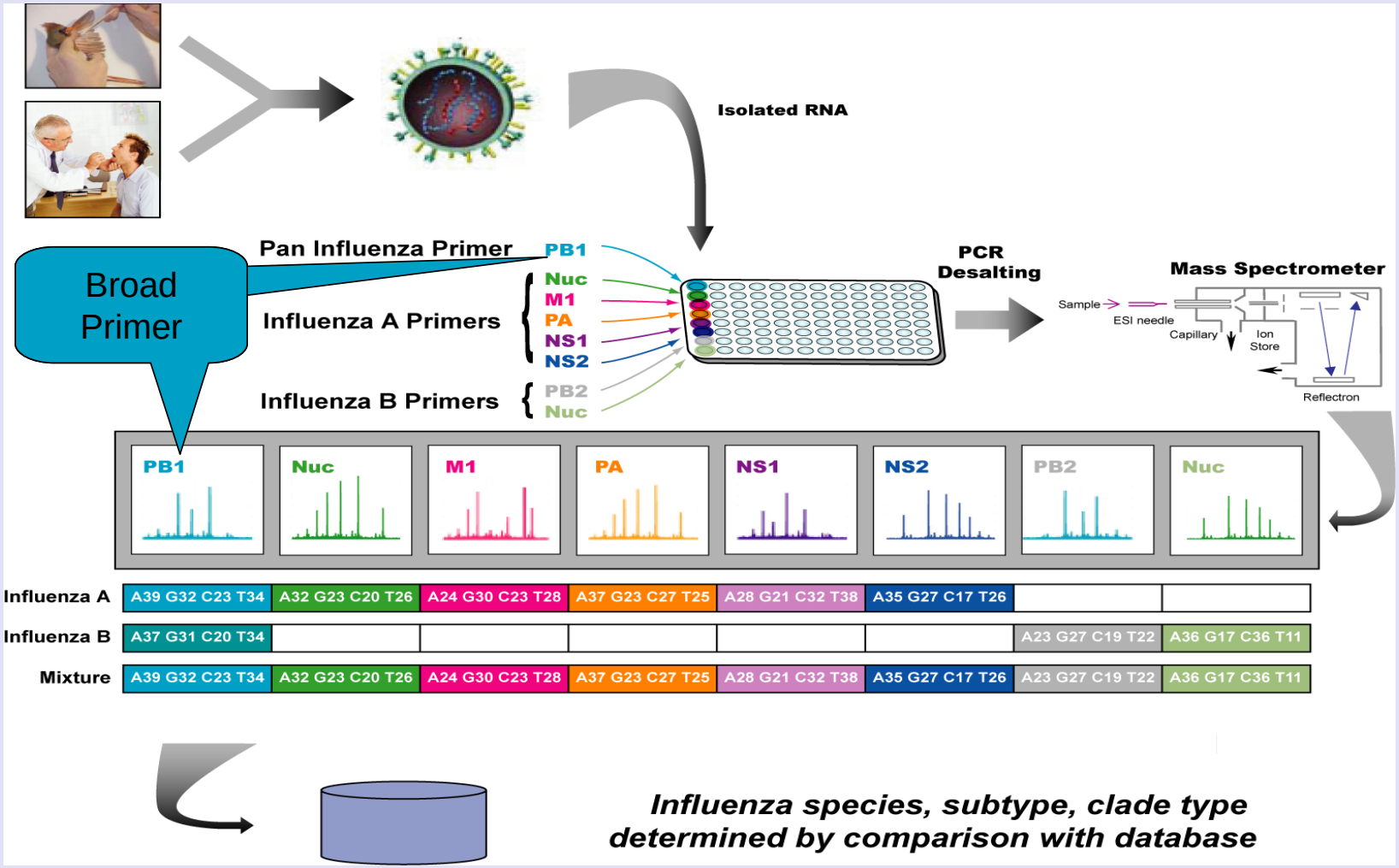

IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

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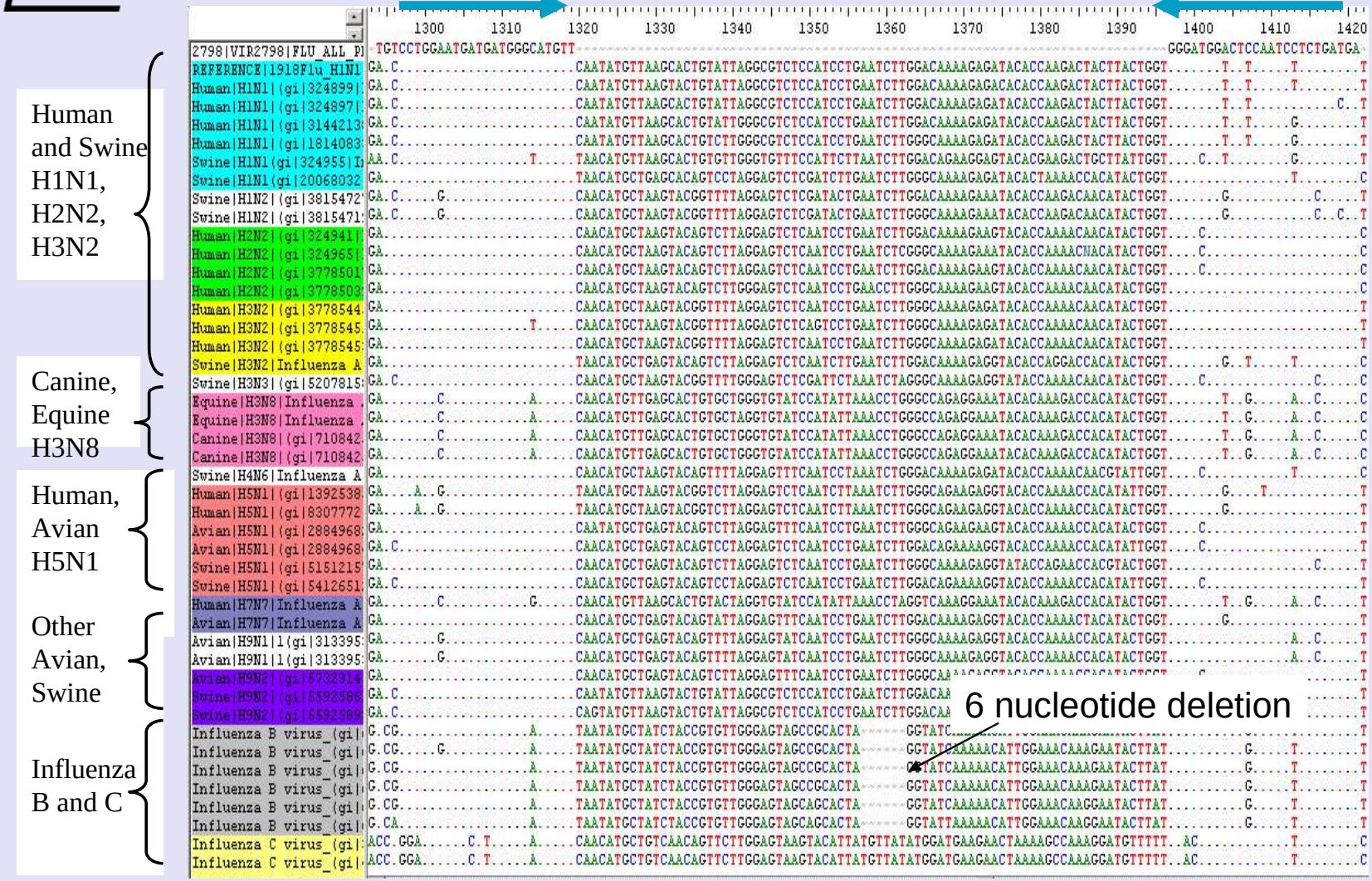
Pan-influenza Primer Polymerase PB1 Primer



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Pan-influenza Primer Polymerase PB1 Primer

PP2798 - Base Composition

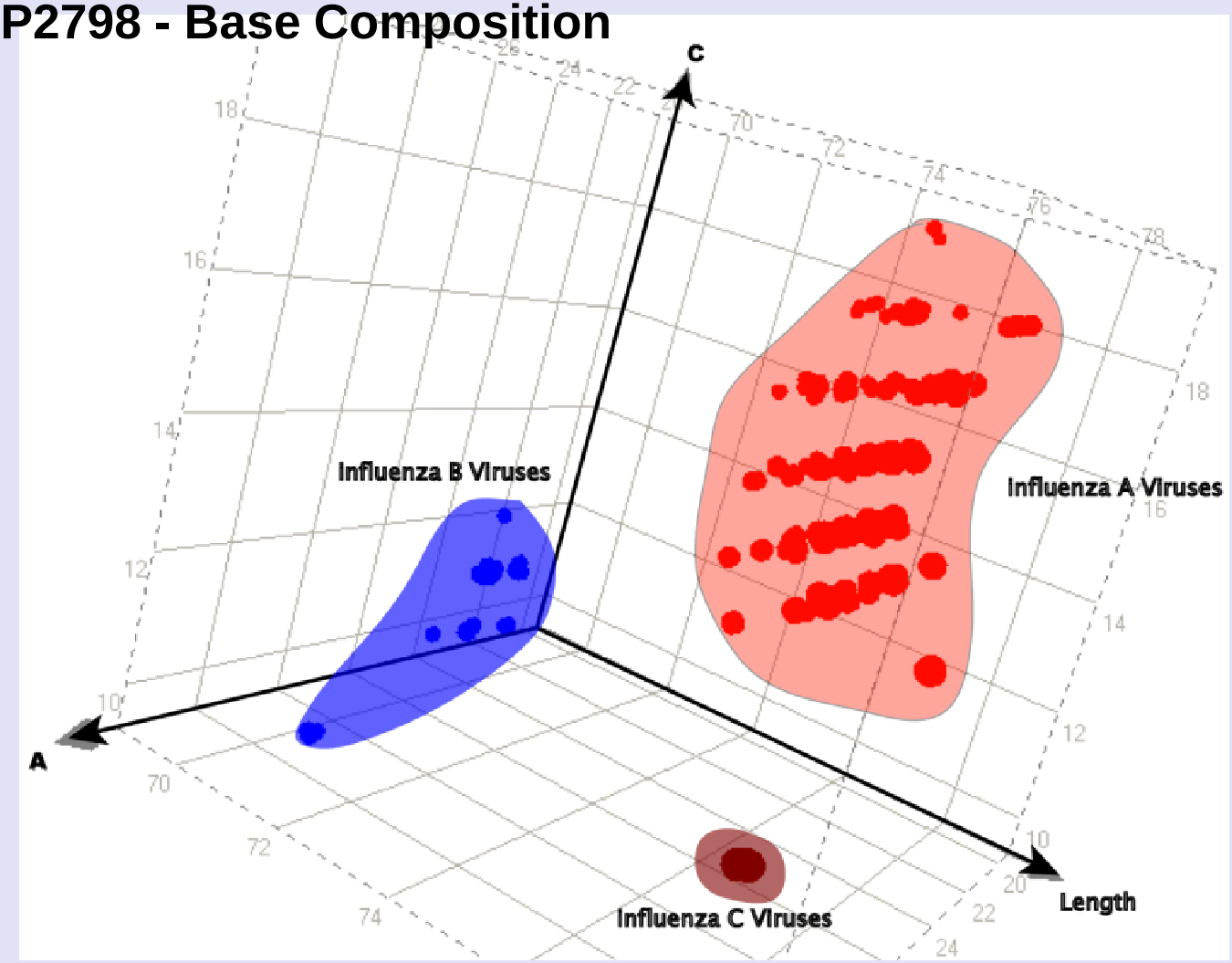

IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

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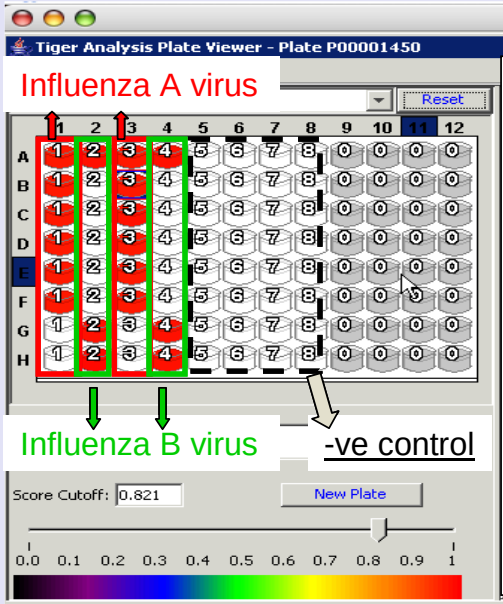
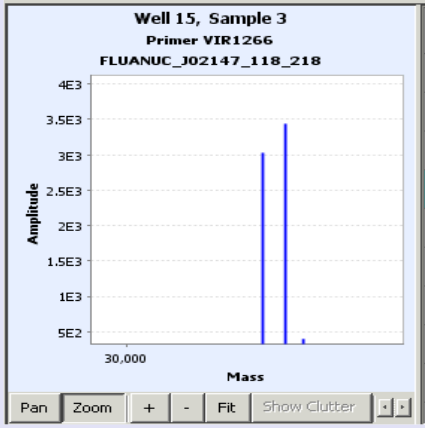
Influenza Virus Assay Results Example



Conclusion

Samples 1 & 3: Influenza A virus (H3N2: A/New York/../2003)

Samples 2 & 4: Influenza B virus



Organism	Sample Score	Basecount	Peak1 Mass	Peak1 Abundance	Peak1 Fit Error	Peak2 Mass	Pe Abun
Influenza A virus|A/NEW YORK/14/2003(H3N2)	0.96539	A26G20C23T32	31051.6 31364.1	3030.0 396.0	0.0 0.0	31225.7 31225.7	3440.0 3440.0
Influenza A virus|A/NEW YORK/15/2003(H3N2)	0.96539	A26G20C23T32	31051.6 31364.1	3030.0 396.0	0.0 0.0	31225.7 31225.7	3440.0 3440.0
Influenza A virus|A/NEW YORK/16/2003(H3N2)	0.96539	A26G20C23T32	31051.6 31364.1	3030.0 396.0	0.0 0.0	31225.7 31225.7	3440.0 3440.0
Influenza A virus|A/NEW YORK/21/2003(H3N2)	0.96539	A26G20C23T32	31051.6 31364.1	3030.0 396.0	0.0 0.0	31225.7 31225.7	3440.0 3440.0
Influenza A virus|A/NEW YORK/22/2003(H3N2)	0.96539	A26G20C23T32	31051.6 31364.1	3030.0 396.0	0.0 0.0	31225.7 31225.7	3440.0 3440.0
Influenza A virus|A/NEW YORK/24/2003(H3N2)	0.96539	A26G20C23T32	31051.6 31364.1	3030.0 396.0	0.0 0.0	31225.7 31225.7	3440.0 3440.0
Influenza A virus|A/NEW YORK/25/2003(H3N2)	0.96539	A26G20C23T32	31051.6 31364.1	3030.0 396.0	0.0 0.0	31225.7 31225.7	3440.0 3440.0
Influenza A virus|A/NEW YORK/26/2003(H3N2)	0.96539	A26G20C23T32	31051.6 31364.1	3030.0 396.0	0.0 0.0	31225.7 31225.7	3440.0 3440.0
Influenza A virus|A/NEW YORK/268/2003(H3N2)	0.96539	A26G20C23T32	31051.6 31364.1	3030.0 396.0	0.0 0.0	31225.7 31225.7	3440.0 3440.0
Influenza A virus|A/NEW YORK/27/2003(H3N2)	0.96539	A26G20C23T32	31051.6	3030.0	0.0	31225.7	3440.0

IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Human Influenza Trial - 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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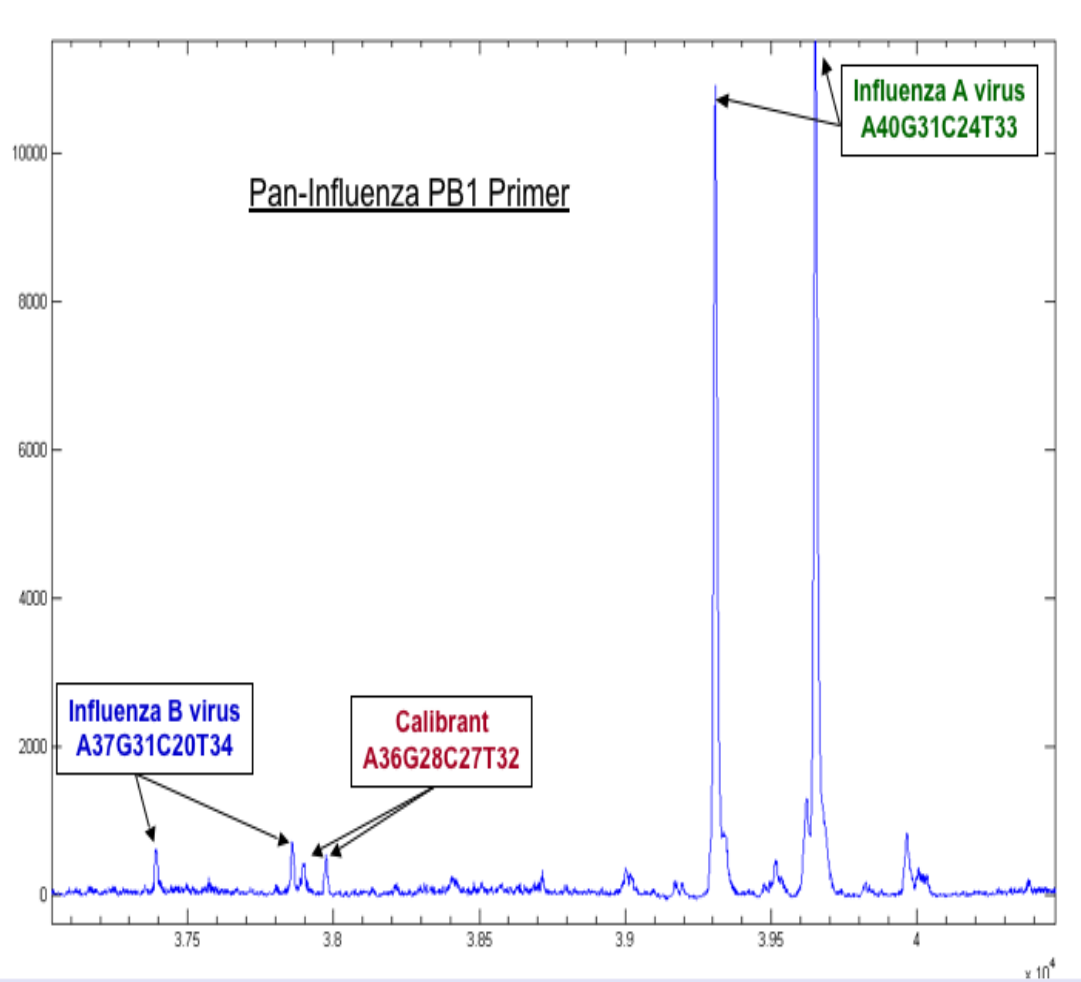
Detection of Mixed Infections



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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

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Avian Flu Detection:

No Change in Assay or Primers

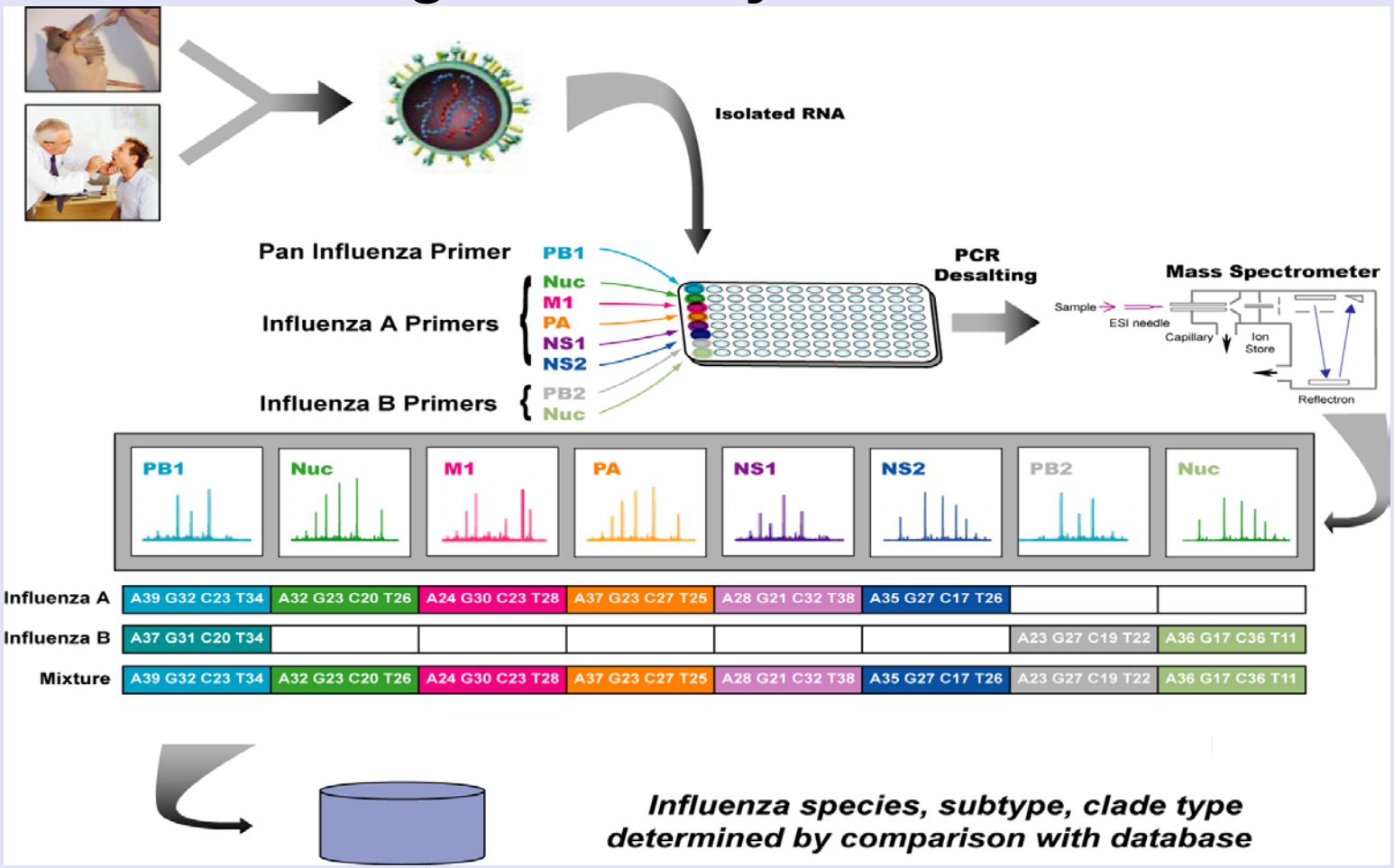

IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

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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,…**
- **Different combinations of H and N subtypes: H12N4, H3N8…**

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

***Same panel was used for human Influenza detection**

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
H6N1	REKN	Bower's Beach, DE	5/25/00	No Detection	No Detection	No Detection	No Detection	No Detection	No Detection

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
- 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**
- Respiratory Virus Surveillance Assay provides a single test platform for 6 families of RNA and DNA respiratory viruses
- 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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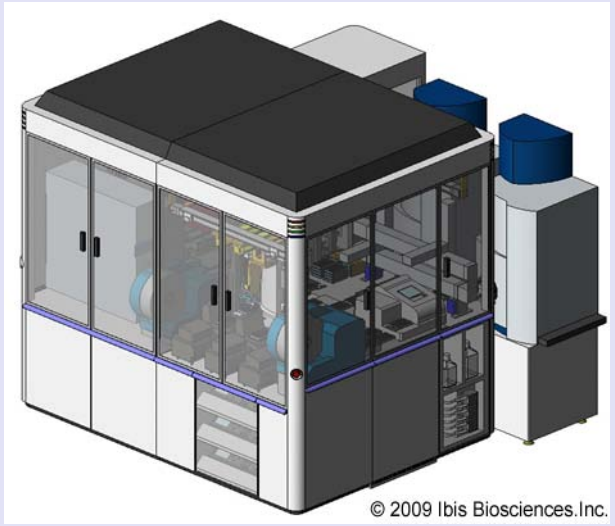
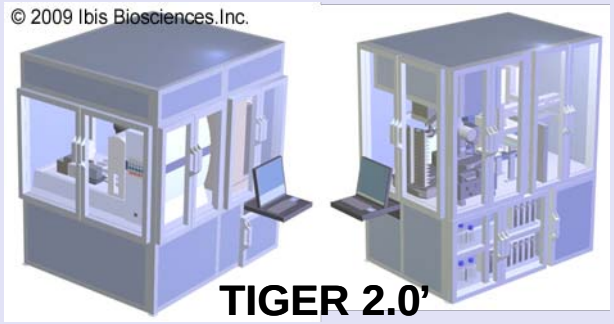
Hardware Overview

System History

TIGER 1.0
2000-2003



TIGER 2.0
2003



TIGER 2.0'
2004-2006

2006-2009

Ibis T6000
2009-

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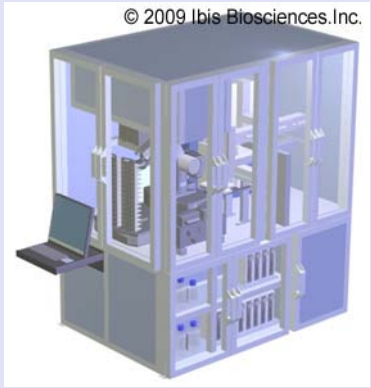
TIGER 1.0

Instrument Formerly Known as TIGER 2.0' - Configuration

- Primary mission is pathogen characterization & bioforensics
- Spatially isolated enclosures
  - A Deck: genome isolation and PCR setup
  - B Deck: PCR, desalt, ESI-TOF, GenX
- Magnetic bead desalting
- Technician transports samples from A Deck to B Deck
- Footprint (A + B) = 54 ft^2

"A Deck"
6' x 5'



"B Deck"
6' x 4'

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Change in Instrument Format

- Rationale – Motivated by discussions with Johns Hopkins and CDC
  - Space is premium in hospital/diagnostic labs
  - Much of “A Deck” function already present in many labs
  - Many different groups/applications use different sample prep protocols
    - CDC core lab model
  - Non-integrated “A Deck” components can be used for other lab functions
  - Lower cost for hardware
- Deployments:
  - CDC in Atlanta, GA
  - FBI in Quantico, VA (2)

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Deployed Systems (T5000) - FBI

- Federal Bureau of Investigation
- Two Ibis T5000™ systems installed in DNA Unit II
- mtDNA forensics analysis
  - Replaces existing sequence-based methods(details to follow)
    - Cost
    - Throughput
    - Heteroplasmy
    - Mixtures
- FBI and Ibis finishing validation package

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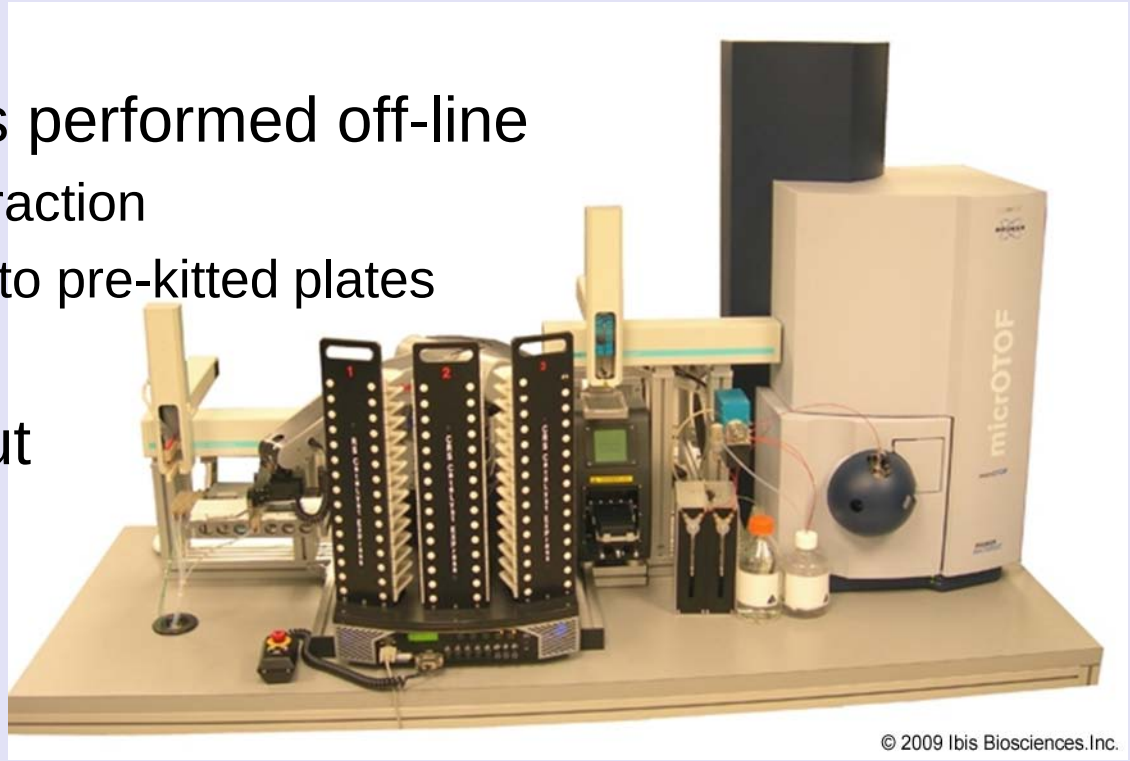
Ibis T5000™

- 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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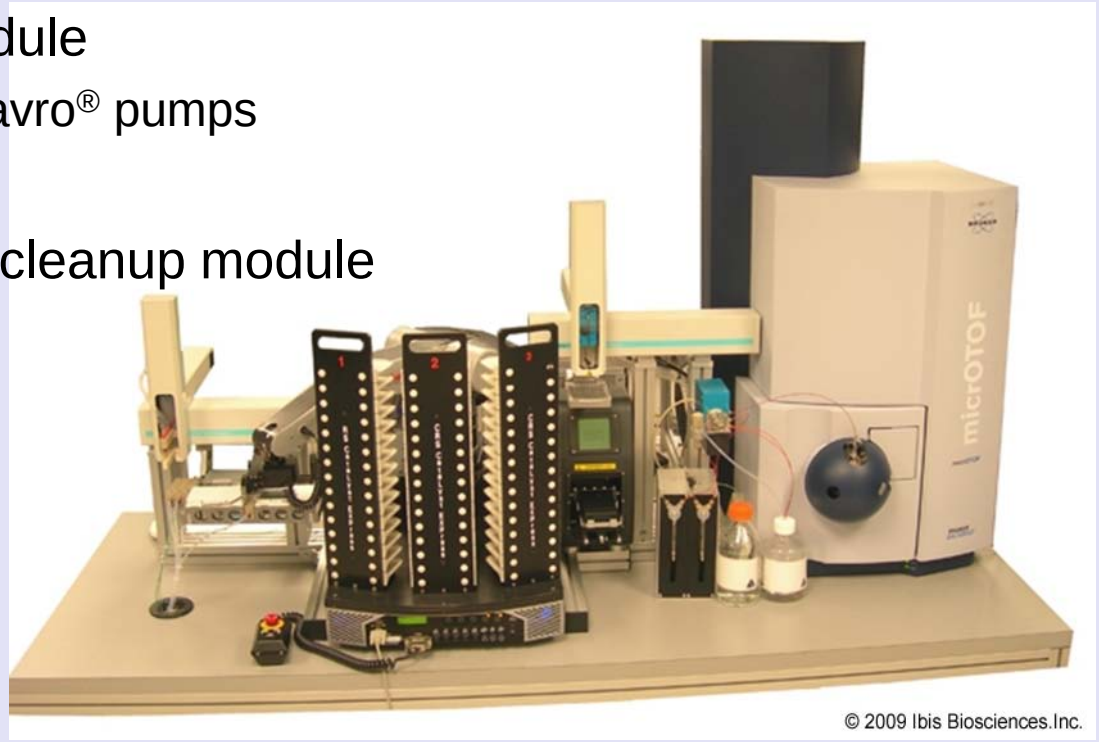
Ibis T5000™ Components

- Bruker Daltonics micrOTOF™
- Thermo CRS robotic arm
  - 3 x 15 plate storage
- LEAP autosampler
- Custom fluidics module
  - Programmable Cavro® pumps
- Heat sealer
- Ibis magnetic bead cleanup module
  - Modified LEAP
  - 8-channel head
  - Shaker
  - Magnetic plate
- Bar code reader
- Computers



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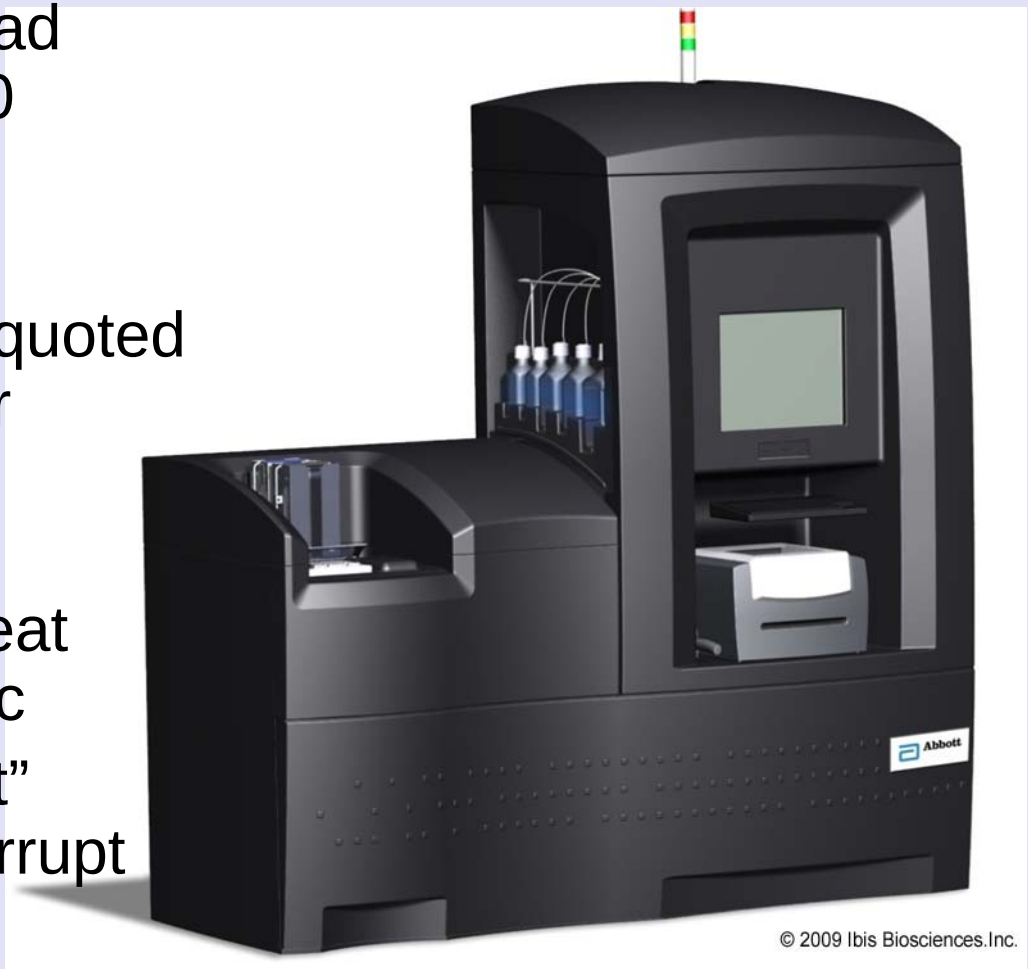
Ibis T6000™

- Key features
  - Remove dependence on complex, high cost 3rd party components
  - Compatibility with existing PCR plates
  - Compatibility with existing cleanup chemistry
  - Accommodate priority “stat” sample
  - Bottom up design with IVD market in mind
    - Rigorously controlled design criteria from the start
  - Support 30 second/well throughput
  - Design and build prototypes such that clinical device manufacturer can build system under FDA-compliant design/manufacturing control

Ibis T6000™ Design

- 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



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