

Technology Transition Workshop

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

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- 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

Technology Transition Workshop 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

Technology Transition Workshop 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

Technology Transition Workshop 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



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Technology Transition Workshop 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

T Globally Important Human Pathogen 🝸 Medically Important Human Pathogen Manual Pathogen Important Plant Pathogen 4 High Potential For Bioengineering Conotic Agent 📚 Toxin CDC Notifiable Agent 🔪 Principal Foodborne Pathogen Emerging Infectious Agent



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Bacteria





Technology Transition Workshop 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!



Technology Transition Workshop 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.

Technology Transition Workshop 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)

Technology Transition Workshop The Ibis Approach to Pathogen ID and Strain-Typing

STEP	

Identify genomic regions for identification: Variable DNA sequences flanked by conserved sequences





Amplify nucleic acids to measure: Use broad-range, unbiased PCR

primers











NJ Technology Transition Workshop Institute Broad Range Priming in Bacteria

STEP	Primers bind to conserved	
1	regions present in ALL	
	(or groups or) bacteria	
	↓	+
Primer pair	CGATTAGATACCCTGCTAGTCC	CGCCTGGGGGAGTACGGCC
E. coli	ACGCCGTAAACGATGTCGACTTGGAGGTTGTGCC-CTTGA-GGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGA	
Cox. burnetii	C	
Leg. pneumophila	ACGCTGTAAACGATGTCAACTAGCTGTTGGTTAT-ATGAAAATAATTAGTGGCGCAGCAAACGCGATAAGTTGAC	
Ricket. prowazekii	ACGCCGTAAACGATGAGTGCTAGATATCGGAGGATTCTCTTTCGGTTTCGCAGCTAACGCATTAAGCACT	
Mycb. tuberculosis	ACGCCGTAAACGGTGGGTACTAGGTGTGGGTTTCCTTCCT	
Trep. pallidum	ACACAGTAAACGATGTACACTAGGTGTTGGGGGCATGAGTCTCGGCGCCGACGCGAACGCATTAAGTGTAC	<mark>T</mark> <mark>T</mark> .
Bacillus anthracis	ACGCCCGTAAACGATGAGTGCTAAGTGTTAGAGGG-TTTCCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTC	
Staph. aureus	ACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGG-TTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACT	<mark>A</mark>
Staph. epidermidis	ACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGG-TTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACT	<mark>A</mark>
Strep. agalactiae	ACGCCGTAAACGATGAGTGCTAGGTGTTAGGCCC-TTTCCCGGGGCTTAGTGCCGCAGCTAACGCATTAAGCACT	<mark>A</mark>
Strep. mutans	ACGCCGTAAACGATGAGTGCTAGGTGTTAGGCCC-TTTCCCGGGGCTTAGTGCCCGGAGCTAACGCAATAAGCACT	<mark>A</mark>
Strep. pneumoniae	ACGCTGTAAACGATGAGTGCTAGGTGTTAGACCC-TTTCCGGGGGTTTAGTGCCGTAGCTAACGCATTAAGCACT	<mark>A</mark>
Strep. pyogenes		<mark>A</mark>

Region varies in different

kinds of bacteria $\Longrightarrow \Delta [A_w G_x C_y T_z]$

Technology Transition Workshop The Polymerase Chain Reaction (PCR)



- 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)



Notional
InstituteTechnology Transition WorkshopNational
InstituteMasses to Base Composition





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



Scale

Weight = 4.6 grams ∴ 2 dimes



Mass spectrum [™]

NJ Technology Transition Workshop National Derived and Expected Base Compositions Primer 356 (RpIB) Expected Products





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Broad Pathogen Detection

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



Technology Transition Workshop 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

Technology Transition Workshop National Institute of Justice Deserved Products (from culture)



All primers of all samples consistent with S. pyogenes



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Direct Analysis of Throat Swab*

*Repeat swab positive on culture for *Streptococcus pyogenes*

NIJ
Institute
of JusticeTechnology Transition WorkshopNational
Institute
of JusticeMulti-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

NJJ Technology Transition Workshop National Institute of Justice Streptococcus pyogenes (Ratio 4/2/1, 1.5 X 10⁶ genomes/swab)



IMAGE COURTESY OF CHRISTIAN MASSIRE, PH.D.

NJ Technology Transition Workshop National Institute **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



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Virus Identification and Typing

National National Institute of Justice Viral Coverage



NIJ Technology Transition Workshop National



IMAGE COURTESY OF CHRISTIAN MASSIRE, PH.D.



Technology Transition Workshop 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

NJ Technology Transition Workshop Institute Justice Ibis T5000[™] Influenza Virus Assay





Influenza species, subtype, clade type determined by comparison with database

NJ Technology Transition Workshop National Institute of Justice Pan-influenza Primer Polymerase PB1 Primer

		lastr.	1300	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420
	2798 VIR2798 FLU ALL P	TGTC	CTGGAATGI	ATGATGGGCA	TGTT							*********	GGGATGGAC	TCCAATCCT	CTGATGA
(REFERENCE 1918F1u HINI	GA.C.			CAATI	ATGTTAAGCAC1	IGTATTAGGCO	TCTCCATCCT	GAATCTTGGAC	AAAAGAGAT	TACACCAAGAC	TACTTACTGGI		. T T.	T
	Human H1N1 (gi 324899	GA.C.			CAATI	ATGTTAAGTACT	IGTATTAGG <mark>C</mark> O	STCTCCATCCT	GAATCTTGGAC	AAAAGAGAG	CACACCAAGAC	TACTTACTGGT	'T.	.TT.	T
Human	Human H1N1 (gi 324897	GA.C.	• • • • • • • • •		CAATA	ATGTTAAGCACI	IGTATTAGGCO	TCTCCATCCT	GAATCTTGGAC	AAAAGAGAT	TACACCAAGAC	TACTTACTGGT	T .	.T	T
Tuman	Human H1N1 (gi 3144213	GA.C.			CAAT!	ATGTTAAGCACI	GTATTGGGCO	TCTCCATCCT	GAATCTTGGAC	AAAAGAGAT	TACACCAAGAC	TACTTACTGGT	<mark>T</mark> .	.TG.	T
and Swine	Human H1N1 (gi 1814083	GA.C.			CAATA	ATGTTAAGCACI	IGTETTEGGEC	STCTCCATCCT	GAATCTTGGGC	AAAAGAGA'	TACACCAAGAC	TACTTACTGGI	T.	.TG.	T
TT1)T1	Swine H1N1(gi 324955 I	AA.C.		<mark>T</mark> .	TAACI	ATGTTAAGCAUT	ICTOTTCCCT	STTTCCATTCT	TAATUTTGGAU	AGAAGGAG	TACACGAAGAC	TGCTTATTGGT	UT.		I
HINI, J	Swine H1N1 (gi 20068032	GA		• • • • • • • • • • •	TAAU	ATGCTGAGCACA	AGTUUTAGGAU	TUTUGATUTT	GAATUTTGGGU	AAAGAGAGA	TACACTAAAAC	CACATACTEGI		· · · · · · · · · · · · · · · · · · ·	
	Swine H1N2 (gi 3815472	GA.C.			CAACI	ATGCTARGIACU	JOITTIIAGGAU	FICILGATACT	GAAICTIGGAC		ACACCAAGAC	AACATACIGGI		••••••	
$\pi 2 \ln 2$, γ	Swine H1N2 (gi 3815471	CA.C.			CAACI	TCCTAAGIACU	GTCTTAGGAU	TCTCALACT	CANTETTECAC	AAAAGAAA	TACACCAAGAC.	AACATACIGGI			
H3N2	Human(HZNZ)(g1(324941)	Cà			CAACI	TCCTAAGTAC	CTCTTAGGAC	TCTCAATCCT	CANTCTCCCCC	AAAAGAAGI	ACACCAAAAC.	NACATACIGGI			
115112	Human H2N2 (g1 324965	CA			CAACI	TCCTAAGTAC	CTCTTAGGAC	TCTCAATCOT	CANTETTECAC	AAAAGAAAI	ACACCAAAAAC.	AACATACTOGI			
	Human(H2N2)(g1)3778501	Ci			CANCI	TCCTAAGTAC	GTCTTRCCA	TCTCAATCCT	CANCETTERCE	000000000000000000000000000000000000000	ACACCAAAAC.	AACATACTOO!			·····
	Human H2N2 (g1 3778503	C)			CAACI	TECTAACTAC	CTTTTACCAC	TCTCANTCCT	CANTETTERCE	1000000	ACACCAMANC.	AACATACTOO!			T
	Human (H3N2) (g1 (3778544	GA		Т	CAACI	TGCTAAGTAC	CETTTTACCAC	TCTCAGTCCT	GAATCTTGGGC	A A A A C A C A T	ACACCAAAAAC	AACATACTEGI			T
	Humon 199821 (g1 3770545	GA			CAAC	TGCTAAGTAC	GTTTTAGGAD	TETEAATEET	GAATCTTGGGC	AAAAGAGA	ACACCAAAAAC	AACATACTGGT			Ţ
	Swine H2N2 Tr fluence	GA			TAAC	TGCTGAGTACA	GTETTAGGAO	TCTCAATCTT	GAATCTTGGAC	AAAGAGG	ACACCAGGAC	CACATACTEGI	G	T T	C
Canine	Swine (H3N2) / mil 5207815	GA.C.			CAAC	TGCTAAGTAC	GTTTTGGGA	TCTCGATTCT	AAATCTAGGGC	AAAGAGGT	TATACCAAAAC	AACATACTGGT	C		.CC
	Fourine [H3N8] [Influenza	GA.	C	A	CAAC	TGTTGAGCACT	GTGCTGGGT	TATCCATATT	AAACCTGGGCC	AGAGGAAAT	ACACAAAGAC	CACATACTGGT	T	.G A.	.cc
Equine J	Equine H3N8 Influenza	GA	C	A.		ATGTTGAGCACI	GTGCTAGGTO	TATCCATATT	AAACCTGGGCC	AGAGGAAAT	ACACAAAGAC	CACATACTGGT	T.	.GA.	C
	Canine H3N8 (gi 710842	GA	C			TGTTGAGCACT	GTGCTGGGTG	TATCCATATT	AAACCTGGGCC	AGAGGAAAT	ACACAAAGAC	CACATACTOGT		.GA.	
H3N8 L	Canine H3N8 (gi 710842	GA	C	A.	CAACA	TGTTGAGCACT	GTGCTGGGTG	TATCCATATT	AAACCTGGGCC	AGAGGAAAT	ACACAAAGAC	CACATACTGGT	T.	.GA.	C
C	Swine H4N6 Influenza A	GA			CAACA	ATGCTAAGTACA	GTTTTAGGAG	TTTCAATCCT	AAATCTGGGAC	AAAAGAGAT	ACACCAAAAC.	AACGTATTGGT	C	T.	C
Human	Human H5N1 (gi 1392538	GA	AG			ATGCTAAGTACO	GGTCTTAGGA	TCTCAATCTT	AAATCTTGGGGC	AGAAGAGGT	ACACCAAAAC	CACATATTGGT	G.	T	T
Trainain,	Human H5N1 (gi 8307772	GA	.AG		TAACA	ATGCTAAGTACO	GGTCTTAGGA	TCTCAATCTT	AAATCTTGGGGC	AGAAGAGGI	ACACCAAAAC	CACATACTGG	G.		T
Avian 🖌	Avian H5N1 (gi 2884968	GA			CAATA	ATGCTGAGTACA	AGTCTTAGGAO	TTTCAATCCT	GAATCTTGGGC	AGAAGAAGI	ACACCAAAAC	CACATACTGGT	C		T
TICN11	Avian H5N1 (gi 2884968	GA.C.			CAACI	ATGCTGAGTACA	AGTCCTAGGAC	TCTCAATCCT	GAATCTTGGAC	AGAAAAGGI	ACACCAAAAC	CACATATTGGT	C		T
HONI	Swine H5N1 (gi 5151215	GA			CAACI	ATGCTGAGTAC!	AGTCTTAGGA0	TCTCAATCCT	GAATCTTGGGGG	AAAAGAGGI	TATACCAGAAC	CACGTACTGGT	····		T
	Swine H5N1 (gi 5412651	GA.C.			CAACA	ATGCTGAGTACA	AGTCCTAGGA0	STCTCAATCCT	GAATCTTGGAC	AGAAAAGGI	TACACCAAAAC	CACATATTGGI	C	• • • • • • • • • • •	T
_	Human H7N7 Influenza A	GA	C	G.	CAACI	ATGTTAAGCACI	IGTACTAGGT0	TATCCATATT	AAACCTAGGTC	AAAGGAAAT	TACACAAAGAC	CACATACTGGT	'T.	.GA.	. C T
Other (Avian H7N7 Influenza A	GA	••••	• • • • • • • • • •	CAACI	ATGCTGAGTACA	AGTATTAGGAC	TTTCAATCCT	GAATCTTGGAC	AAAAGAGGI	ACACCAAAAC	TACATACTGGT	G.	• • • • • • • • • • • • •	T
	Avian H9N1 1(gi 313395	: GA	·		CAACI	ATGCTGAGTACA	AGTTTTAGGAC	TATCAATCCT	GAATCTTGGGC	AAAAGAGGI	TACACCAAAAC	CACATACTGGI	· · · · · · · · · · · ·	A.	.CT
Avian, ≺	Avian H9N1 1(gi 313395	: GA			CAACA	ATGCTGAGTACA	RGTTTTAGGAU	TATCAATCCT	GAATETTGGGC	AAAAGAGGI	TACACCAAAAC	CACATACTGGT		· · · · · · · A.	.CT
Cruina	Avian H9N2 (gi 5732314	GA			CAACI	ATGUTGAGTAUA	RETETTAGGAL	TTTCAATCUT	GAATUTTGGGC		01.01.1.000001.	retre retretter			· · · · · · · · · ·
Swine C	Swine H9N2 (gi 5592586	GA.C.			CAATA	ATGTTAAGTACT	IGTATTAGGUU	FUTURATUUT	GAATUTTGGAU	ii 6 r	ามเตโตเ	otide	dala	tion	· · · · · · · · · · · · · · · · · · ·
C	Swine H9N2] (gi 5592589	GA.C.			UAG12	AIGITAAGIAU	CTCTTCCCA	TUTULATUUT	GAAICIIGGAC		lucic	onac	ucic		
	Influenza B virus_(gi)	16.06.		· · · · · · · · · A ·	TAAII	AIGCIAICIACO	Jeleiieeeee	TAGLUGUAUT	AGGIA		TROCALACAA	10112100001			
Tuffurnes	Influenza B virus_(gi)	0.00.			TAAT	TGCTATCTAC	CTCTTCCCA	TAGUUGUAUT	A GGIA	CAAAAACA	TTCCALACAA	AGAAIACIIAI	********	. G	
iniiuenza j	Influenza B virus_(gi)	C CC		A.	TAATI	TCCTATCTACC	CTCTTCCCAC	TAGECGEACT	A CCTA	CAAAAACA	TTCCANACAA	ACAATACTIAI		C T	
B and C	Influenza B virus_(gi)	C CC.			TAAT	TGCTATCTAC	CTCTTCCCA	TACCACCACT	CCTA	CAAAAACA	TTCCINICAN	CCMATACTIAI		G T	I
	Influenza B virus_(gi)	C C2	••••••	A.	TAAT	TCCTATCTAC	CTCTTCCCAC	TAGCAGCAUT	CCTA	TAAAAACA	TTCCALACAA	CCMATACITAT CCMMTACTTAT		C T	
	Influenza B virus_(gi)	ACC C	24	гт а	CAACI	TECTETCIAC	CTTCTTCCAC	TABCAGCACI	ATCTTATATCC	TCIICII	TIBOAAACAA	ACCATCTTTT	30		
	Influenza (virus (gi)	ACC C	24	т а	CAACI	TECTETCAACA	CTTCTTCCAC	TANGTACATT	ATCTTATATOC	TCAACAAC	TAAAAGCCAA	ACCATCTTTT	10		
	inituenza t virus (gi)	14200.0	**** * * * * * *		unnur	1.001010HH0	101101100AC	anno monti	and a mini our	a vanonau	- manooona	and out of the the	· · · · · · · · · · · · · · · · · · ·		



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

National
Influenza Virus Assay Results Example

00	Image: Constraint of the second se											
Influenza A virus Image: structure viewed Place Powork Structure Image: structure												
	Well 15, Sample 3 Primer VIR1266	Organism	Sample Score	Basecount	Peak1 Mass	Peak1 Abundance	Peak1 Fit Error	Peak2 Mass	Pe Abun			
	FLUANUC_J02147_118_218	Influenza A virus A/NEW YORK/14/2003(H3N2)	0.96539	A26G20C23T32	31051.6	3030.0	0.0	31225.7	3440.0			
4E3 ·			-		31051.6	3030.0	0.0	31225.7	3440.0			
3.5E3		Influenza A virus A/NEW YORK/15/2003(H3N2)	0.96539	A26G20C23T32	31364.1	396.0	0.0	31225.7	3440.0			
352	Influenza A virus A/NEW YORK/16/2003(H3N2)		0.96539	A26G20C23T32	31051.6	3030.0	0.0	31225.7	3440.0			
JES 1					31364.1	395.0	0.0	31225.7	3440.0			
# 2.5E3		Influenza A virus A/NEW YORK/21/2003(H3N2)	0.96539	A26G20C23T32	31364.1	396.0	0.0	31225.7	3440.0			
		Influenza A virus A/NEW YORK/22/2003(H3N2)	0.96539	A26G20C23T32	31051.6	3030.0	0.0	31225.7	3440.0			
₹					31364.1	396.0	0.0	31225.7	3440.0			
1.5E3 ·	· · · · · · · · · · · · · · · · · · ·	Influenza A virus A/NEW YORK/24/2003(H3N2)	0.96539	A26G20C23T32	31364.1	396.0	0.0	31225.7	3440.0			
163					31051.6	3030.0	0.0	31225.7	3440.0			

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Influenza A virus A/NEW YORK/25/2003(H3N2) 0.96539 A26G20C23T32 31364.1 396.0 5E2 31051.6 3030.0 Influenza A virus/A/NEW YORK/26/2003(H3N2) 0.96539 A26G20C23T32 31364.1 396.0 30,000 31051.6 3030.0 Mass Influenza A virus A/NEW YORK/268/2003 (H3N2) 0.96539 A26G20C23T32 31364.1 396.0 Pan Zoom Fit 4 1 3030.0 31051.6 Influenza & visual &/NEW VODK/27/2003/H3N2) 026620623732 0 06530

National Institute of Justice

Technology Transition Workshop Human Influenza Trial - Blinded Samples

Sou	Source		Collection Dates	Sample type	No. of Samples
Naval I Research	Naval Health Research Center		1999-2005	Throat swabs, nasal swabs, nasal washes	317
Johns H University Cer	lopkins ⁄ Medical nter	Baltimore, MD	2003-2005	Nasal aspirates	229
NY State Hea	Dept. of alth	Throughout NY	1999-2005	Nasal aspirates, BAL, tracheal aspirates, throat swabs	100
TX State Hea	Dept. of alth	Throughout TX	2005-2006	Throat swabs, nasal washes	10
Тс	otal				656
-Correctly identified all Influenza A types					nfluenza
			Sensit	ivity	96.8%
× 4 40 LION	Specif	icity	97 5%		

≻149 H3N2
≻34 H1N1 **-67 Influenza B**

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

NIJ Technology Transition Workshop Detection of Mixed Infections



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

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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

NJ Technology Transition Workshop National Institute of Justice Avian Flu Detection:

No Change in Assay or Primers





Technology Transition Workshop 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

NJTechnology Transition WorkshopNational
InstituteIbis 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					

J Technology Transition Workshop National Institute of Justice 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 \bullet
 - 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.



Technology Transition Workshop



Hardware Overview



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0ST5000

2006-2009

System History



TIGER 1.0 2000-2003

TIGER 2.0'

2004-2006

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TIGER 2.0 2003

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NJ Technology Transition Workshop "T.I.G.E.R."



TIGER 1.0

Technology Transition Workshop 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 $(A + B) = 54 \text{ ft}^2$





"B Deck" 6' x 4'

NJ Technology Transition Workshop 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)

NJ Technology Transition Workshop National Institute **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



Technology Transition Workshop 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



Technology Transition Workshop Institute of Justice 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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Institute
of JusticeIbis 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



Technology Transition Workshop 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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Technology Transition Workshop

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