Genomics Impact on Infectious Diseases

Jonathan Zenilman MD

Johns Hopkins University

March 2, 2012

Conflicts--None

end-stage renal disease in the United States. Bethesda, Md.: Nation-

Welcome to the Genomic Era

Alan E. Guttmacher, M.D., and Francis S. Collins, M.D., Ph.D.

To him who devotes his life to science, nothing can give more happiness than increasing the number of discoveries, but his cup of joy is full when the results of his studies immediately find practical applications.

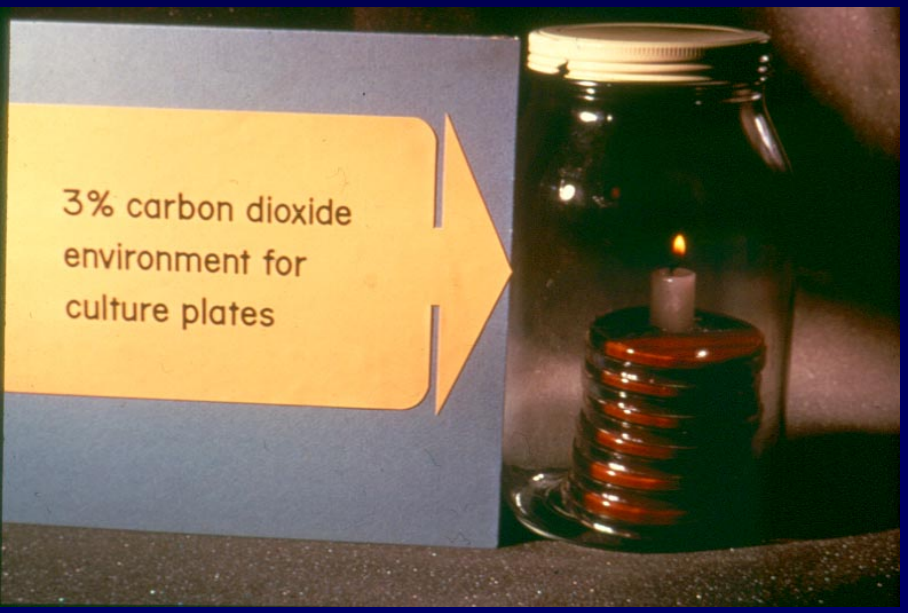
— Louis Pasteur

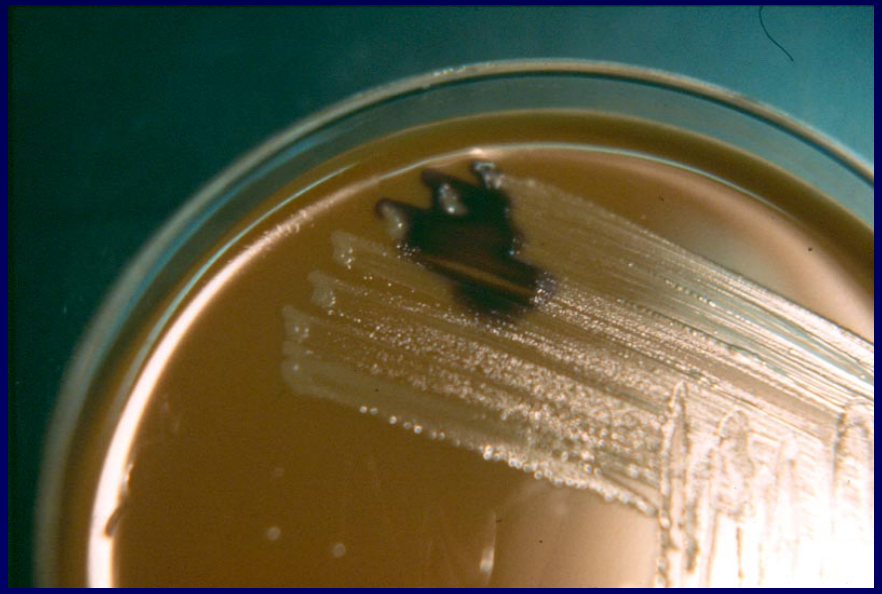
announcement (available at http://www.genome.gov/11006929) that it had achieved the last of the project's original goals, the complete sequencing of the human genome. The extent and pace of progress in genomics are suggested by the fact that this achievement occurred 11 days shy of the 50th anni-

versary of the publication of Watson and Crick's

NEJM Sept 4, 2003

Bacterial Diagnosis until 2000





Problems with Cultures

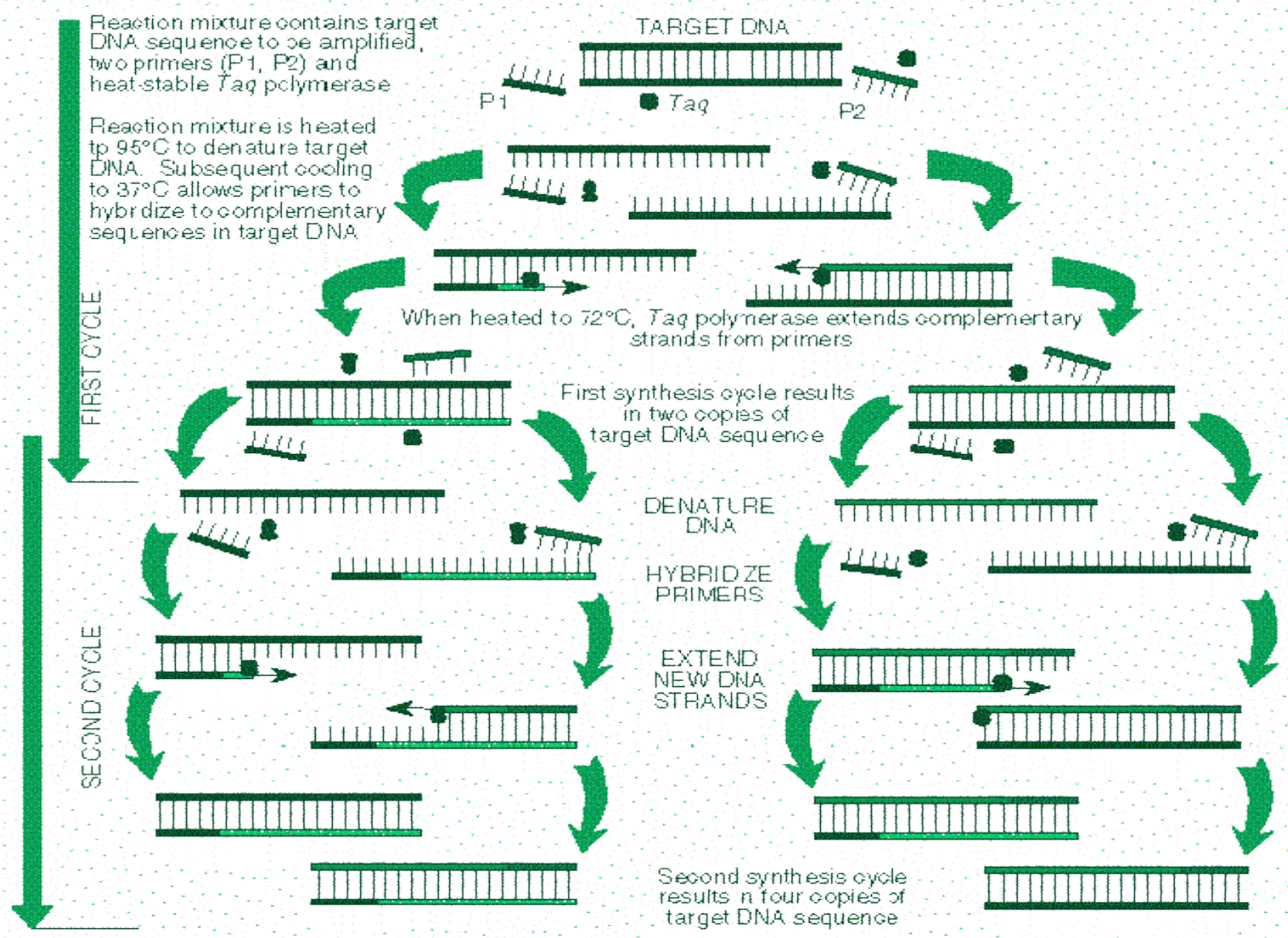
- Cultures take 24-48 hours to process
- Quantitative Cultures take longer
- Cultures are prone to overgrowth
- Are there molecular approaches?

Genomics Diagnostics--Principles

- Detect DNA
- Amplification via PCR—Impact on Sensitivity
- Bacterial DNA have unique 16S ribosome DNA elements
- March to libraries
- Link to detection system
- Specificity can be a problem

ORNL-DWG 91M-17475

DNA Amplification Using Polymerase Chain Reaction



Source: *DNA Science*, see Fig. 13.

Accelerated Progress

- Nucleic Acid Diagnostics Commercialized in 1990s (STDs, HIV viral load)
- Non cultivable pathogens identified
- 2001 attacks—Major investments
- Simultaneous HGP and Sequence projects
- ~2000 organisms fully sequenced

Current Trends

- Commercialization of Discovery
- Rapid Clinical Diagnostics
- Genomics as Clinical Management Tools
- Bacterial Population Genomics and Impact
- Host Genomics and Susceptibility
- Microbiome Projects
- Expert and Benchtop systems

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 MAY 15, 2003 VOL. 348 NO. 20

A Novel Coronavirus Associated with Severe Acute Respiratory Syndrome

Thomas G. Ksiazek, D.V.M., Ph.D., Dean Erdman, Dr.P.H., Cynthia S. Goldsmith, M.S., Sherif R. Zaki, M.D., Ph.D., Teresa Peret, Ph.D., Shannon Emery, B.S., Suxiang Tong, Ph.D., Carlo Urbani, M.D.,* James A. Comer, Ph.D., M.P.H., Wilina Lim, M.D., Pierre E. Rollin, M.D., Scott F. Dowell, M.D., M.P.H., Ai-Ee Ling, M.D., Charles D. Humphrey, Ph.D., Wun-Ju Shieh, M.D., Ph.D., Jeannette Guarner, M.D., Christopher D. Paddock, M.D., M.P.H.T.M., Paul Rota, Ph.D., Barry Fields, Ph.D., Joseph DeRisi, Ph.D., Jyh-Yuan Yang, Ph.D., Nancy Cox, Ph.D., James M. Hughes, M.D., James W. LeDuc, Ph.D., William J. Bellini, Ph.D., Larry J. Anderson, M.D., and the SARS Working Group†

ABSTRACT

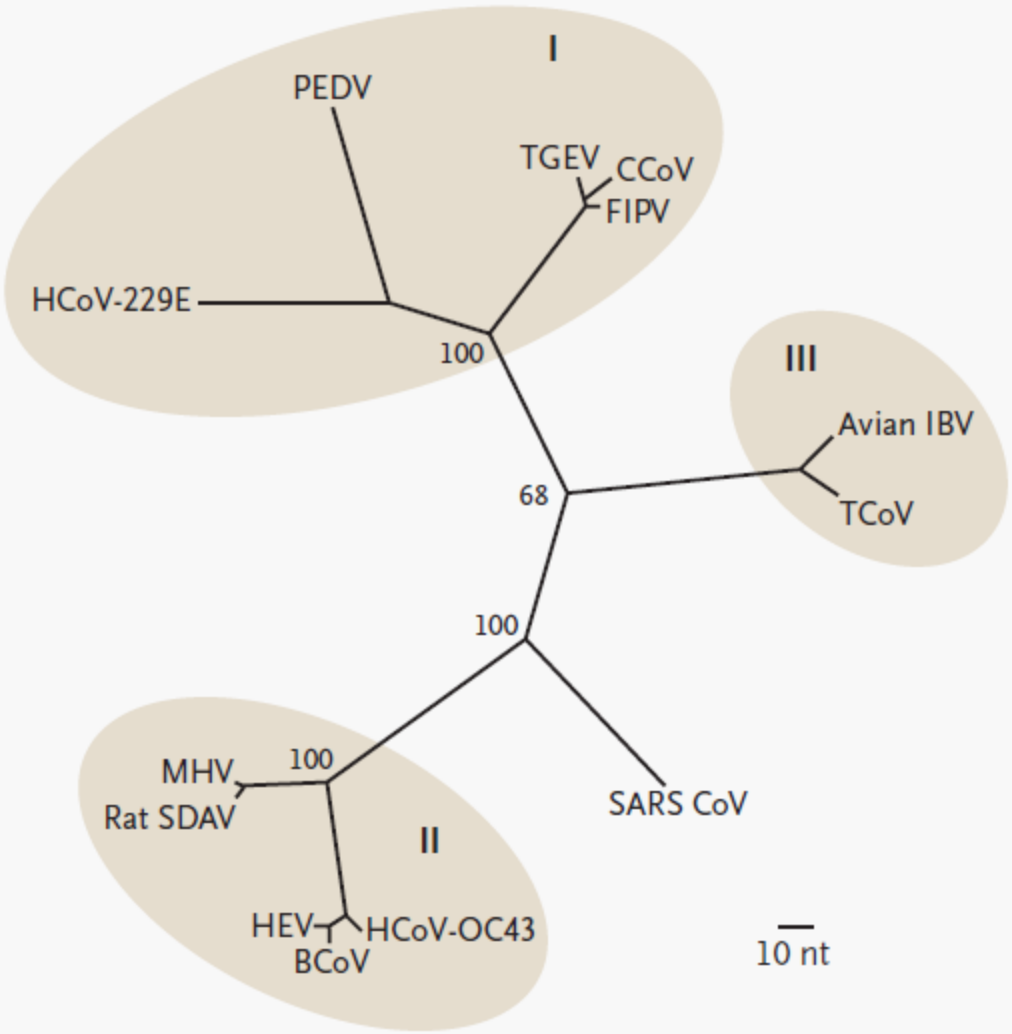


Figure 3. Estimated Maximum-Parsimony Tree Based on the Sequence Alignment of 405 Nucleotides of the Coronavirus Polymerase Gene Open Reading Frame 1b (Nucleotide Numbers 15173 to 15578 Based on Bovine Coronavirus Complete Genome Accession Number NC_003045) Comparing SARS Coronavirus with Other Human and Animal Coronaviruses.

The three major coronavirus antigenic groups (I, II, and III), represented by

Nucleic Acid Amplification Tests

- NAAT tests are the dominant mode of gonococcal and chlamydia testing
- Can be used in genital and non-genital samples-eg urine, self administered swabs-field applications
- Screening in field settings, schools, jails etc
- No transport issues
- Turnaround 24 hours
- Multiplex
- BUT-cant detect resistance

Untreated Gonococcal and Chlamydial Infection in a Probability Sample of Adults

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Susan M. Rogers, PhD
Heather G. Miller, PhD
William C. Miller, MD, PhD
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Context The prevalence and distribution of gonococcal and chlamydial infections in the general population are poorly understood. Development of nucleic acid amplification tests, such as the ligase chain reaction assay, provides new opportunities to estimate the prevalence of untreated infections in the population.

Objective To estimate the overall prevalence of untreated gonococcal and chlamydial infections and to describe patterns of infection within specific demographic subgroups of the young adult population in Baltimore, Md.

Design and Setting Cross-sectional behavioral survey based on a probability sample of Baltimore households with collection of urine specimens between January 1997 and September 1998.

Participants A total of 728 adults aged 18 to 35 years completed the interview portion of the study, and 579 of these respondents also provided a urine specimen adequate for testing.

Main Outcome Measure Prevalence of untreated infection, as measured by the

UNTREATED INFECTION WITH

Table 2. Estimated Prevalence of Untreated Gonococcal and Chlamydial Infections by Race and Sex: 1997-1998 Baltimore STD and Behavior Survey*.

Table 2. Estimated Prevalence of Untreated Gonococcal and Chlamydial Infections by Race and Sex: 1997-1998 Baltimore STD and Behavior Survey*

	Black		Other Race		
	Women	Men	Women	Men	Total
Unweighted sample size	193	126	142	118	579
Neisseria gonorrhoeae and/or *Chlamydia trachomatis*					
No. of cases (unweighted)†	21	14	8	6	49‡
Prevalence (weighted), % (SE)§	15.0 (3.7)	6.4 (2.1)	1.3 (0.5)	2.8 (1.3)	7.9 (1.6)
N gonorrhoeae					
No. of cases (unweighted)†	12	10	8	3	33
Prevalence (weighted), % (SE)§	9.3 (3.3)	5.3 (2.0)	1.3 (0.5)	1.3 (0.9)	5.3 (1.4)
C trachomatis					
No. of cases (unweighted)†	10	4	0	4	18
Prevalence (weighted), % (SE)§	6.4 (2.2)	1.1 (0.7)	0	2.4 (1.3)	3.0 (0.8)

*STD indicates sexually transmitted disease. Estimates are based on age-eligible respondents who provided urine specimens for *N gonorrhoeae* and *C trachomatis* testing (ligase chain reaction assay). The estimates are weighted to account for differing probabilities of selection and poststratification adjustments to match US Census marginals. The estimates presented differ slightly from preliminary estimates for sexually experienced subjects presented at the International Society for Sexually Transmitted Diseases Research meetings.[25] In addition to the difference in population definition (all subjects vs subjects with sexual experience), subsequent comparison of laboratory records and the preliminary analysis file revealed 1 instance in which a subject who tested positive for gonococcal infection was mistakenly coded as positive for both pathogens in the preliminary analysis file.

†Unweighted case counts are not appropriate for making inferences about the prevalence of infection in populations since they do not take account of the differing probabilities of selection of households and individuals.

‡Includes 2 cases that were positive for both infections.

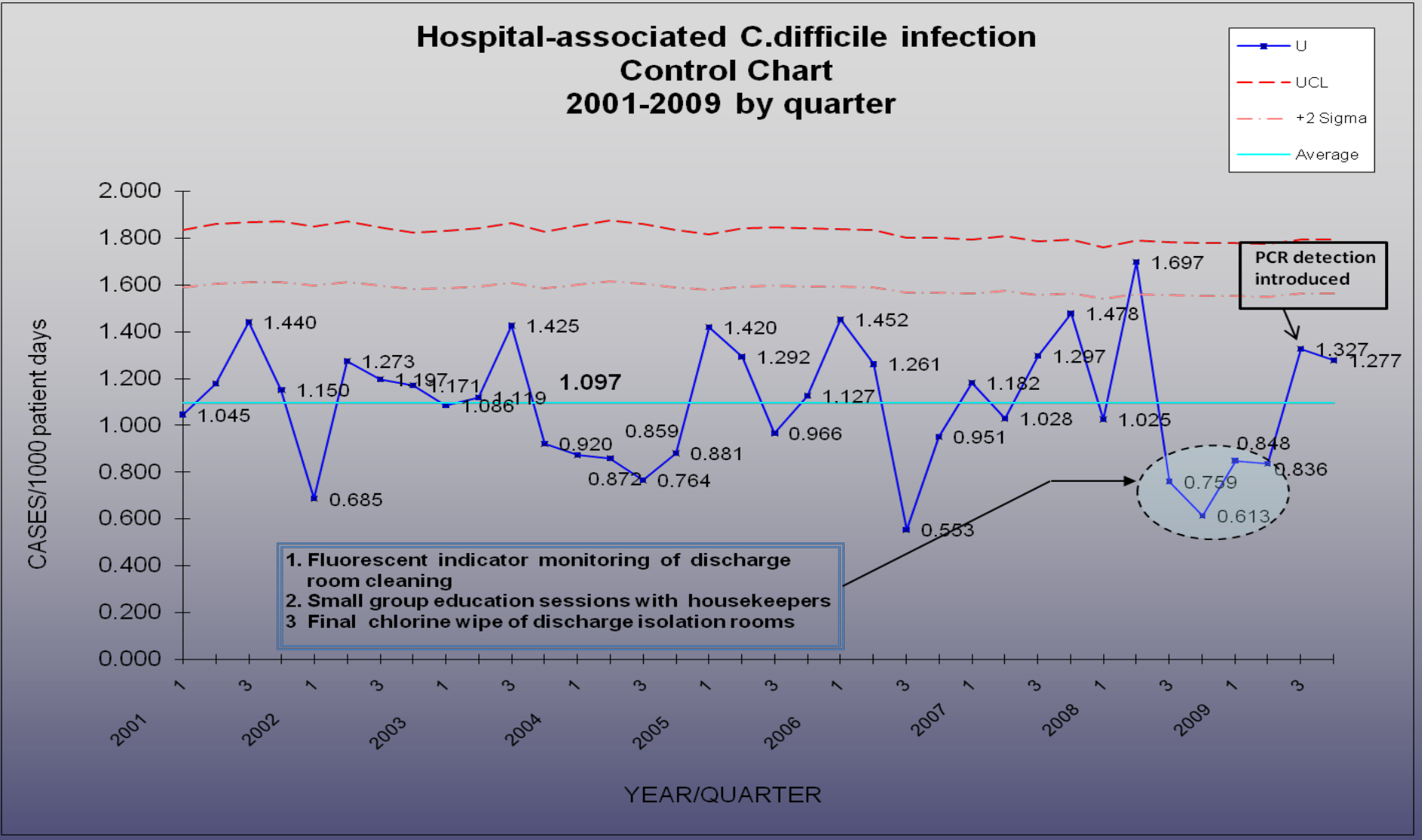
§SEs were calculated from weighted data using statistical algorithms that take account of impact of complex sample design on variance estimates.

Turner, C. F. et al. JAMA 2002;287:726-733

Diagnostic Progression of C Difficile

- Culture—Takes days and is non-specific
- Toxin assay –Stool filtrate in tissue culture
- ELISA assay—Sensitivity ~80%
- Current—PCR of toxinA/toxinB genes—potential 6 hour turnaround

BUT—what happens when you start using the new tests?


Hospital-associated C.difficile infection
Control Chart
2001-2009 by quarter
U
UCL
+2 Sigma
Average
PCR detection introduced
1.097
1. Fluorescent indicator monitoring of discharge room cleaning
2. Small group education sessions with housekeepers
3 Final chlorine wipe of discharge isolation rooms
CASES/1000 patient days
YEAR/QUARTER
2.000
1.800
1.600
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
2001
2002
2003
2004
2005
2006
2007
2008
2009
1.045
1.440
1.150
0.685
1.273
1.197
1.171
1.086
1.119
1.425
0.920
0.872
0.859
0.764
0.881
1.420
1.292
0.966
1.127
1.452
1.261
0.553
0.951
1.182
1.028
1.297
1.478
1.025
1.697
0.759
0.613
0.848
0.836
1.327
1.277

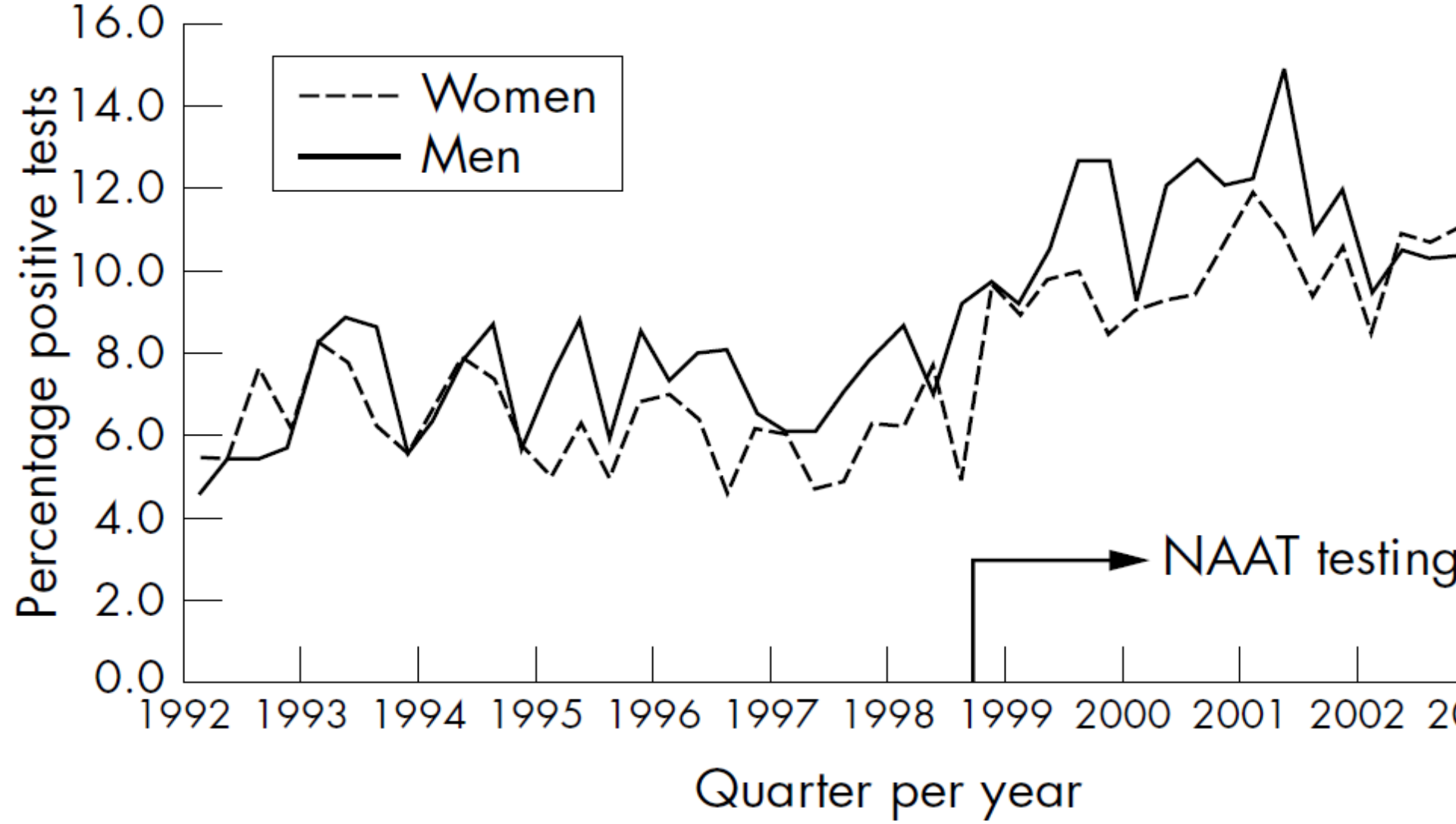


Figure 1 Percentage positive chlamydia tests per yearly quarter Royal Infirmary Edinburgh GUM dataset for 1992 to 2003, separ for males and females; date of change from culture to NAAT for re

JCM
Journals.ASM.org

Comparison of the Idaho Technology FilmArray System to Real-Time PCR for Detection of Respiratory Pathogens in Children

Virginia M. Pierce,[a,b,d] Michael Elkan,[b,d] Marilyn Leet,[c,d] Karin L. McGowan,[c,d] and Richard L. Hodinka[b,d]

Division of Infectious Diseases, Department of Pediatrics,[a] Clinical Virology Laboratory,[b] Clinical Microbiology Laboratory,[c] and Department of Pathology and Laboratory Medicine,[d] Children's Hospital of Philadelphia and University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA

The FilmArray Respiratory Panel (RP) multiplexed nucleic acid amplification test (Idaho Technology, Inc., Salt Lake City, UT) was compared to laboratory-developed real-time PCR assays for the detection of various respiratory viruses and certain bacterial pathogens. A total of 215 frozen archived pediatric respiratory specimens previously characterized as either negative or positive for one or more pathogens by real-time PCR were examined using the FilmArray RP system. Overall agreement between the FilmArray RP and corresponding real-time PCR assays for shared analytes was 98.6% (kappa = 0.92 [95% confidence interval (CI), 0.89 to 0.94]). The combined positive percent agreement was 89.4% (95% CI, 85.4 to 92.6); the negative percent agreement was 99.6% (95% CI, 99.2 to 99.8). The mean real-time PCR threshold cycle (C_T) value for specimens with discordant results was 36.46 ± 4.54. Detection of coinfections and correct identification of influenza A virus subtypes were comparable to those of real-time PCR when using the FilmArray RP. The greatest comparative difference in sensitivity was observed for adenovirus; only 11 of 24 (45.8%; 95% CI, 27.0 to 64.9) clinical specimens positive for adenovirus by real-time PCR were also positive by the Film-

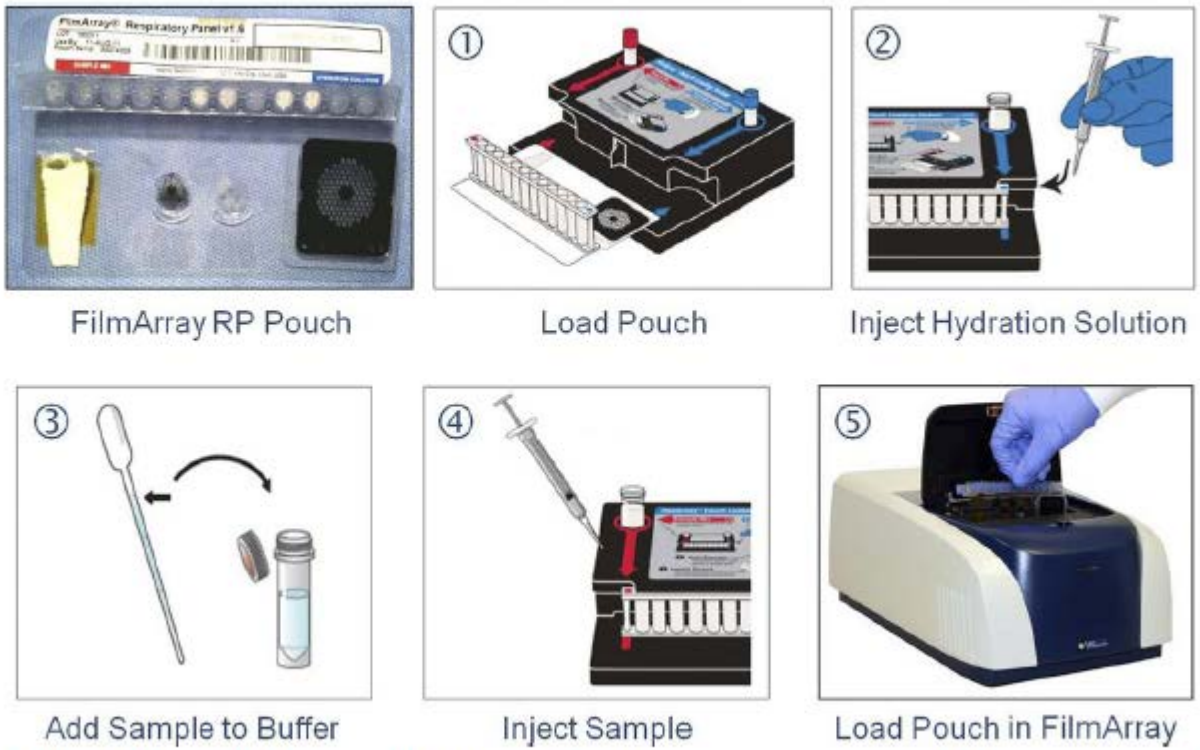


FIG 1 Illustration of the FilmArray RP pouch and the steps involved in processing a specimen for testing using the FilmArray system.

Detecting Undetectable/Hard to detect organisms

- Bartonella and other fastidious bacteria (eg TB)
- HPV viruses
- T. palliduma and LGV in lesions
- Newly discovered organisms

The Expansion of the Microbiological Spectrum of Brain Abscesses with Use of Multiple 16S Ribosomal DNA Sequencing

Mouhamad Al Masalma,[1] Fabrice Armougom,[1] W. Michael Scheld,[4] Henri Dufour,[2] Pierre-Hugues Roche,[3] Michel Drancourt,[1] and Didier Raoult[1]

[1]Pôle des Maladies Infectieuses, Assistance Publique-Hôpitaux de Marseille and URMITE, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 6236, Institut pour la Recherche et le Développement 198, Université de la Méditerranée, [2]Service de Neurochirurgie, Hôpital de la Timone, Assistance Publique-Hôpitaux de Marseille, and [3]Service de Neurochirurgie, Hôpital Sainte-Marguerite, Assistance Publique-Hôpitaux de Marseille, Marseille, France; and [4]Department of Internal Medicine, Division of Infectious Diseases and International Health, University of Virginia Health System, Charlottesville, Virginia

(See the editorial commentary by DiGiulio and Relman on pages 1179–81)

20 patients with brain abscess
Cultures=22 strains; PCR=72 strains
27 species not previously seen in brain abscess
1 subject had 16 strains

JID 2009; 48:1169

Antimicrobial Resistance

- Genomics can rapidly detect antimicrobial resistance
- You need to know what you are looking for
- Can be used as rapid screens
- High utility in tracking outbreaks, identifying clones

Quinolone Resistance–Determining Region Mutations and *por* Type of *Neisseria gonorrhoeae* Isolates: Resistance Surveillance and Typing by Molecular Methodologies

Julie A. Giles,[1] Jason Falconio,[2,a] Jeffrey D. Yuenger,[1] Jonathan M. Zenilman,[1] Michael Dan,[3] and Margaret C. Bash[2]

[1]Division of Allergies and Infectious Disease, Johns Hopkins University School of Medicine, Baltimore, and [2]Division of Bacterial, Parasitic and Allergenic Products, Center for Biologics Evaluation and Research, United States Food and Drug Administration, Bethesda, Maryland; [3]Infectious Disease Unit, Edith Wolfson Hospital, Tel Aviv, Israel

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Quinolone resistance is increasing rapidly in *Neisseria gonorrhoeae* and is a significant public health problem

Table 3. Mutations in the quinolone resistance–determining regions (QRDRs) of *Neisseria gonorrhoeae* strains isolated in Israel from January 2000 through October 2001.

Fluoroquinolone susceptibility	No. of isolates	MIC of ciprofloxacin, μg/mL	QRDR mutations, by gene and amino acid		
			In *gyrA*[a]		In *parC*,[b]
			aa 91	aa 95	aa 86
CipR[c]	39	2–16	TCC→TTC	GAC→AAC	GAC→AAC
	2	4–8	TCC→TTC	GAC→GGC	GAC→AAC
	1	8	TCC→TTC	*wt*	GAC→AAC
CipI[d]	1	0.125	*wt*	GAC→AAC	*wt*
CipS[e]	37	.002–.016	*wt*	*wt*	*wt*

NOTE. CipI, intermediately resistant to ciprofloxacin; CipR, resistant to ciprofloxacin; CipS, susceptible to ciprofloxacin; *wt*, wild type.

[a] All isolates were *wt* at loci coding aa 92–94 of *gyrA*.
[b] All isolates were *wt* at loci coding aa 85 and 87–91 of *parC*.
[c] MIC of ciprofloxacin, ⩾1 μg/mL.
[d] MIC of ciprofloxacin, ⩾0.125 μg/mL.
[e] MIC of ciprofloxacin, <0.125 μg/mL.

Syphilis –T pallidum cannot be cultured –Genomics has facilitated understanding the epidemiology of resistance

The Presence of the 23S rRNA Gene Mutation in *T. pallidum* Samples Collected from Sites in the United States and Ireland from 1912 through 2003.

Table 1. The Presence of the 23S rRNA Gene Mutation in *T. pallidum* Samples Collected from Sites in the United States and Ireland from 1912 through 2003.

Geographic Site	Date Sample Collected	Samples with Mutation/ Total Amplifiable Samples
		no./total no. (%)
San Francisco	1999–2002 2003	1/25 (4) 11/30 (37)
Seattle	2001–2003	3/23 (13)
Baltimore	1998–2000	2/19 (11)
Dublin	2002	15/17 (88)
Historical strains from multiple locations	1912–1987	1/18 (6)

Lukehart SA et al. N Engl J Med 2004;351:154-158.

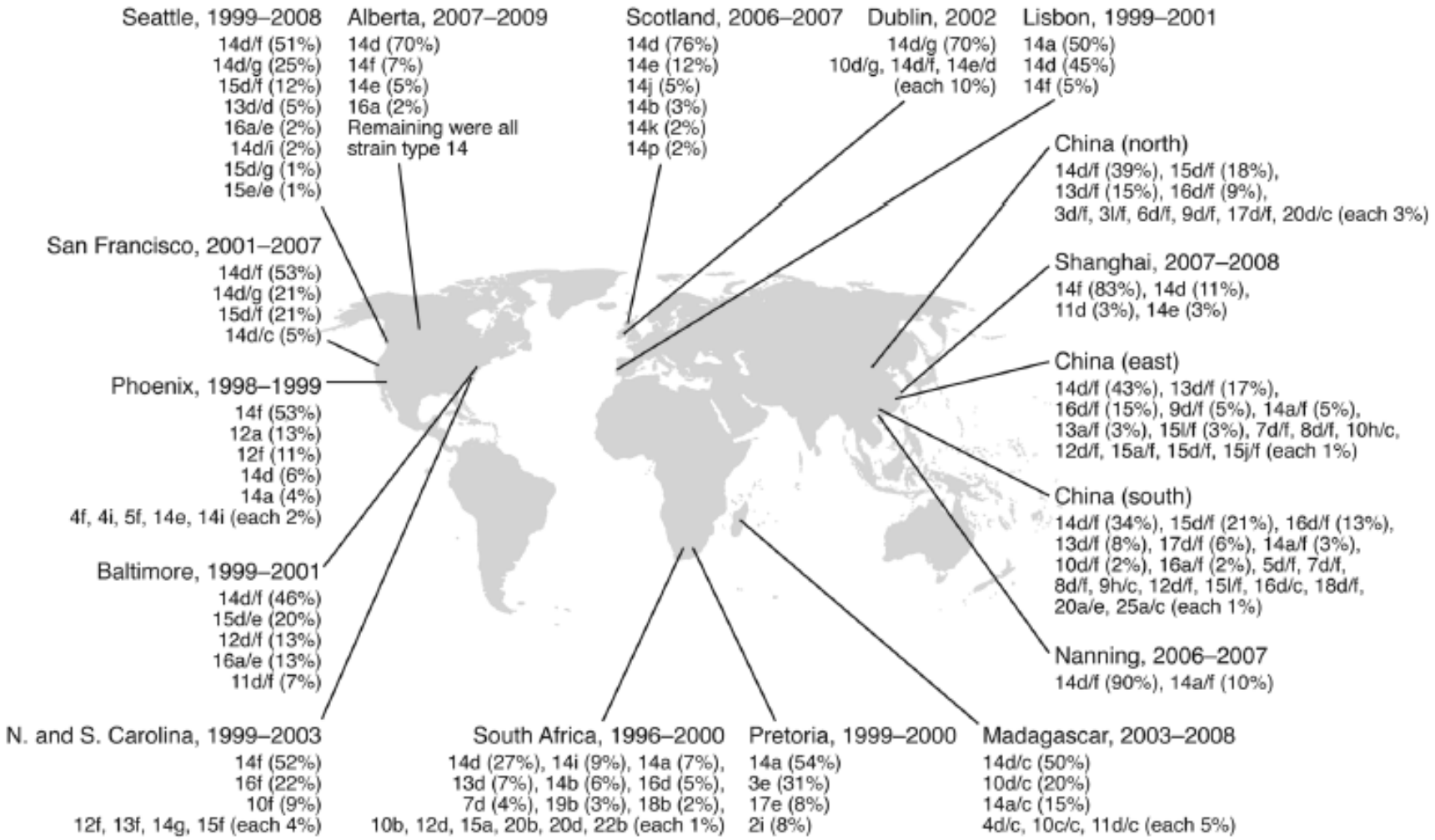

Figure 3

T. pallidum strain types identified throughout the world. The strain type information, years of collection, and the frequency of each strain type from each location are based on information in references 126, 127, 129–131, 133–135, 149, and 150.

J Clin Invest 2011;121:4584

ESTABLISHED IN 1812 SEPTEMBER 9, 2010 VOL. 363 NO. 11

Rapid Molecular Detection of Tuberculosis and Rifampin Resistance

Catharina C. Boehme, M.D., Pamela Nabeta, M.D., Doris Hillemann, Ph.D., Mark P. Nicol, Ph.D., Shubhada Shenai, Ph.D., Fiorella Krapp, M.D., Jenny Allen, B.Tech., Rasim Tahirli, M.D., Robert Blakemore, B.S., Roxana Rustomjee, M.D., Ph.D., Ana Milovic, M.S., Martin Jones, Ph.D., Sean M. O'Brien, Ph.D., David H. Persing, M.D., Ph.D., Sabine Ruesch-Gerdes, M.D., Eduardo Gotuzzo, M.D., Camilla Rodrigues, M.D., David Alland, M.D., and Mark D. Perkins, M.D.

Sensiotivity and Specificity >98% for both TB detection and Susceptibilty

Rapid Turnaround (2 hours)

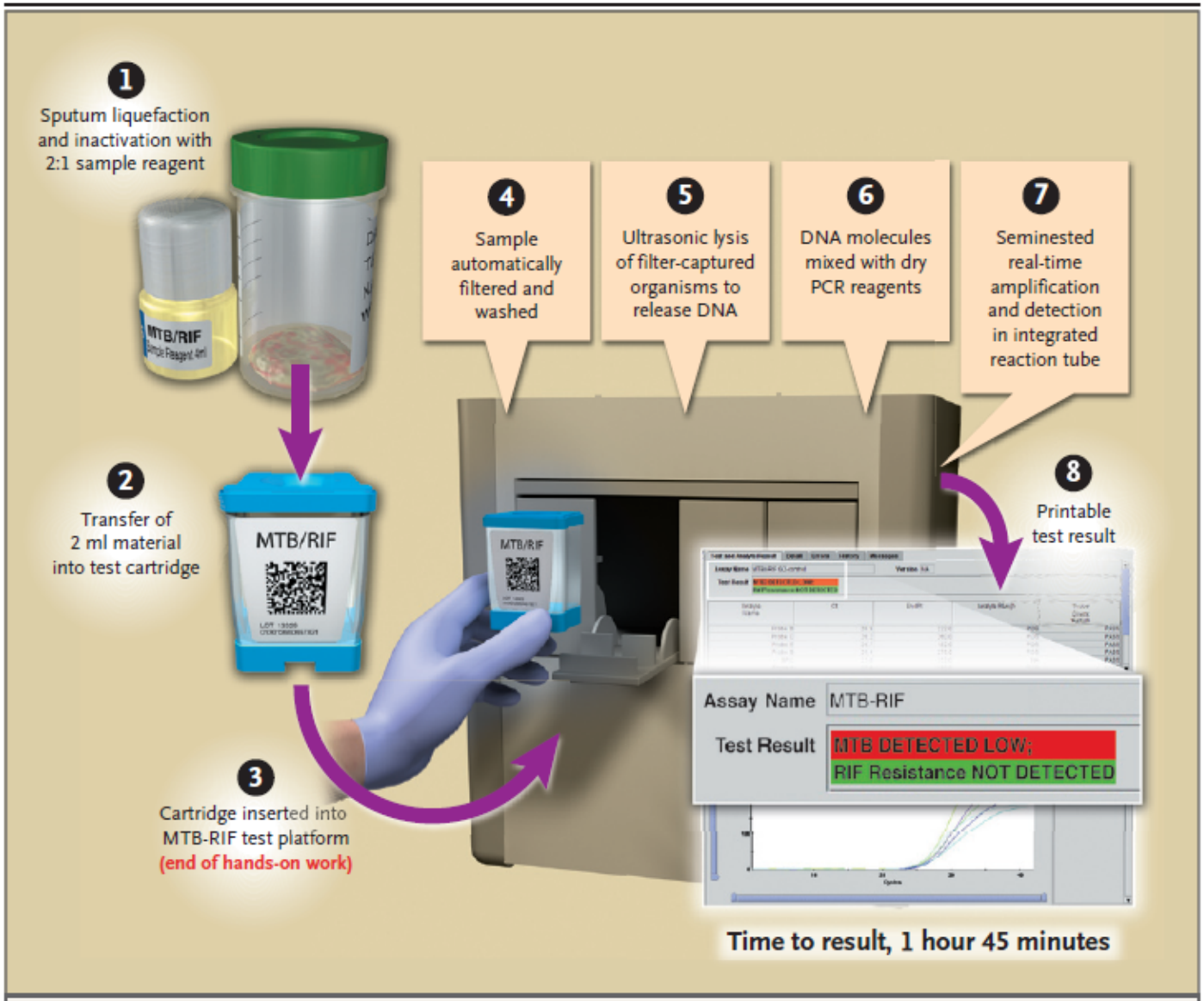

Figure 2. Assay Procedure for the MTB/RIF Test.

Two volumes of sample treatment reagent are added to each volume of sputum. The mixture is shaken, incubated at room temperature for 15 minutes, and shaken again. Next, a sample of 2 to 3 ml is transferred to the test cartridge, which is then loaded into the instrument. All subsequent steps occur automatically. The user is provided with a printable test result, such as "MTB detected; RIF resistance not detected." PCR denotes polymerase chain reaction.

AAC
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High-Level Cefixime- and Ceftriaxone-Resistant *Neisseria gonorrhoeae* in France: Novel *penA* Mosaic Allele in a Successful International Clone Causes Treatment Failure

Magnus Unemo,[a] Daniel Golparian,[a] Robert Nicholas,[b] Makoto Ohnishi,[c] Anne Gallay,[d] and Patrice Sednaoui[e]

WHO Collaborating Centre for Gonorrhoea and Other STIs, Department of Laboratory Medicine, Microbiology, Orebro University Hospital, Orebro, Sweden[a]; Department of Pharmacology, University of North Carolina, Chapel Hill, North Carolina, USA[b]; National Institute of Infectious Diseases, Tokyo, Japan[c]; Institut de Veille Sanitaire, Saint-Maurice, France[d]; and Institut Alfred Fournier, Centre National de Référence des Gonocoques, Paris, France[e]

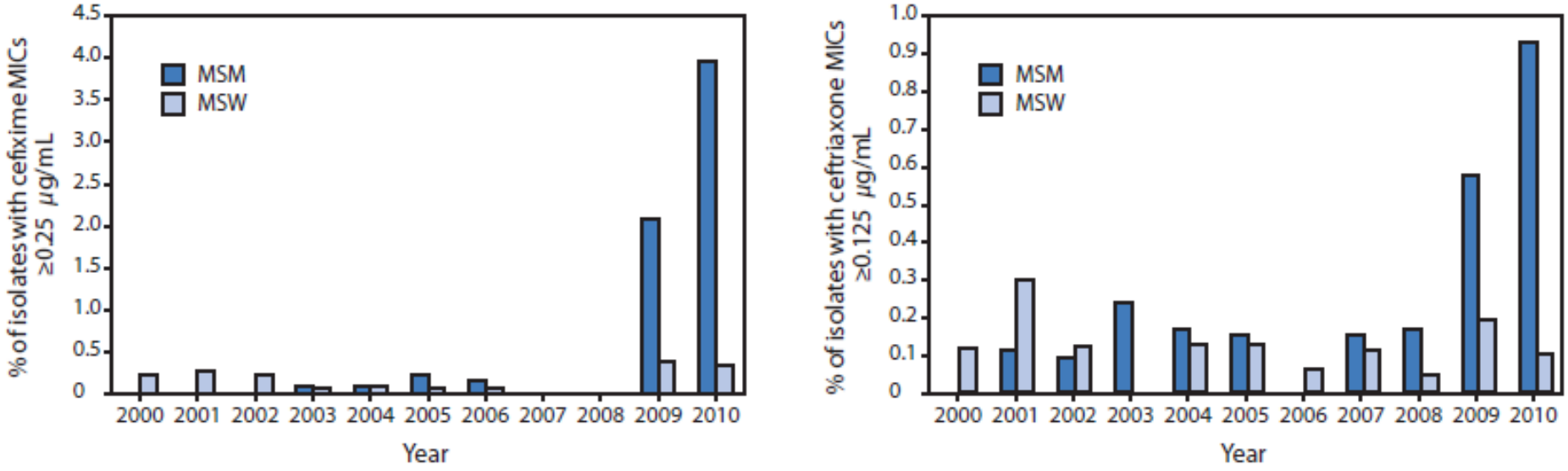
Morbidity and Mortality Weekly Report

Cephalosporin Susceptibility Among *Neisseria gonorrhoeae* Isolates — United States, 2000–2010

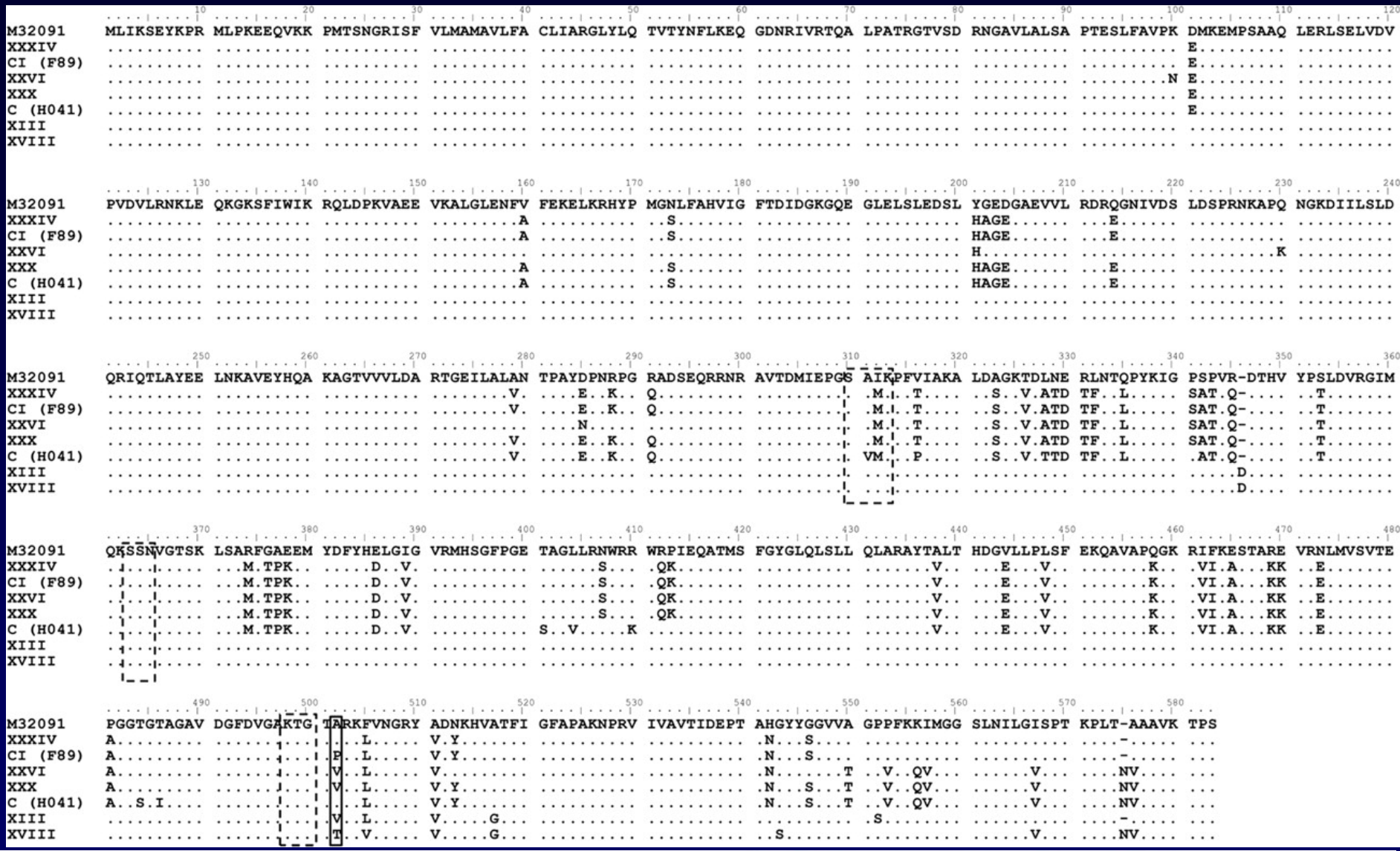
Neisseria gonorrhoeae is a major cause of pelvic inflammatory disease, ectopic pregnancy, and infertility, and it can facilitate human immunodeficiency virus (HIV) transmission (*1*). Emergence of gonococcal resistance to penicillin and tetracycline occurred during the 1970s and became widespread during the early 1980s. More recently, resistance to fluoroquinolones developed. Resistance was documented first in Asia, then emerged in the United States in Hawaii followed by other

2000–2010 were analyzed. Cefixime susceptibilities were not determined during 2007–2008 because cefixime was unavailable in the United States during that period. Decreased antibiotic susceptibility for cefixime or ceftriaxone is defined by the Clinical and Laboratory Standards Institute (CLSI) as MICs ≥0.5 μg/mL; criteria for cefixime and ceftriaxone resistance in *N. gonorrhoeae* have not been defined (*6*). Because few isolates exhibited decreased susceptibility and

FIGURE 2. Percentage of gonorrhea isolates with cefixime MICs ≥0.25 μg/mL and ceftriaxone MICs ≥0.125 μg/mL, by sex of sex partner — Gonococcal Isolate Surveillance Project, United States, 2000–2010



Abbreviations: MICs = minimum inhibitory concentrations; MSM = men who have sex with men; MSW = men who have sex exclusively with women.



Unemo M et al. Antimicrob. Agents Chemother. 2012;56:1273-1280

Antimicrobial Agents and Chemotherapy

L'inquiétante émergence de «superbactéries»

Mots clés : Résitance, Bactérie, Antibiotique, INDE, PAKISTAN, GRANDE-BRETAGNE

Par Tristan Vey

12/08/2010 | Mise à jour : 00:01 Réactions (199)

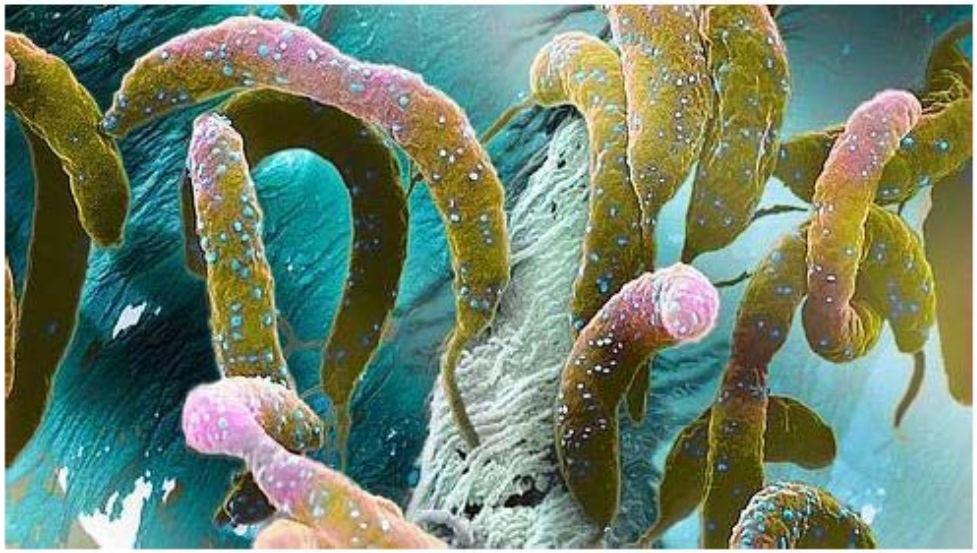


Photo d'une colonie de bactéries d'eau prise en 2008. Crédits photo : ASSOCIATED PRESS

De nombreux cas de patients infectés par une famille de bactéries très résistantes aux antibiotiques usuels ont été découverts en Grande-Bretagne. La propagation rapide et massive de cette souche, isolée pour la première fois en Inde en 2008, inquiète la communauté médicale.

Le Figaro , Paris
Aug 12, 2010

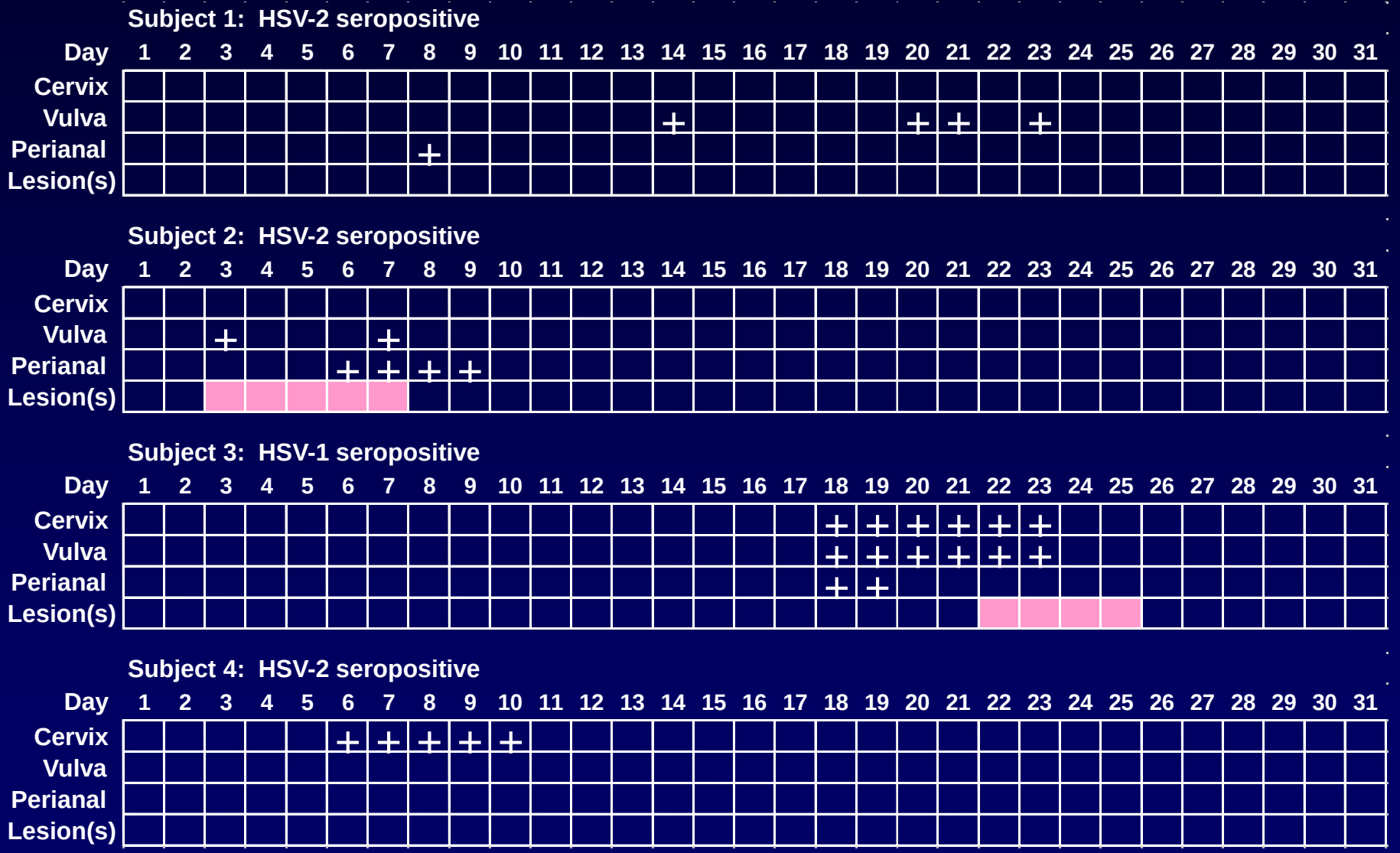
Facilitating Epidemiology

Subclinical Herpes Viral Shedding –Old Model

- >90% of persons with genital HSV-2 shed virus asymptomatically
- Present 1%-10% of asymptomatic days in persons who have recurrent herpes due to HSV-2
- Responsible for most transmission

Wald A *NEJM* 1995.

Viral Shedding Patterns in Women



Wald A *NEJM* 1995.

Genital Shedding of Herpes Simplex Virus Among Symptomatic and Asymptomatic Persons With HSV-2 Infection

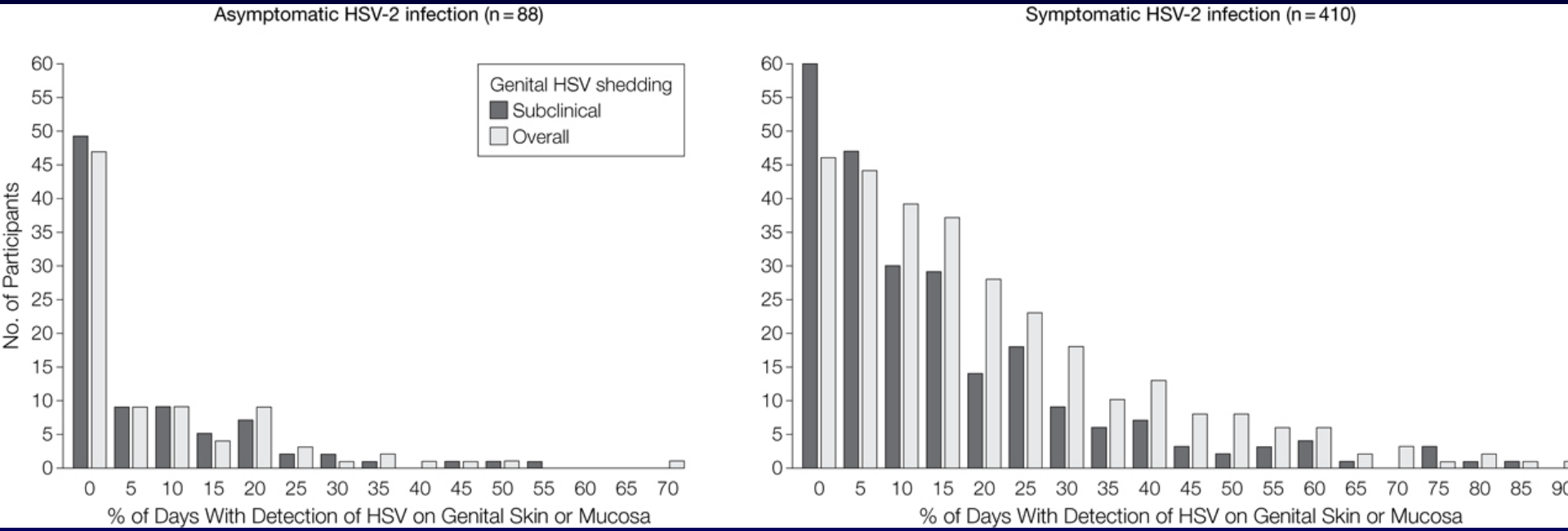
Elizabeth Tronstein, MPH
Christine Johnston, MD, MPH
Meei-Li Huang, PhD
Stacy Selke, MA
Amalia Magaret, PhD
Terri Warren, ANP
Lawrence Corey, MD
Anna Wald, MD, MPH

Context Since herpes simplex virus type 2 (HSV-2) antibody tests have become commercially available, an increasing number of persons have learned that they have genital herpes through serologic testing. The course of natural history of HSV-2 in asymptomatic, seropositive persons is uncertain.

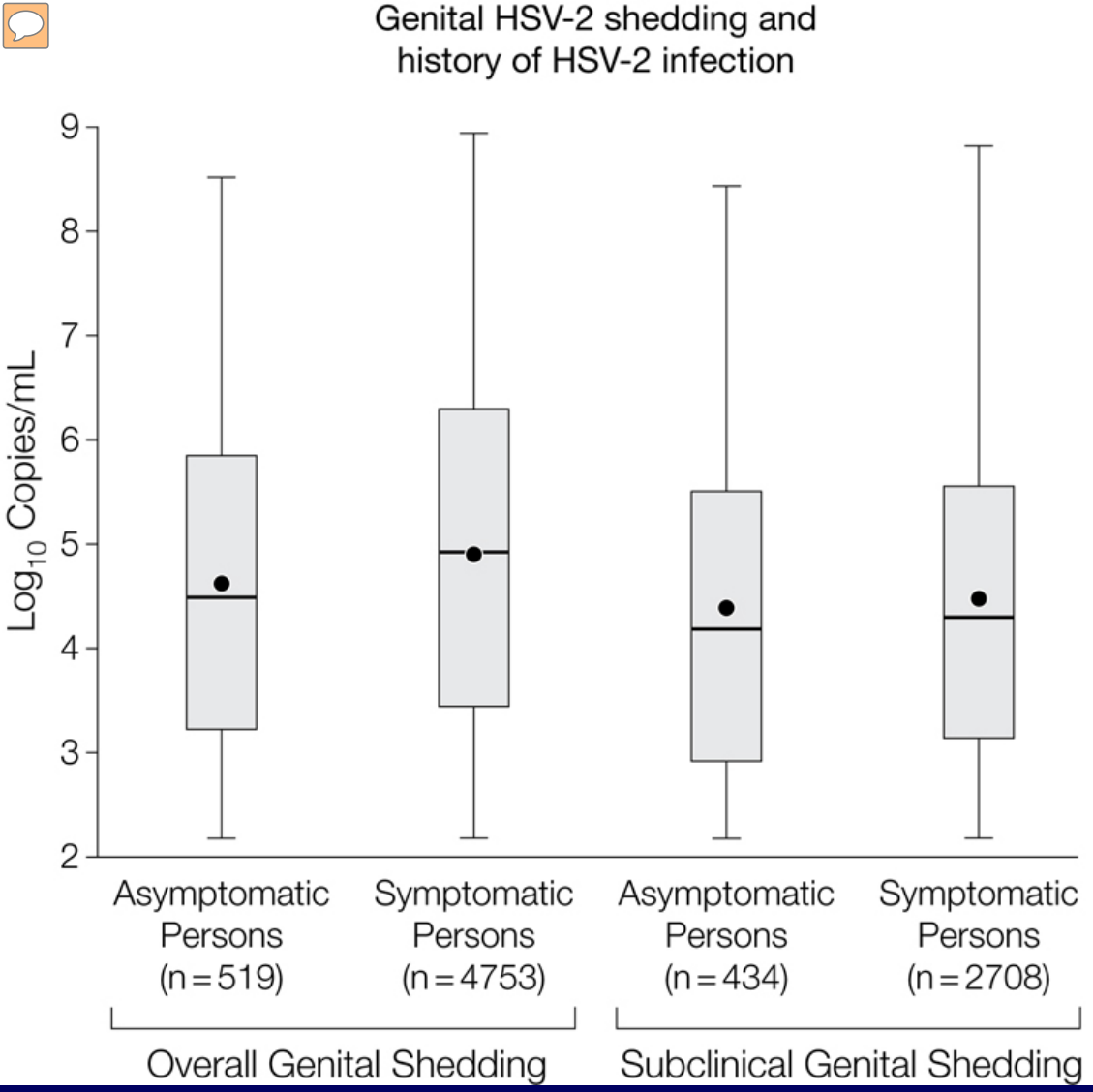
Objective To evaluate the virologic and clinical course of HSV genital shedding among individuals with symptomatic and asymptomatic HSV-2 infection.

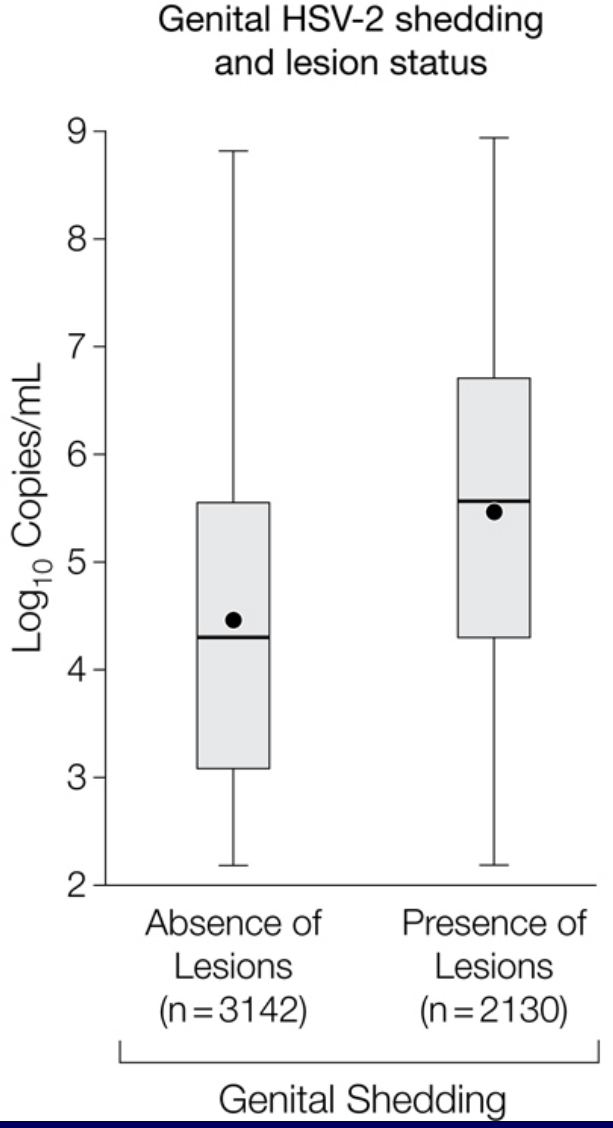
Design, Setting, and Participants Cohort of 498 immunocompetent HSV-2–seropositive persons enrolled in prospective studies of genital HSV shedding at the University of Washington Virology Research Clinic, Seattle, and Westover Heights Clinic, Portland, Oregon, between March 1992 and April 2008. Each participant obtained daily self-collected swabs of genital secretions for at least 30 days.

Figure 1. Distribution of Genital Shedding Rate Among Asymptomatic and Symptomatic Infection Groups



Tronstein, E. et al. JAMA 2011;305:1441-1449





Tronstein, E. et al. JAMA 2011;305:1441-1449

ORIGINAL ARTICLE

Triple-Reassortant Swine Influenza A (H1) in Humans in the United States, 2005–2009

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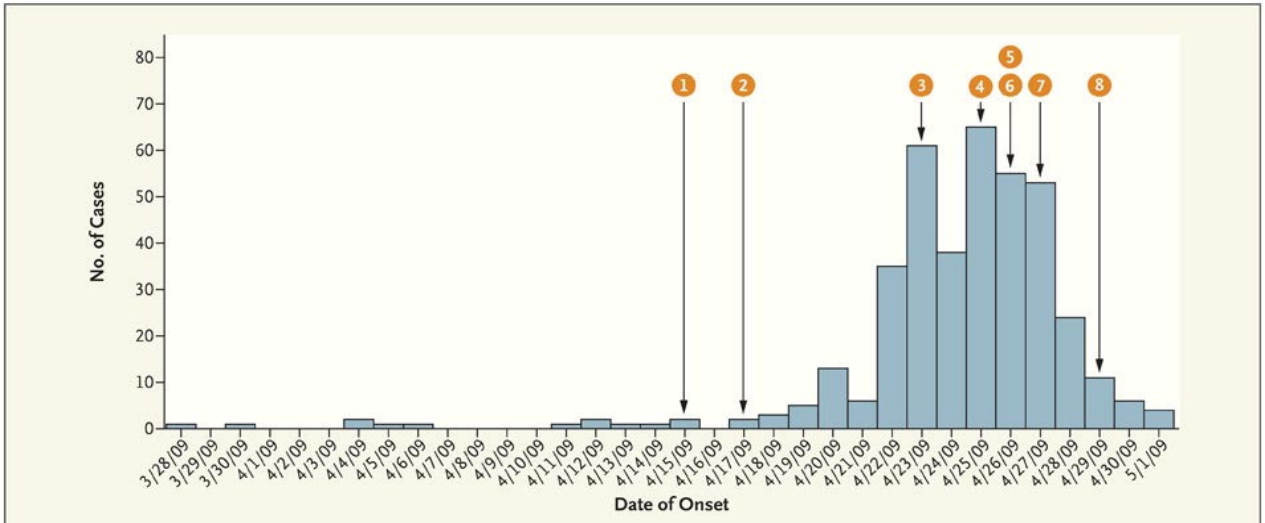
Epidemiologic Curve of Confirmed Cases of Human Infection with Swine-Origin Influenza A (H1N1) Virus with Known Date of Illness Onset in the United States (March 28–May 5, 2009).



1. April 15, 2009 — CDC identifies S-OIV from specimen taken from Patient 1.
2. April 17, 2009 — CDC identifies S-OIV from specimen taken from Patient 2 and the U.S. government notifies World Health Organization (WHO) of Patients 1 and 2 per International Health Regulations.
3. April 23, 2009 — CDC conducts first press briefing related to outbreak.
4. April 25, 2009 — WHO declares public health emergency of international concern.
5. April 26, 2009 — WHO raises global pandemic alert to phase 3, characterized by sporadic cases or small clusters of disease caused by human–animal transmission of an influenza reassortant virus.
6. April 26, 2009 — United States declares public health emergency.
7. April 27, 2009 — WHO raises global pandemic alert to phase 4, characterized by human-to-human transmission of an animal or human–animal influenza reassortant virus able to cause "community-level outbreaks."
8. April 29, 2009 — WHO raises global pandemic alert to phase 5, characterized by human-to-human transmission of the virus in at least two countries in one WHO region.

Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team. N Engl J Med 2009;360:2605-2615.

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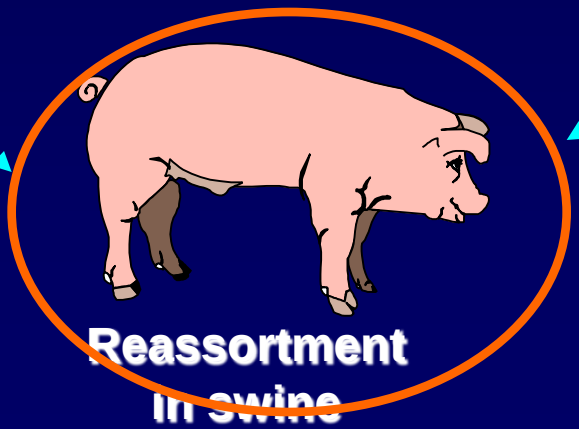
Influenza Transmission to Humans

Avian virus

Avian virus

Reassortment in humans

Avian virus



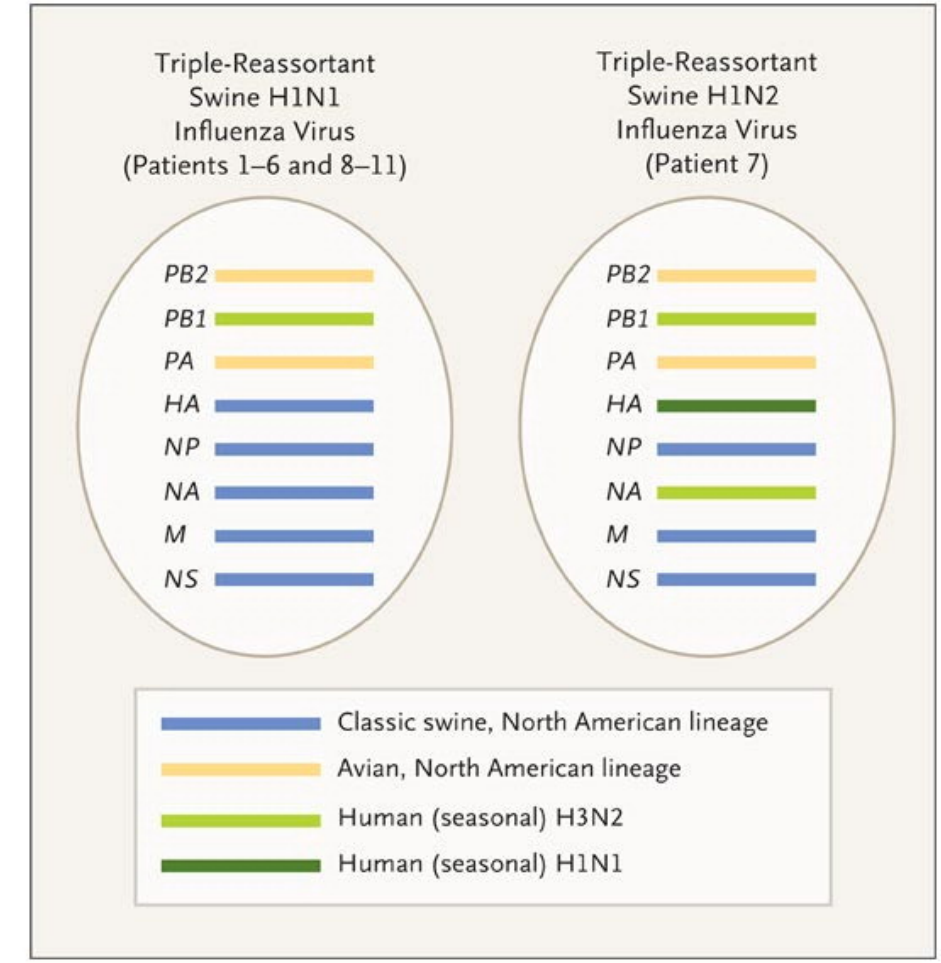
Reassortment in swine

Human virus

CDC

Centers for Disease Control and Prevention

Genetic Components of Triple-Reassortant Swine Influenza A (H1) Viruses Isolated from 11 Patients between December 2005 and February 2009 in the United States.



Shinde V et al. N Engl J Med 2009;360:2616-2625.

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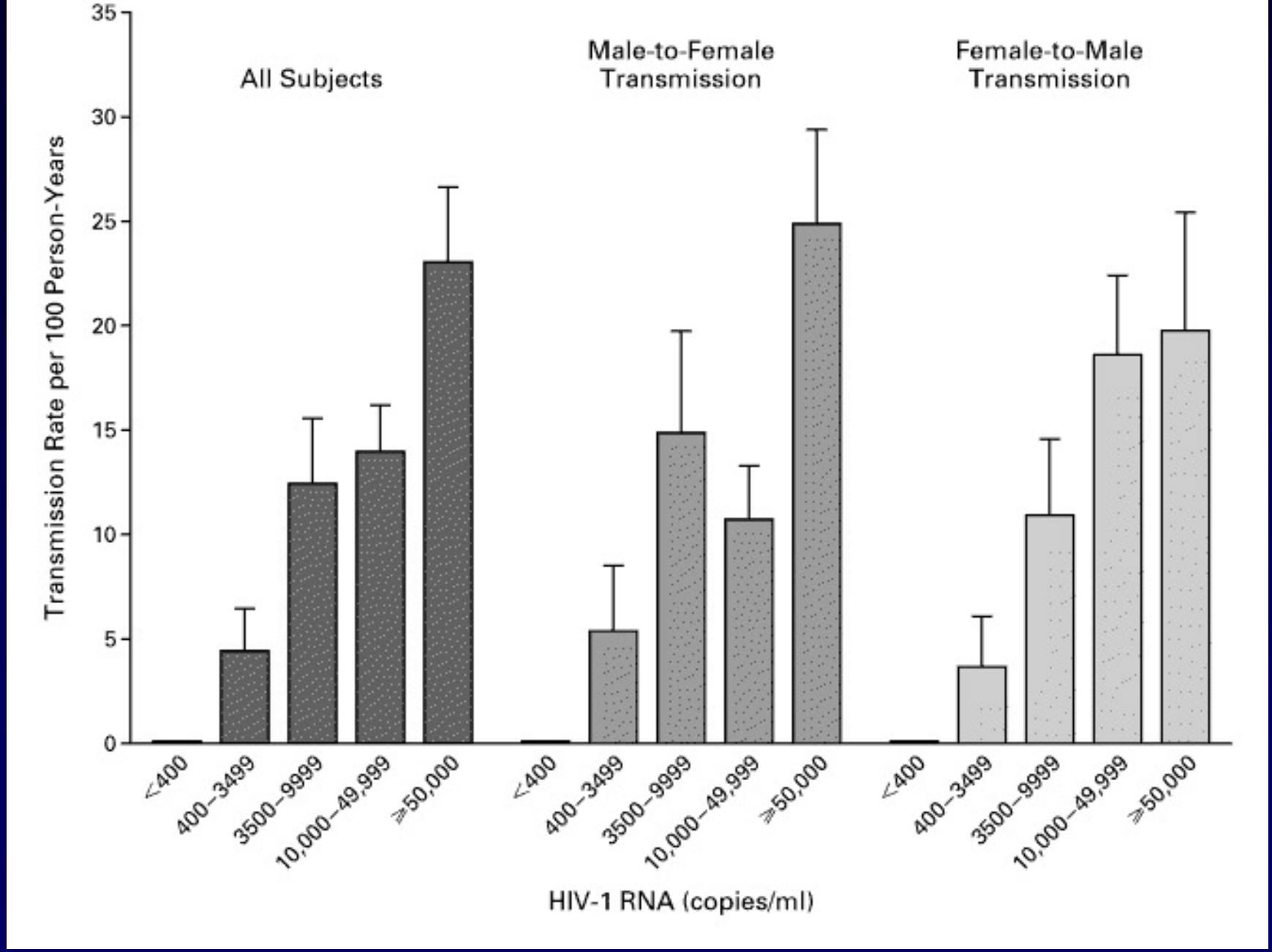
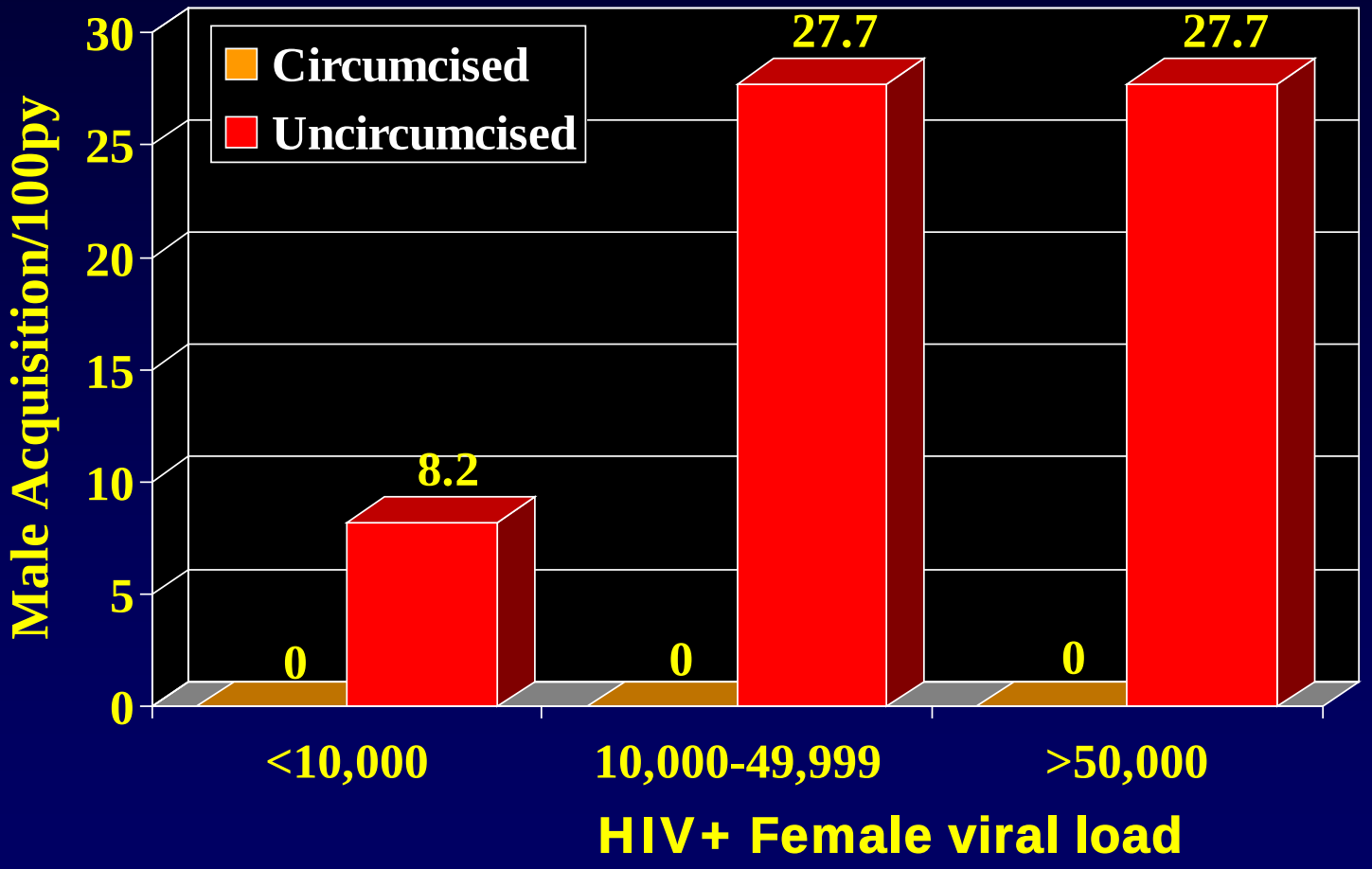
HIV
Where Genomics Guides
Epidemiological Investigation
Understanding Transmission
Interventions
Therapy

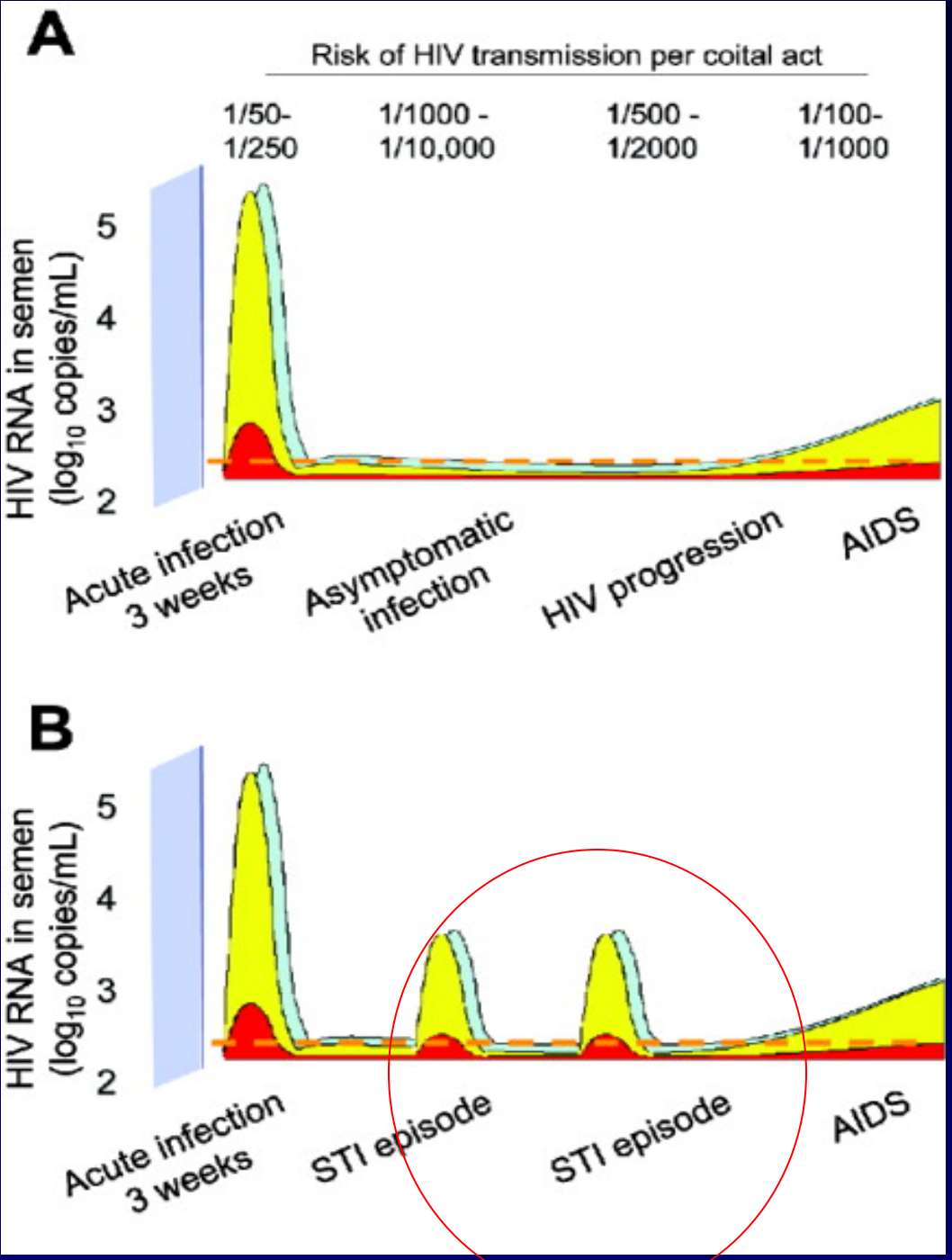


Quinn TC et al. N Engl J Med 2000;342:921-929.

Female-to-male HIV transmission in HIV-discordant couples, by circumcision status in Rakai, Uganda



Gray *et al* *AIDS* 2000



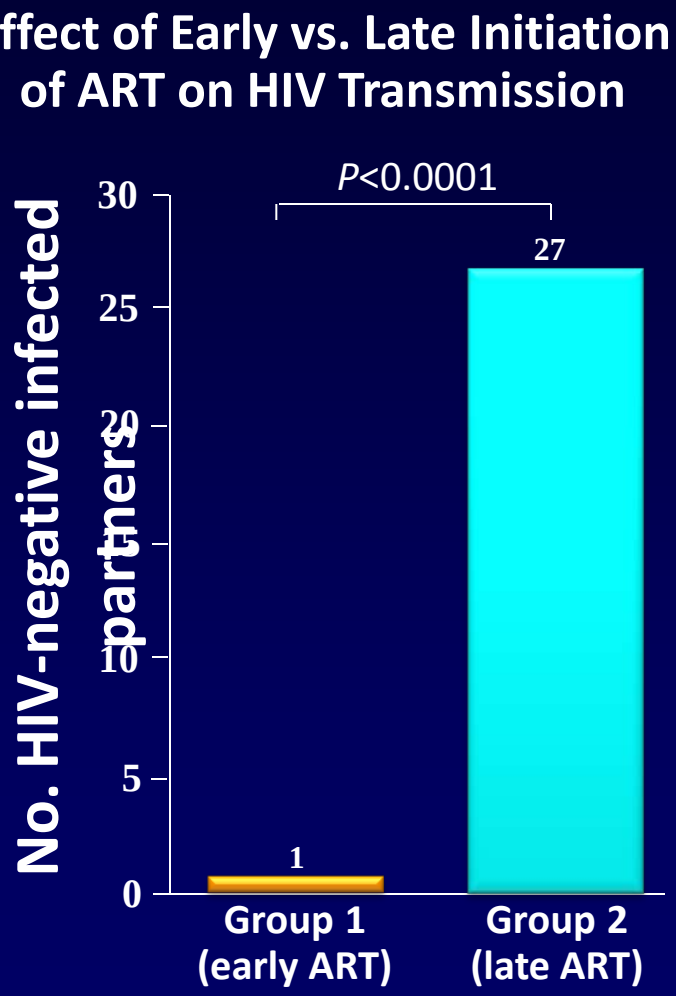
Cohen MS, et al. J Infect Dis. 2005 May 1;191(9):1391-3.

Interventions

- Detecting Acute HIV cases
- Circumcision
- Treatment to Prevent Transmission

ART, Serodiscordant Couples, and HIV Transmission: Study Results

- ART initiation substantially protected HIV-negative sexual partners from acquiring HIV infection
  - **Group 1:** Early treatment group—only 1 partner infected by the HIV-infected participant, with a 96% reduction in risk of HIV infection
  - **Group 2:** Late treatment group—27 partners infected by the HIV-positive participant
- The difference was highly statistically significant (P<0.0001)



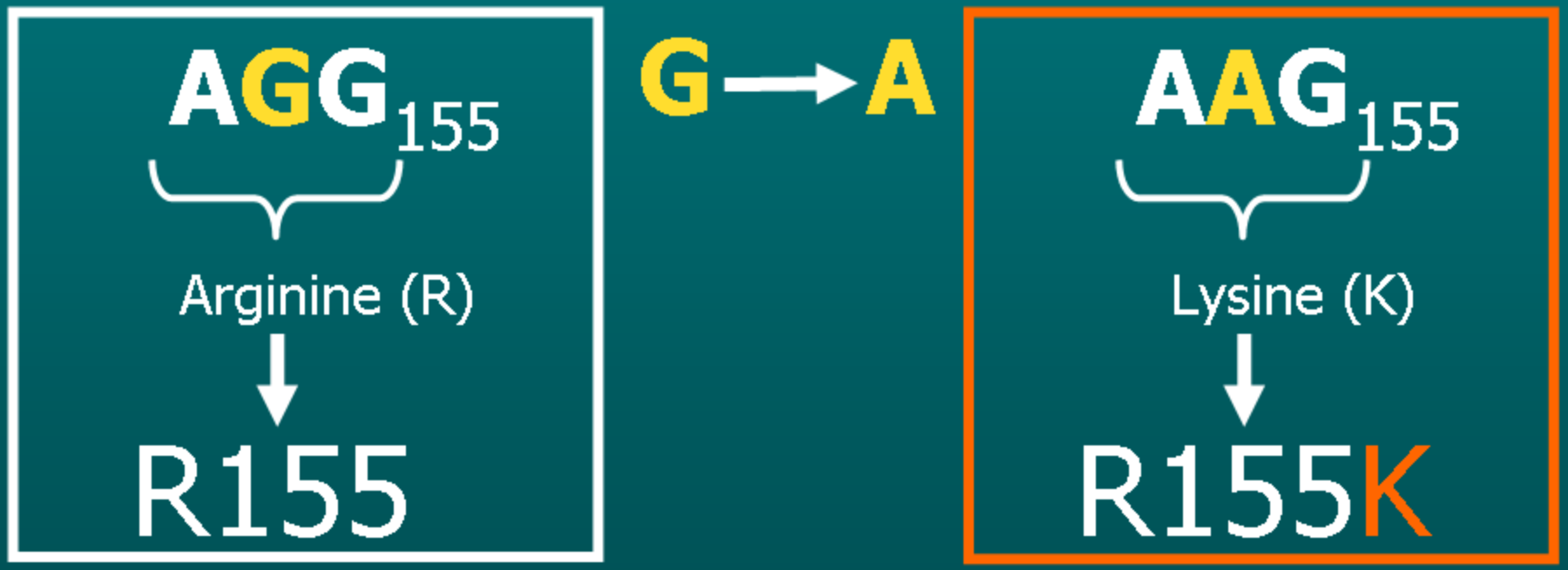
Source: Cohen MS, et al. *N Engl J Med.* 2011;365:493-505.

Hepatitis C

- Genomics Guide Detection and Therapy
- Resistance is genomically defined (similar to HIV)
- Therapy strategies based on genomic testing
- Host susceptibiity genomically defined

Nucleotide Changes, Result In Codon Changes That Can Confer Resistance To A Drug

Example: Codon 155 of the HCV Protease



Consensus "wild type" amino acid

Resistant variant amino acid

Lack Of Cross-Resistance Between Peg-IFN/RBV &/Or A Combination Of Antiviral Agents May Provide An Opportunity For Elimination Of Resistant Variants

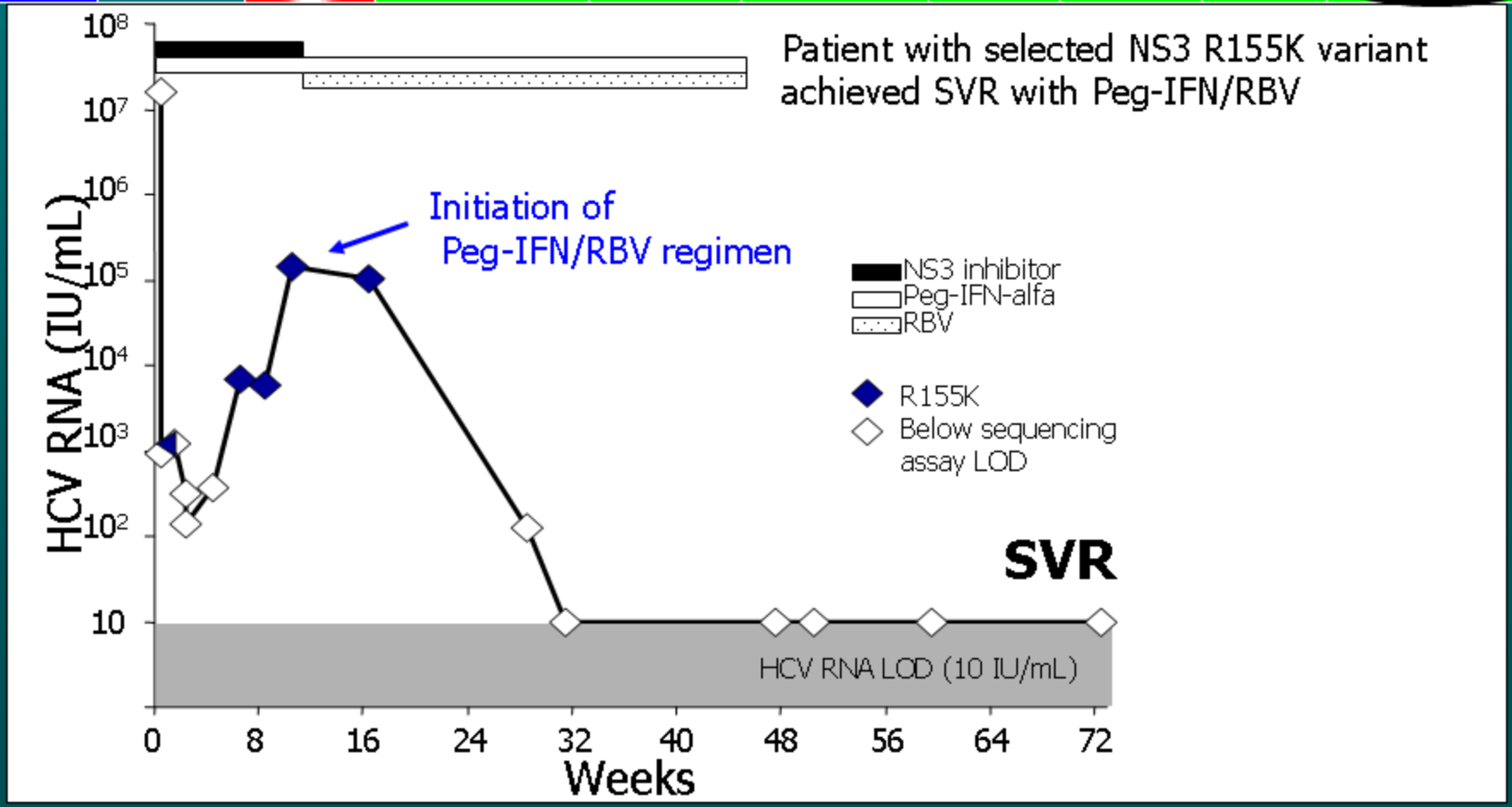
Target	Variant	NS3 Linear	NS3 Macrocyclic	NS5A inhibitor	NS5B nucleoside	NS5B Palm	NS5B Thumb	NS5B Finger	IFN	RBV
NS3 Protease	V36M	R	S	S	S	S	S	S	S	S
	T54A	R	S	S	S	S	S	S	S	S
	R155K	R	R	S	S	S	S	S	S	S
	A156T	R	R	S	S	S	S	S	S	S
	D168V	S	R	S	S	S	S	S	S	S
NS5A	L28V	S	S	R	S	S	S	S	S	S
	Y93H	S	S	R	S	S	S	S	S	S
NS5B	S282T	S	S	S	R	S	S	S	S	S
	C316Y	S	S	S	S	R	S	S	S	S
	M414T	S	S	S	S	R	S	S	S	S
	R422K	S	S	S	S	S	R	S	S	S
	M423T	S	S	S	S	S	R	S	S	S
	P495S	S	S	S	S	S	S	R	S	S

S = Susceptible (< 4 fold shift in HCV replicon EC50)

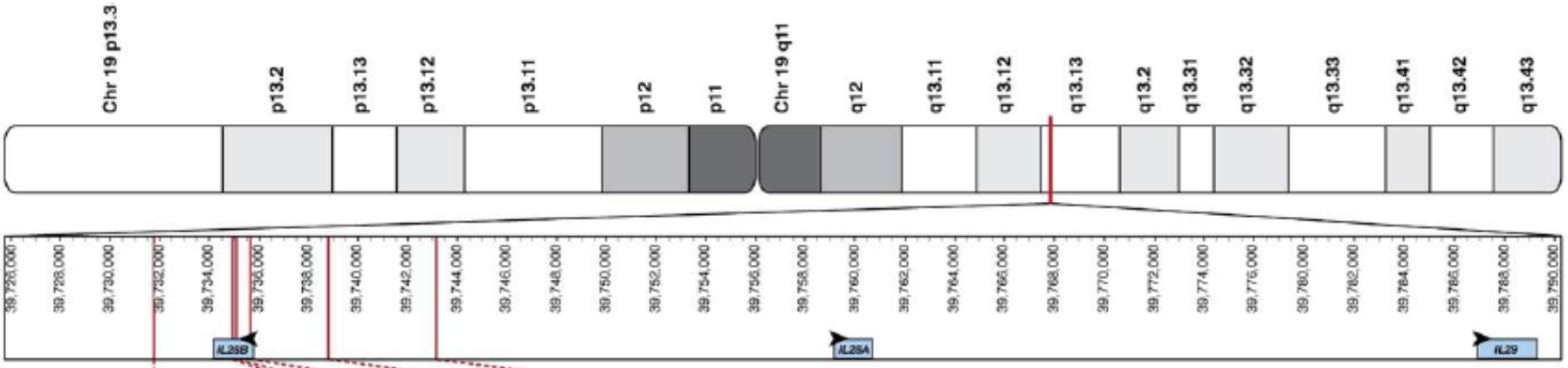
R = Resistant (>4 fold increase in EC50)

Patients With NS3 Inhibitor-Resistant Variants Can Respond To Peg-IFN/RBV

Target	Variant	NS3 Linear	NS3 Macrocyclic	NS5A inhibitor	NS5B nucleoside	NS5B Palm	NS5B Thumb	NS5B Finger	IFN	RBV
NS3	R155K	R	S	S	S	S	S	S	S	S



Unpublished data, example from TVR PROVE2 study



	Study population ancestry		GWAS Chip	rs12980275	rs11881222	rs8103142 (K70R)	rs28416813	rs12979860	rs8099917
Ge et al HCV GT 1	European African	GWAS	Illumina Human610	●	-	●	-	●	●
	Asian Hispanic	Genetic mapping				●	●		
Suppiah et al HCV GT 1	European	GWAS	Illumina Infinium HumanHap300/CNV370	-	-	-/●	-	-/●	●
		Genetic mapping		●		●			●
Tanaka et al HCV GT 1	Asian	GWAS	Affymetrix Human SNP Array 6.0	●	-	-	-	-	●
		Genetic mapping		●	●	●	●		●
Rauch et al HCV GT 1/2/3/4	European	GWAS	Illumina Human1M-Duo/ HumanHap550/Human610W	●	-	-/●	-	-/●	●
		Genetic mapping				●	●		

● SNP found to be associated with SVR
● SNP not found to be associated with SVR
- Chip did not contain SNP
-/● SNP was not found to be associated with SVR in the overall analysis, however ≥ 1 GWAS platform did not have probes to test for this SNP

Figure 1. SNPs in IFN-λ gene cluster associated with HCV control. The IFN-λ gene cluster is shown in the *top panel*, indicating its position on chromosome 19. In the *second panel*, the positions of the relevant SNPs corresponding to the text and published data are indicated in relation to the IFN-λ gene cluster.[10,12–14] *IL28B* is upstream and in reverse orientation compared with *IL28A*. The *third panel* depicts the genomic structure of *IL28B*, including its 5 exons, intervening introns, and flanking putative regulatory regions. *Vertical lines* denote the position of individual SNPs that are

HCV.[16,17] These results indicate the involvement of the ... This distribution of alleles could account for the high

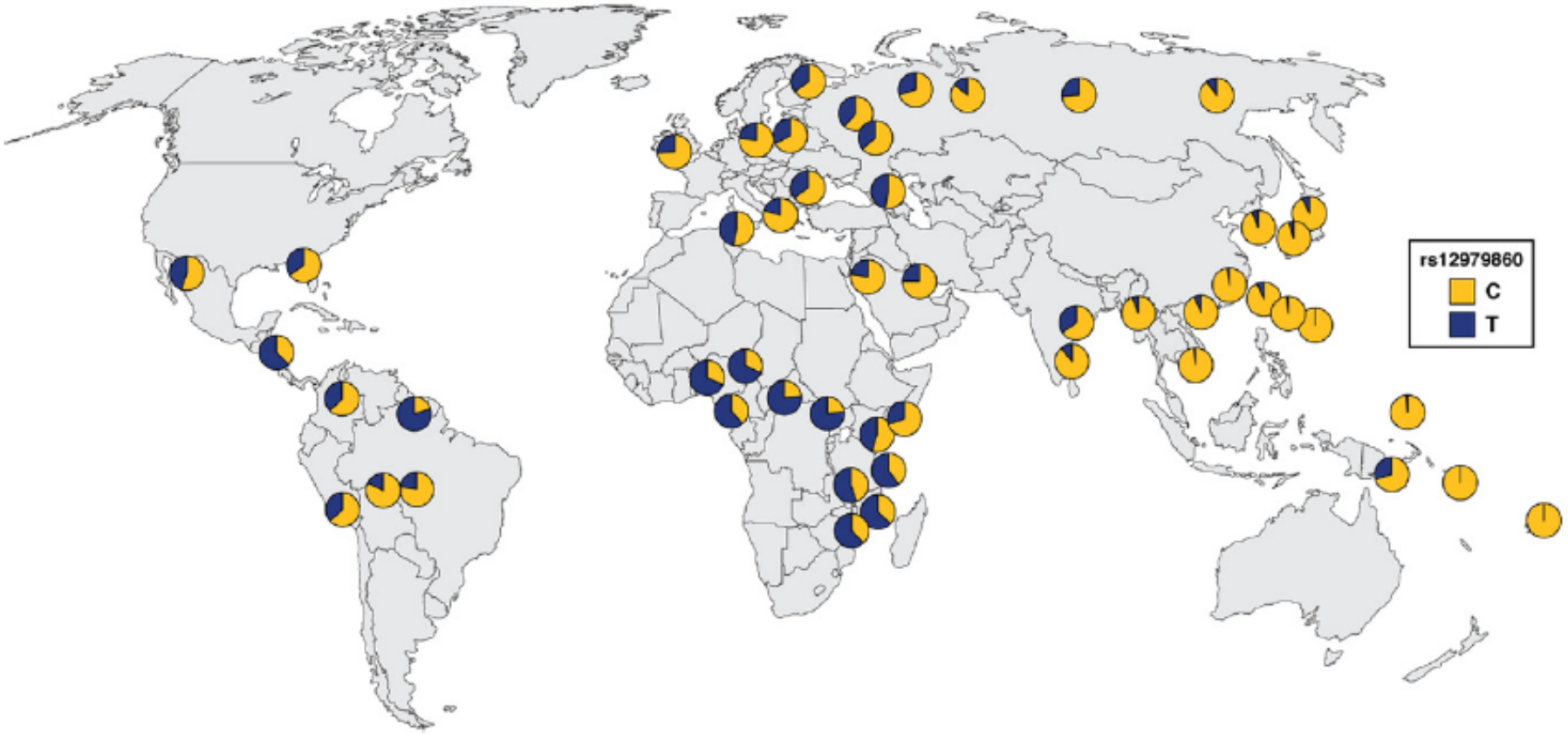
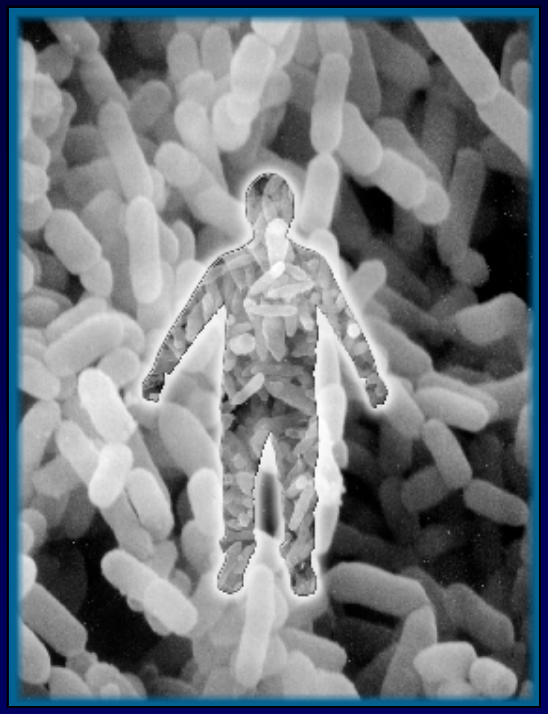


Figure 4. Allele frequencies of the SNP rs12979860 among different ethnic populations. Thomas et al genotyped the rs12979860 SNP in 2371 subjects from 51 distinct populations. The frequency map shows the proportional prevalence of the C (associated with HCV clearance) and T (associated with persistence) alleles. People in East and Southeast Asia have the lowest frequency of the alleles associated with HCV persistence, people in Europe have intermediate incidence, and people in sub-Saharan Africa have the highest frequency. Adapted with permission from Macmillan Publishers Ltd: Nature, Thomas et al, © 2009.[15]

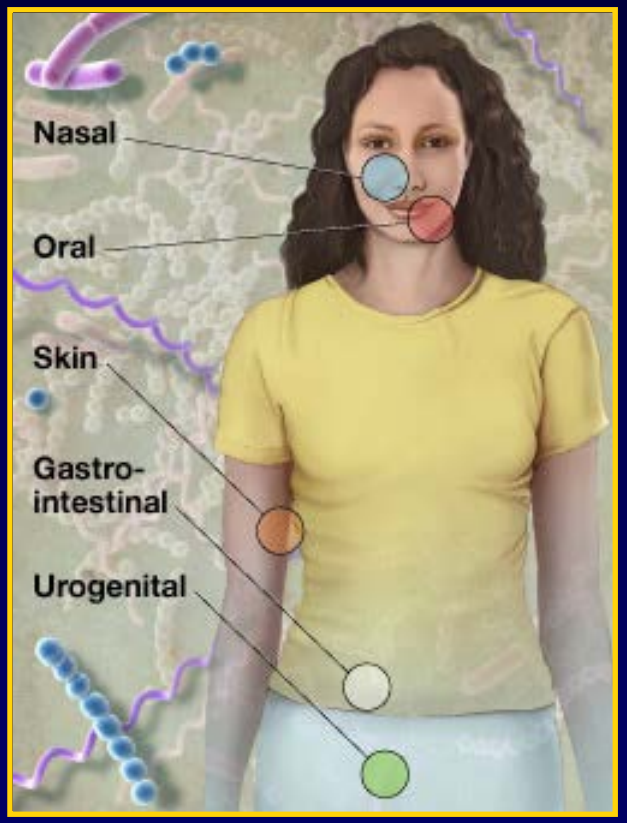
The Human Microbiota

- Our adult bodies harbor ~10 times more microbial cells than human cells – a significant number of these species have not been successfully grown in culture
- The “human genome” is an amalgam of human genes and the genes of our microbial partners
- Our microbial partners carry out many metabolic reactions that are not encoded in the human genome and are necessary for health
- A number of studies have suggested that various disease states are associated with microbial community disturbance
- Without understanding the interactions between our human and microbial genomes, it is impossible to obtain a complete picture of our biology



The NIH Human Microbiome Project

$



- Determining whether individuals share a core human microbiome
- Understanding whether changes in the human microbiome can be correlated with changes in human health
- Developing the new technological and bioinformatic tools needed to support these goals

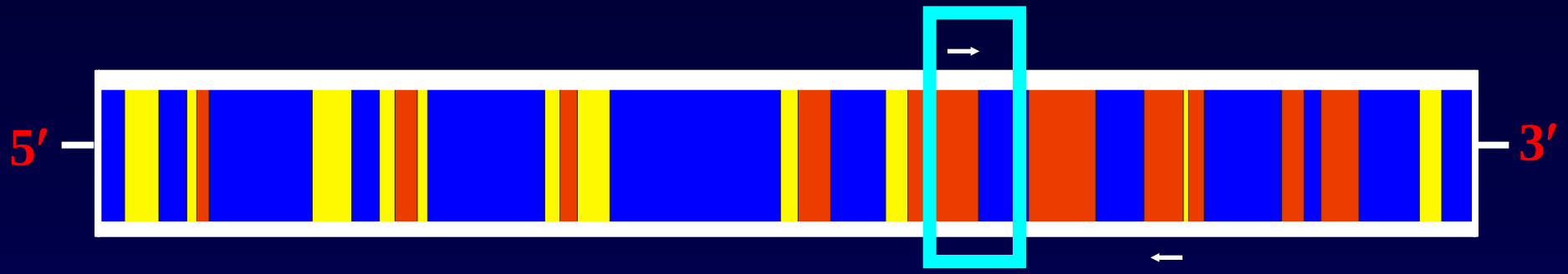
NIH Human Microbiome Project is only one of several international efforts

Critical questions

- How do we acquire and maintain our microbial communities?
- How resilient is our microbiome in response to stress?
- Can we use this information to devise ways to intentionally manipulate our microbiome (probiotics, immunization) to promote health and/or to prevent or treat various diseases?
- How do genotype, environmental exposures, and physiological status affect microbiome composition?

KEY CONCEPT—MICROBIOLOGICAL COMMUNITY

16S rRNA Gene

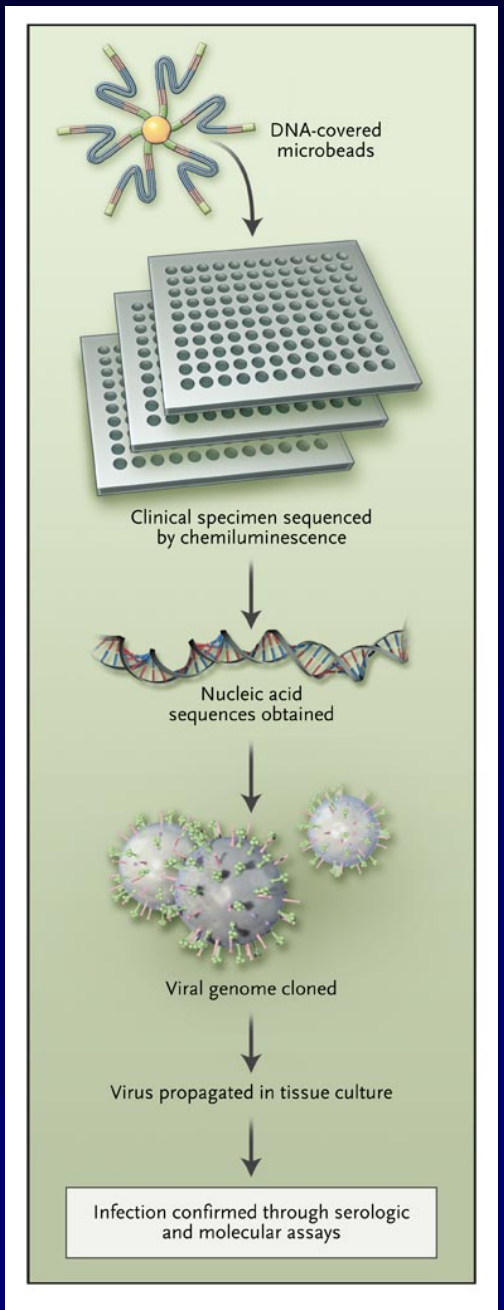


Highly Conserved Regions (99% Identity)

Conserved Regions (93-95% Identity)

Hypervariable Regions

Use of High-Throughput DNA Pyrosequecing for Pathogen Discovery



Whitley R. N Engl J Med 2008;358:988-989

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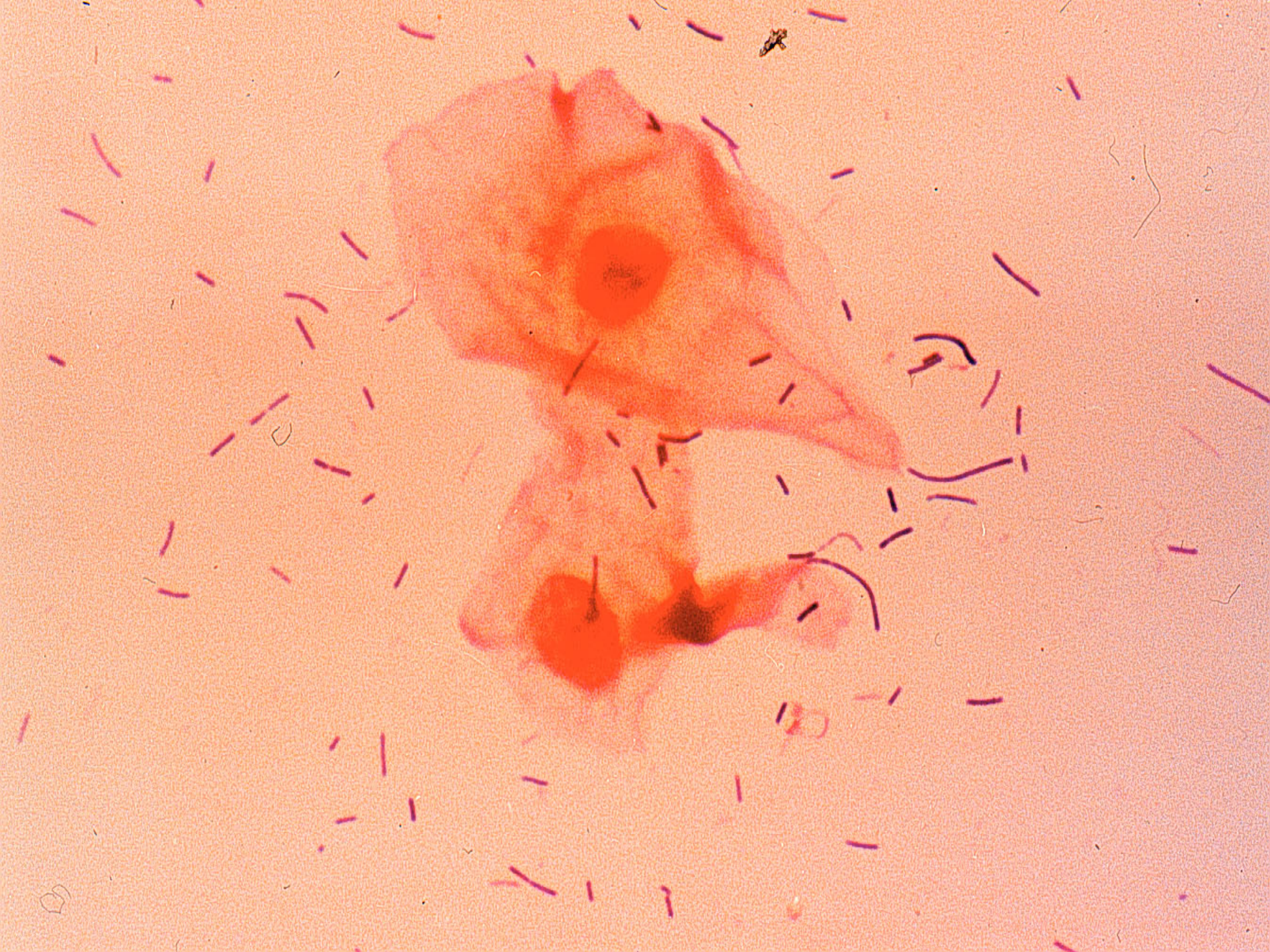
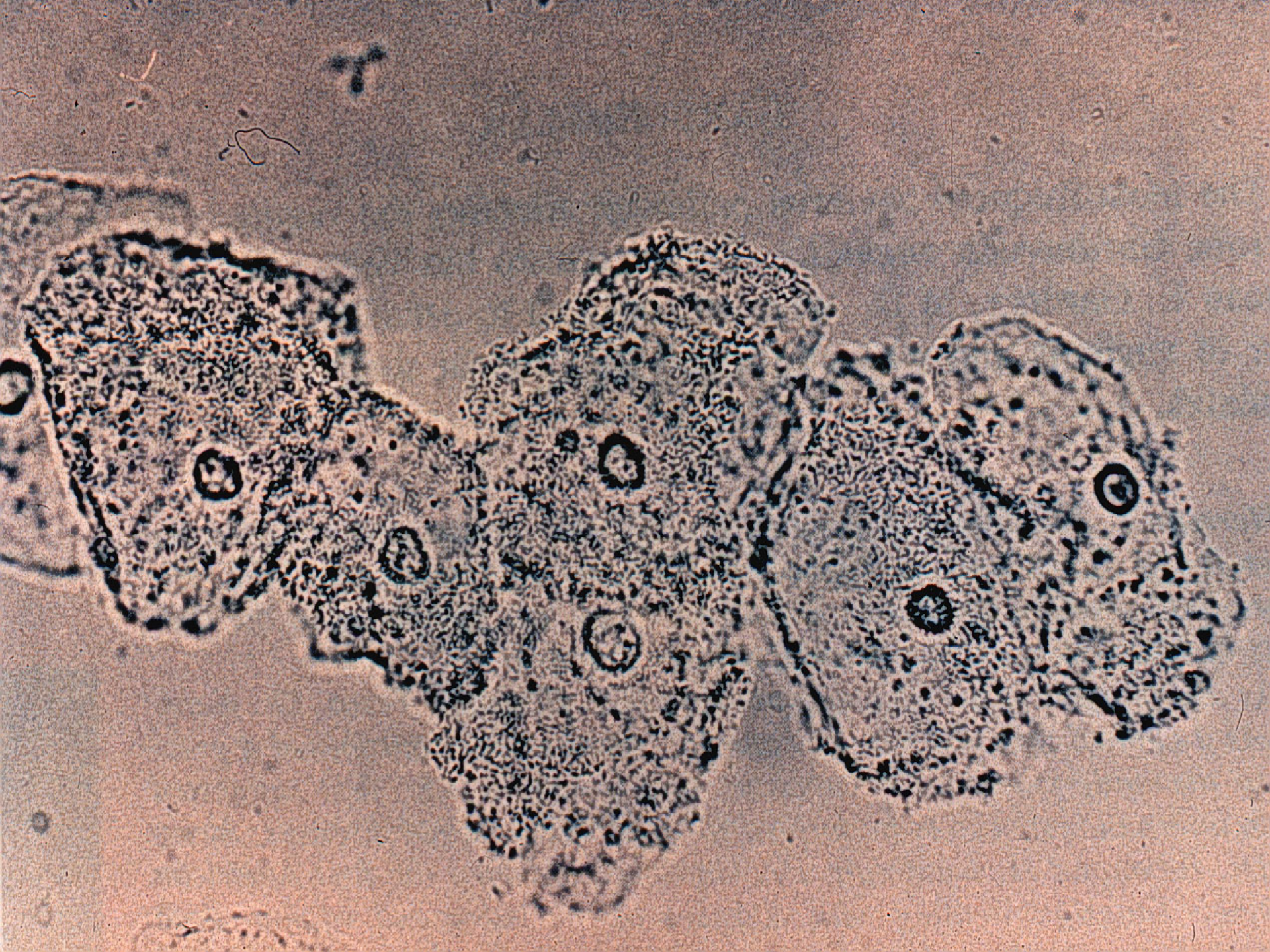
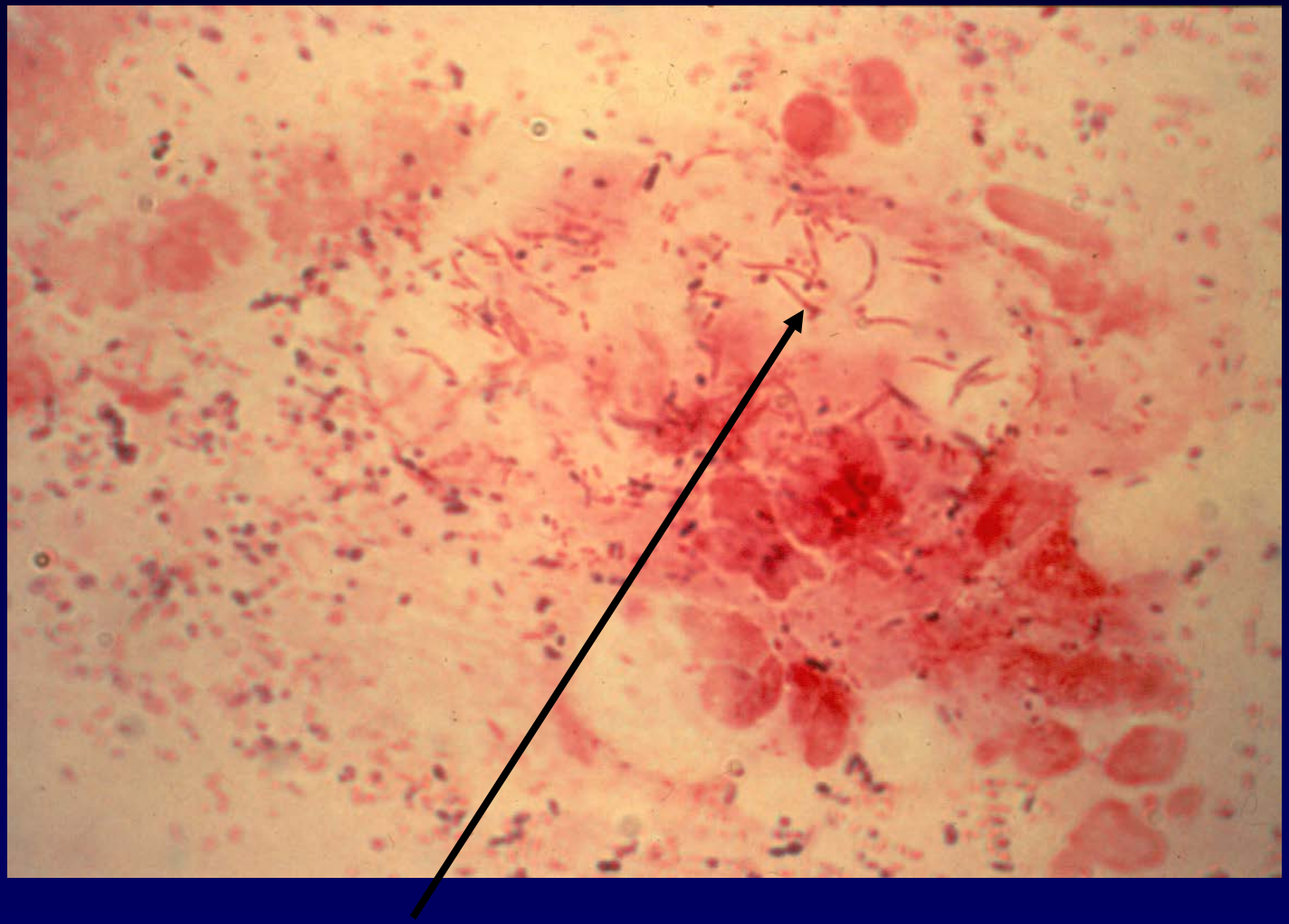
Bacterial Vaginosis

- Ecologic disturbance of vaginal flora
- Not an STD
- Dx based on clinical criteria or gram stain

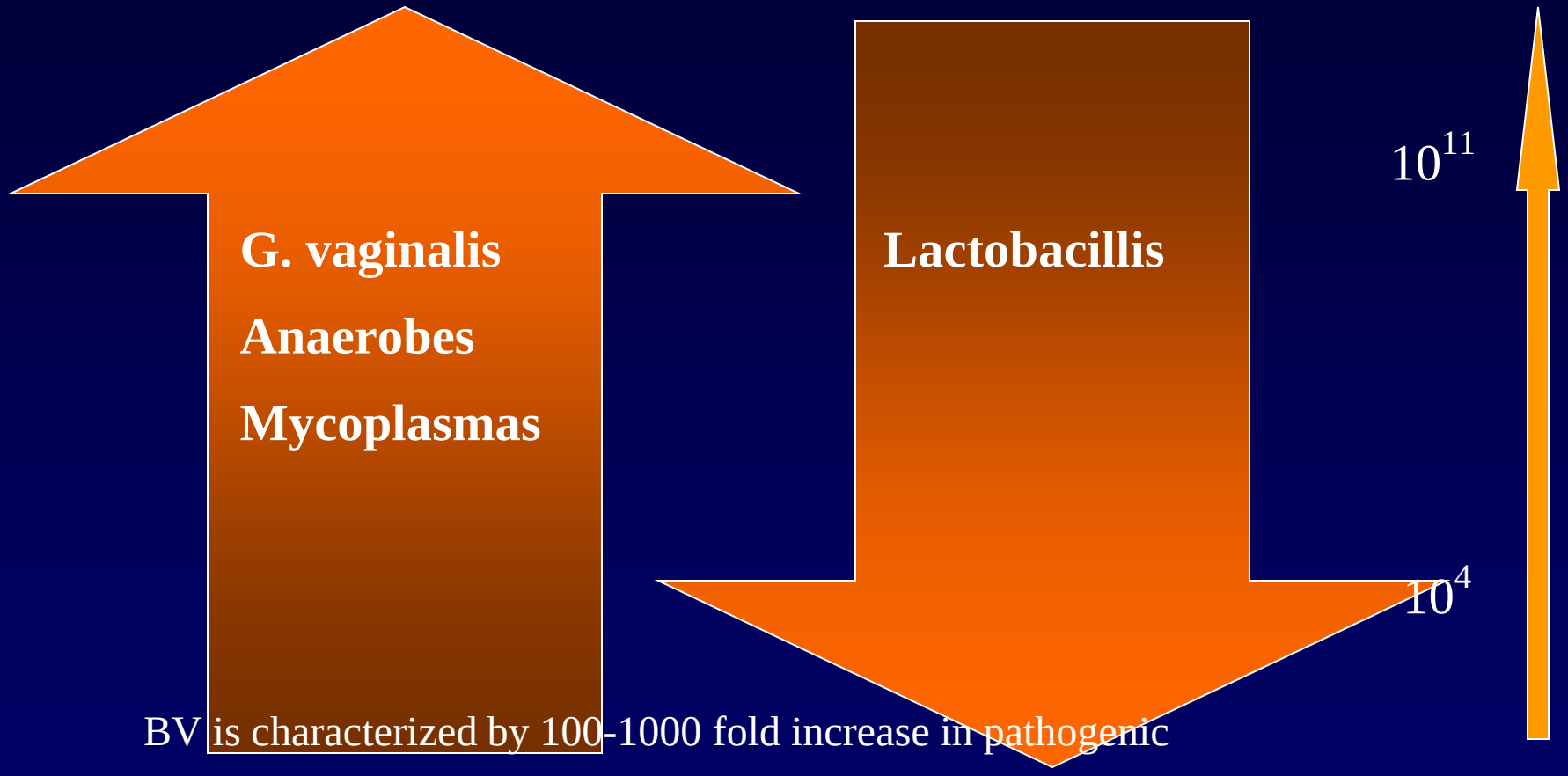




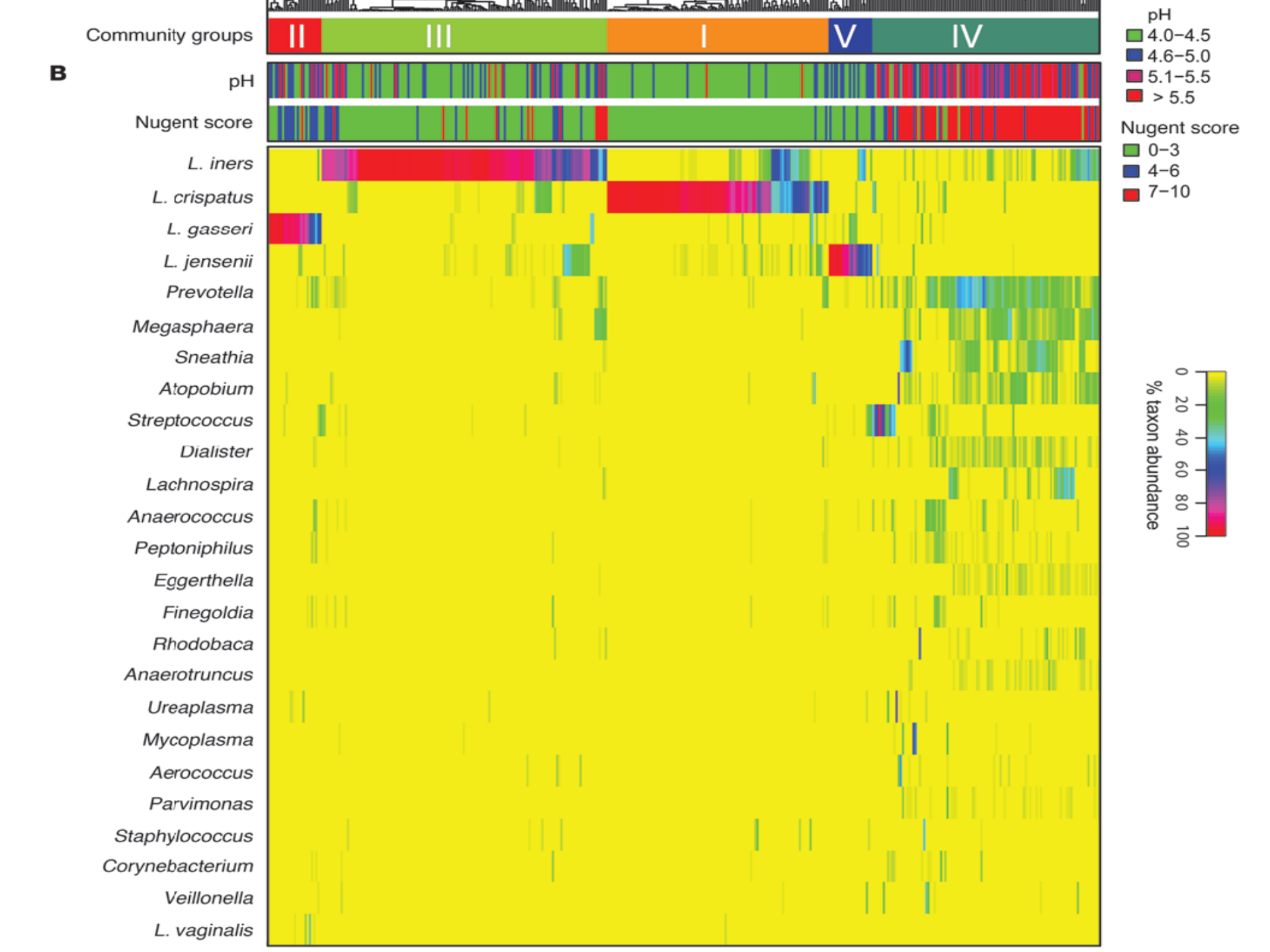


Vaginal Gram stain with fusobacillary forms

Microbial Shifts Occurring in BV



BV is characterized by 100-1000 fold increase in pathogenic bacteria. Lactobacilli concentrations decrease substantially


B
Community groups
II
III
I
V
IV
pH
Nugent score
L. iners
L. crispatus
L. gasseri
L. jensenii
Prevotella
Megasphaera
Sneathia
Atopobium
Streptococcus
Dialister
Lachnospira
Anaerococcus
Peptoniphilus
Eggerthella
Finegoldia
Rhodobaca
Anaerotruncus
Ureaplasma
Mycoplasma
Aerococcus
Parvimonas
Staphylococcus
Corynebacterium
Veillonella
L. vaginalis
pH
4.0–4.5
4.6–5.0
5.1–5.5
> 5.5
Nugent score
0–3
4–6
7–10
% taxon abundance
0
20
40
60
80
100

Chronic Wounds Impact

- Wounds have direct medical cost impact ($25 Billion)
- Wounds have substantial indirect cost benefit
  - Self image
  - Economic impact on family members
  - Disability
- There is little evidence basis for care that is provided
- Most research is dressing directed
- Little pathogenesis research is being done—OPPORTUNITY!!

Infection in Chronic Wounds— Current state of art

Definitions of infection in Chronic Wounds

Colonization versus Infection

Clinical— erythema, advancing border, purulent drainage, “you know it when you see it”

– Quantitative Culture– >100,000 CFU/Gm of tissue


cm
WOUNDMATRIX™ Measuring Guide
www.woundmatr
Discard after single use
Date 7/27/05
378997
Patient Initials/Number TAE
LLE 5days Post 6mFT

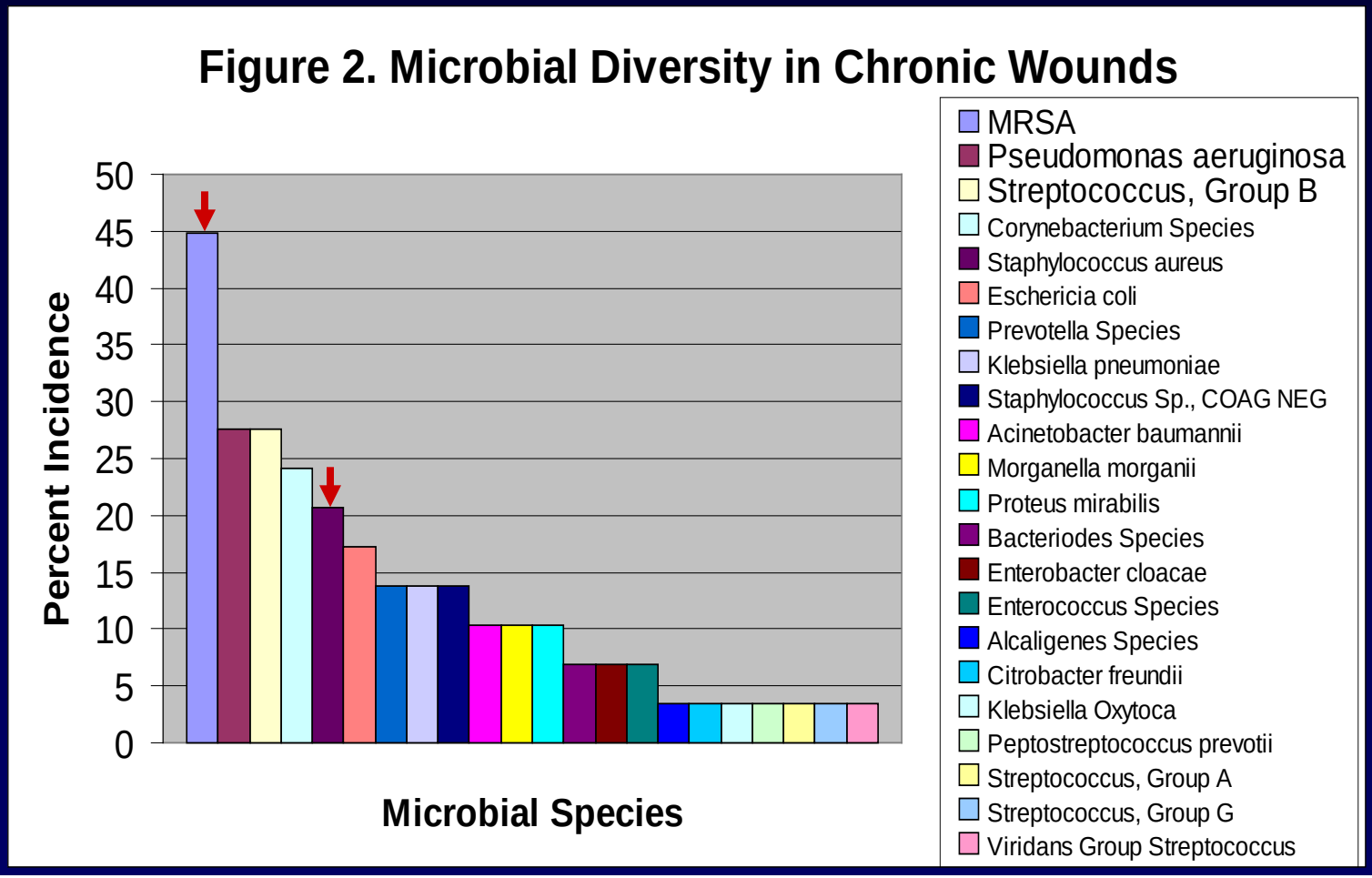
Problems with Cultures

- Cultures take 24-48 hours to process
- Quantitative Cultures take longer
- Cultures are prone to overgrowth
- Are there molecular approaches?

JHU Wound Research Program objectives

- Describe prevalence of bacterial species in chronic wounds in a tertiary wound care clinic.
- Assess microbial burden by: qualitative culture, quantitative culture, and bacterial DNA (real-time polymerase chain reaction (RT-PCR)
- Compare microbial populations found at 2 different locations within a single chronic wound (standardize methodology).
- Preliminary investigation of DNA footprints of microbes in wound tissue

Prevalence of bacterial species by quant culture

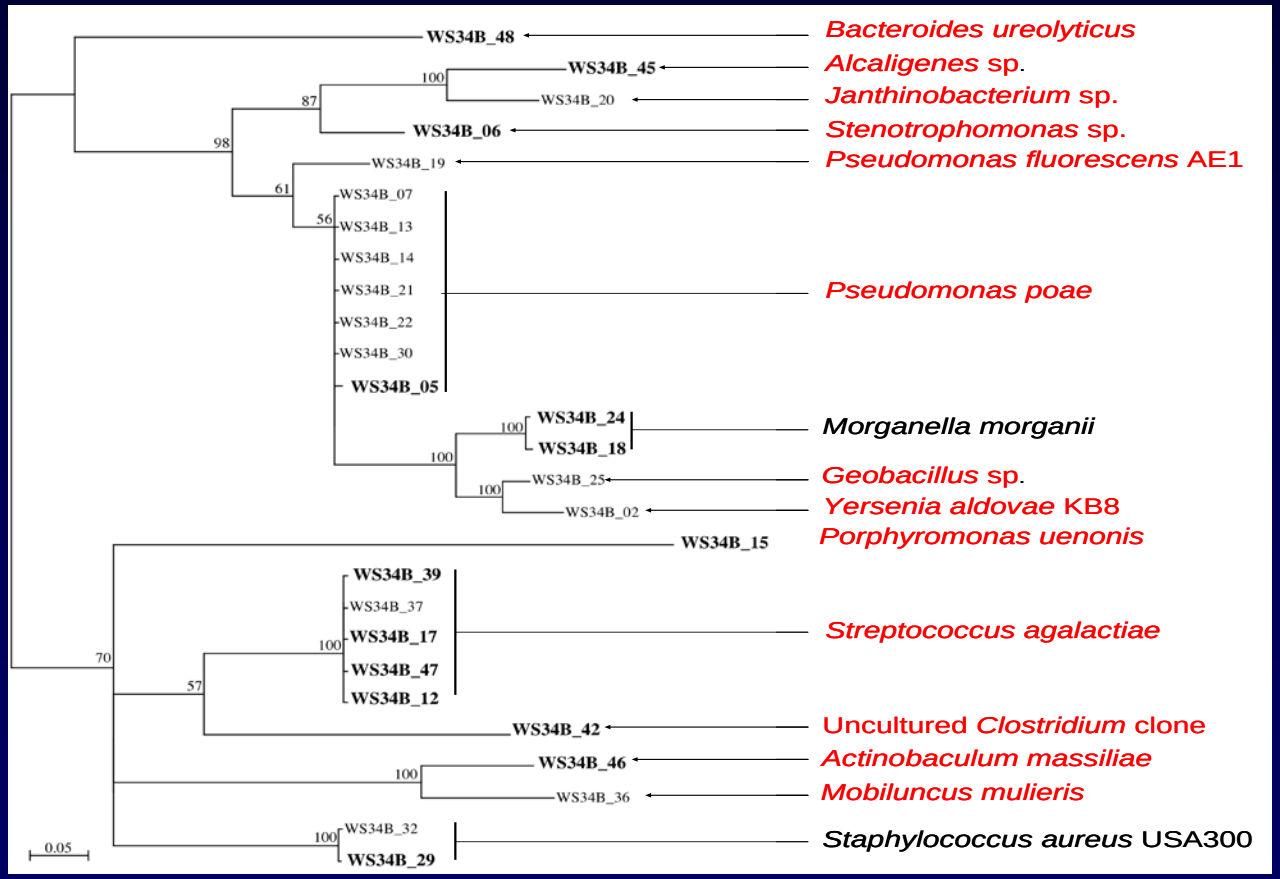


- **MRSA (44.8%) , *Pseudomonas aeruginosa* (27.6%), Group B Streptococcus (27.6%).**

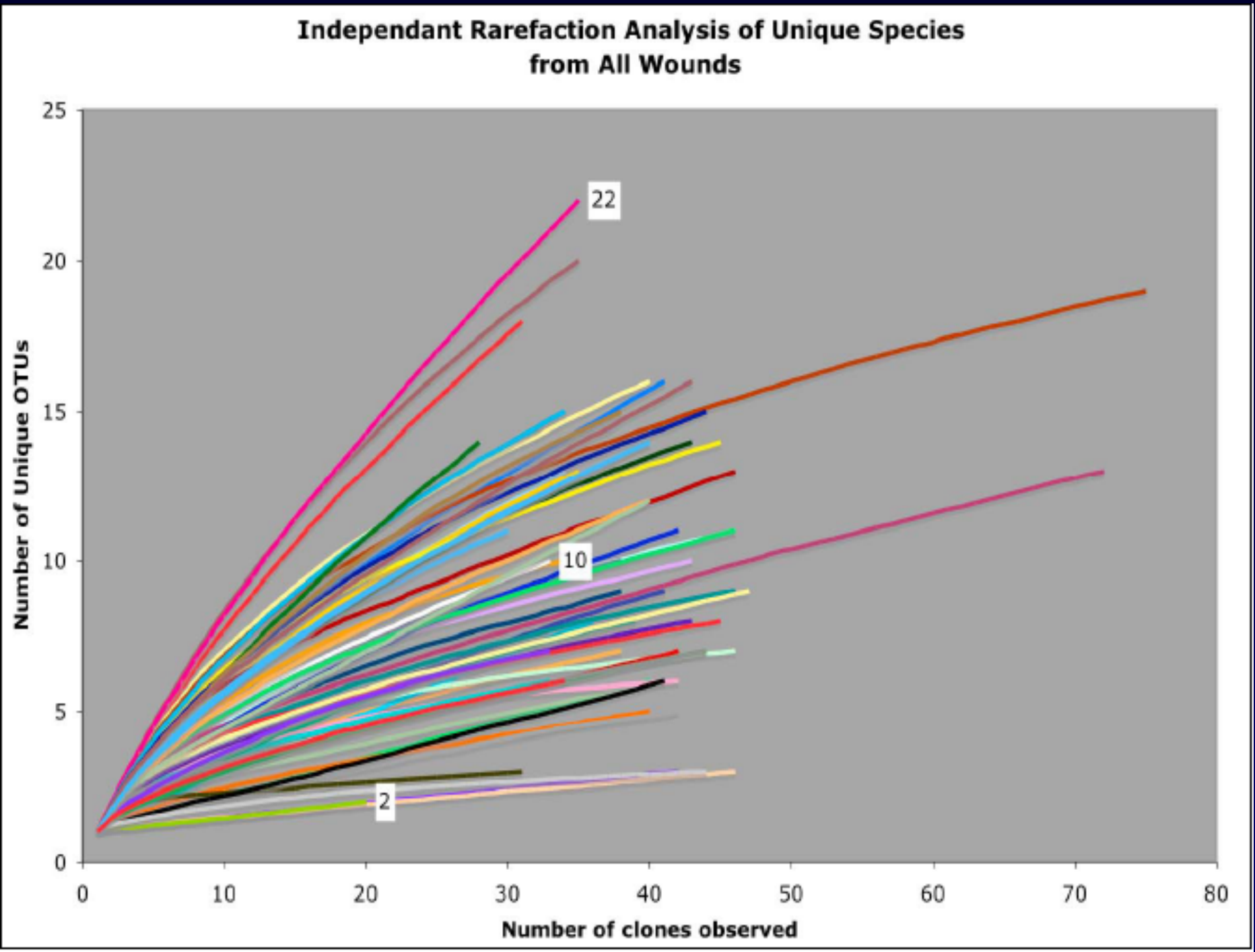
Prevalence of bacterial species

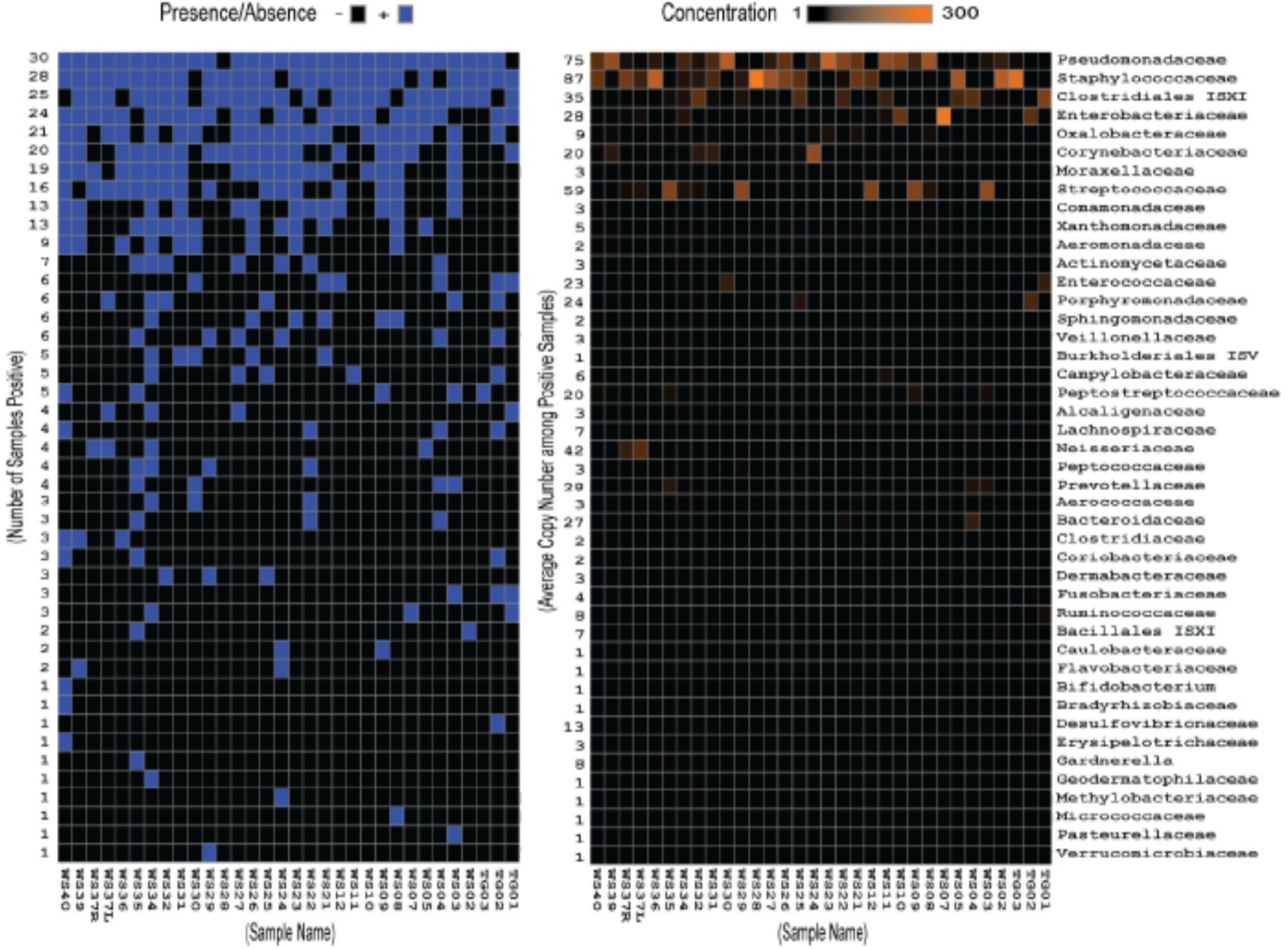
- 97% of wounds cultured had at least one organism, 60% three or more
- MRSA (44.8%) , *Pseudomonas aeruginosa* (27.6%), Group B Streptococcus (27.6%).
- 19/22 samples positive for MRSA had ≥ 10^5 CFU/g organisms.
- 11/14 of negative qualitative results were positive on quantitative microbiology (78.6%, 95%CI 49.2%-95.3%).

Preliminary 16S DNA clone libraries suggest that wounds contain many more species of organisms than recovered by culture



- **16S-based phylogenic tree from a single chronic wound.**
- **Organisms labeled in red were not recovered by quantitative microbiology.**


Independant Rarefaction Analysis of Unique Species from All Wounds
Number of Unique OTUs
Number of clones observed
0
5
10
15
20
25
0
10
20
30
40
50
60
70
80
22
10
2


Presence/Absence
-
+
(Number of Samples Positive)
30
28
25
24
21
20
19
16
13
13
9
7
6
6
6
6
5
5
5
4
4
4
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1
1
1
1
1
M540
M539
M537R
M537L
M536
M535
M534
M532
M531
M530
M529
M528
M527
M526
M525
M524
M523
M522
M521
M512
M511
M510
M509
M508
M507
M505
M504
M503
M502
Z003
Z002
Z001
(Sample Name)
Concentration
1
300
(Average Copy Number among Positive Samples)
75
87
35
28
9
20
3
59
3
5
2
3
23
24
2
3
1
6
20
3
7
42
3
29
3
27
2
2
3
4
8
7
1
1
1
13
3
8
1
1
1
1
1
Pseudomonadaceae
Staphylococcaceae
Clostridiales ISXI
Enterobacteriaceae
Oxalobacteraceae
Corynebacteriaceae
Moraxellaceae
Streptococcaceae
Comamonadaceae
Xanthomonadaceae
Aeromonadaceae
Actinomycetaceae
Enterococcaceae
Porphyromonadaceae
Sphingomonadaceae
Veillonellaceae
Burkholderiales ISV
Campylobacteraceae
Peptostreptococcaceae
Alcaligenaceae
Lachnospiraceae
Neisseriaceae
Peptococcaceae
Prevotellaceae
Aerococcaceae
Bacteroidaceae
Clostridiaceae
Coriobacteriaceae
Dermabacteraceae
Fusobacteriaceae
Ruminococcaceae
Bacillales ISXI
Caulobacteraceae
Flavobacteriaceae
Bifidobacterium
Bradyrhizobiaceae
Desulfovibrionaceae
Erysipelotrichaceae
Gardnerella
Geodermatophilaceae
Methylobacteriaceae
Micrococcaceae
Pasteurellaceae
Verrucomicrobiaceae
M540
M539
M537R
M537L
M536
M535
M534
M532
M531
M530
M529
M528
M527
M526
M525
M524
M523
M522
M521
M512
M511
M510
M509
M508
M507
M505
M504
M503
M502
Z003
Z002
Z001
(Sample Name)

Molecular Microbiology: New Dimensions for Cutaneous Biology and Wound Healing

Jo M. Martin[1], Jonathan M. Zenilman[2] and Gerald S. Lazarus[3]

OPEN ACCESS Freely available online

PLoS one

Community Analysis of Chronic Wound Bacteria Using 16S rRNA Gene-Based Pyrosequencing: Impact of Diabetes and Antibiotics on Chronic Wound Microbiota

Lance B. Price[1]*, Cindy M. Liu[1,2], Johan H. Melendez[3], Yelena M. Frankel[3], David Engelthaler[1], Maliha Aziz[1], Jolene Bowers[1], Rogan Rattray[1], Jacques Ravel[4], Chris Kingsley[1], Paul S. Keim[1,2], Gerald S. Lazarus[3], Jonathan M. Zenilman[3]

RESEARCH LETTERS

Defining Wound Microbial Flora: Molecular Microbiology Opening New Horizons

Yelena M. Frankel, MD, MPH
Johan H. Melendez, MS
Nae-Yuh Wang, PhD
Lance B. Price, PhD
Jonathan M. Zenilman, MD
Gerald S. Lazarus, MD

Conclusions from Metagenomics

- Microbial Diversity was significantly lower in those patients treated with antimicrobials
- High proportion of anaerobes and non cultivables
- Pyrosequencing validated RT/PCR and culture results –when latter were positive; i.e. was more sensitive
- Genomics data suggest that anaerobes are critically important and this may represent synergistic infections
- This is DNA only—Need to do RNA transcriptome and Host

Conclusions

- Genomics has impacted
  - Discovery of new pathogens
  - Detection
  - Understanding the epidemiology
  - Guiding therapy and interventions
  - Understanding resistance
  - Understanding host susceptibility

Conclusions

- Genomic Methods are rapidly replacing traditional microbiology
- “Cellphone Paradigm” in appropriate settings

Conclusions

- The Microbiome is an ecological concept that is leading to new understanding of infectious diseases based on “microbial community” concepts