

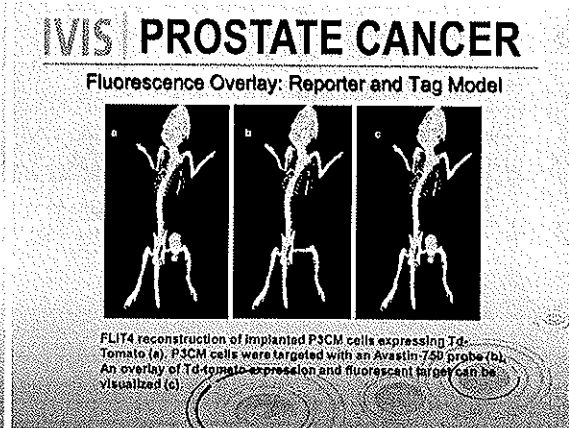
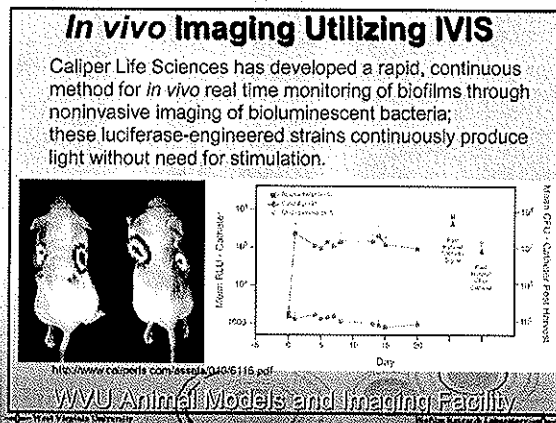
BIOFILMS IN A NEW DIRECTION:
IN VITRO/IN VIVO "SWITCH"

1. Infant Ventilator-Endotrach-Lung Model
2. TriPhasic PLUS Wound Model
3. Silver Wound Dressings
4. IVIS – Real-Time Imaging with Bioluminescent Bacteria

Vol. III
PINK BOOK

Abstracts/Posters/PowerPoint Summary
2009-2010

John G. Thomas, Ph.D. (HCLD) and Staff
Director, International University Biofilm Research Consortium
Department of Pathology
West Virginia University School of Medicine
Honorary Visiting Professor, Cardiff University, Wales, UK
Visiting Professor, National University of Singapore



The *in vitro* – *in vivo* SWITCH.
Real time imaging using bioluminescent bacteria (see Section C)

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 - A. Endotrach Functionality: I-VEL (NICU)**
 - B. Chronic Wounds and Dressings: TriPhasic PLUS Wound Model & Preclinical Trials**
 - C. Biofilms and Tumors: The Link**
 - D. Probiotics**
 - E. Global Tracking: Antibiotic Resistance**
 - F. Oral Care**
 - G. Education: eWeb and conferences**
- V. Summary & Conclusion**
- VI. Complete Abstract List**

Section I. Introduction/Executive Summary.

- Our first Posters/Abstract Books (Blue & Green) focused upon the research supported by Tyco/Covidien. The clinical area of Translational Research was VAP (Ventilator Associated Pneumonia) and potential impact of a silver coated endotrachs, first called “Guardian”, and then called, “Sentinel”.
- Two Posters/Abstract Booklets were constructed. The first, the “Blue Book” and the second, the “Green Book” and each described advancing research in the years respectively 2006 to 2007, then 2007 to 2009.
- Here, we focus on the post-Tyco/Covidien Era and transition from Adult VAP to 1) Pediatric and/or Infant VAP, 2) chronic wounds and dressing with silver (Triphasic PLUS Wound Model), 3) the use of probiotics, 4) the evolution of biofilms as tumors or vice versa, and 5) a Global Tracking Network for antibiotic resistance focusing on MRSA.
- The organization and design utilized here is to highlight the five areas of Translational Research just described. As in the Green and Blue Books, the Introduction will highlight certain PowerPoint presentations, which compliment the posters/abstracts and lead to their Purpose, Hypothesis, Objectives, and complementary information where necessary.
- Of primary significance, is the expansion of our data assessment and samples to include organisms and collaborate areas from around the globe emphasizing the six regions of the World Health Organization Globe and colleagues and investigators who have similar international passion for translational research in the areas of VAP, chronic wounds, probiotics, and tumors and *Candida albicans* (CJ – Hong Kong).

- Finally, it is important to note that the translational research presented here was often driven by preliminary and/or pilot studies, and the ultimate goal of this research was to be awarded with an NIH/Federal Grant or Industrial support of significance for West Virginia University.

SECTION II. Summary of Key Scientific Observations for Translational Research:

“Globalization,” Tumors and Cancer.

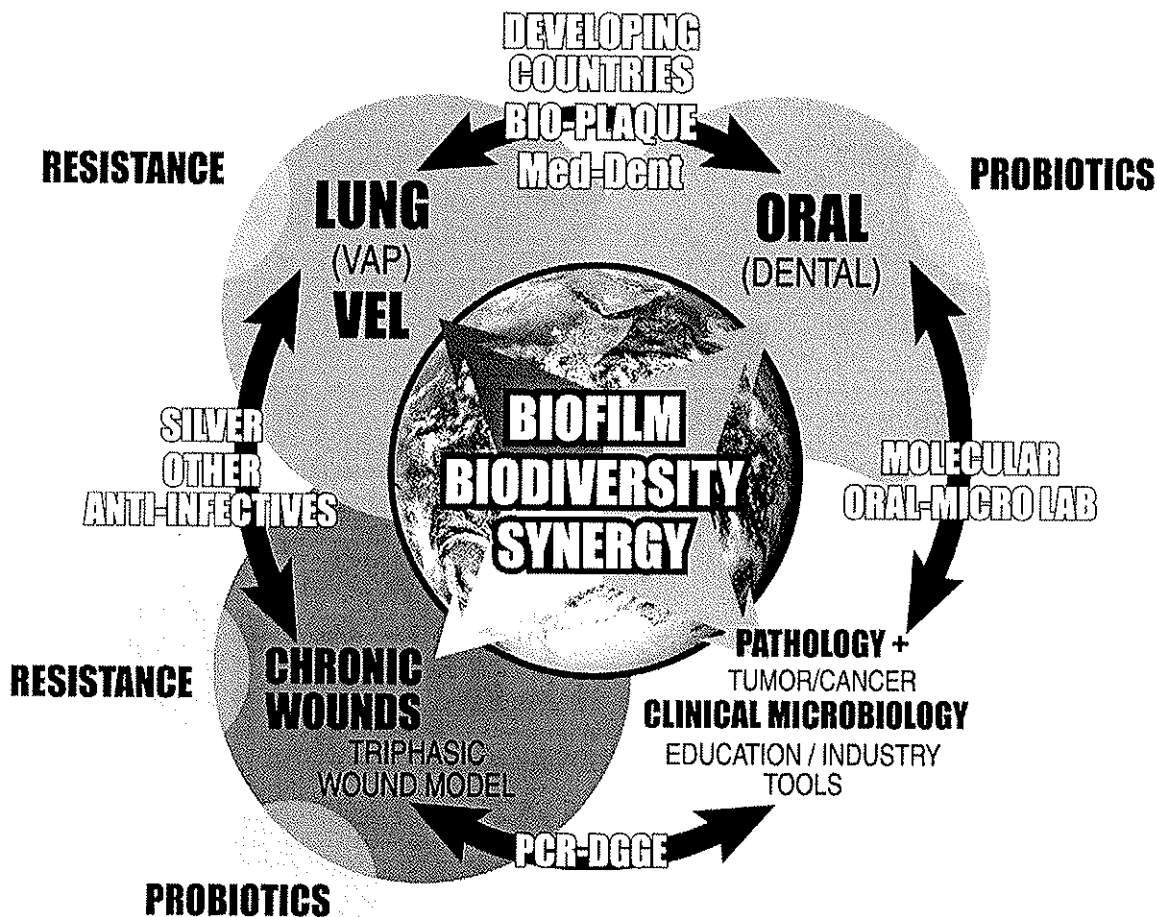
Includes:

- Six logos defining our translational research and global interface.
- Recent expansion particularly below the Equator with the inclusion of South African and Queensland University Health Center in Australia (IUBRC).
- These were natural extensions of the global education and collaboration maximized by tours in the UK and the Asia Pacific Theaters (V).
- Ultimate Goal: Include sites from each of the 6 WHO regions: Globalization

Logos (Mission Based)

1. Translational Research
2. Educational, Global/ E-Commerce (21st Symposium)
3. International University Biofilm Research Consortium
4. International Antibiotic Resistance
5. Cancer
6. WHO

TRANSLATIONAL RESEARCH: Bench to Bedside Bench to Clinical Microbiology



Prof. John G. Thomas, Director
www.hsc.wvu.edu/som/pathology/thomas/



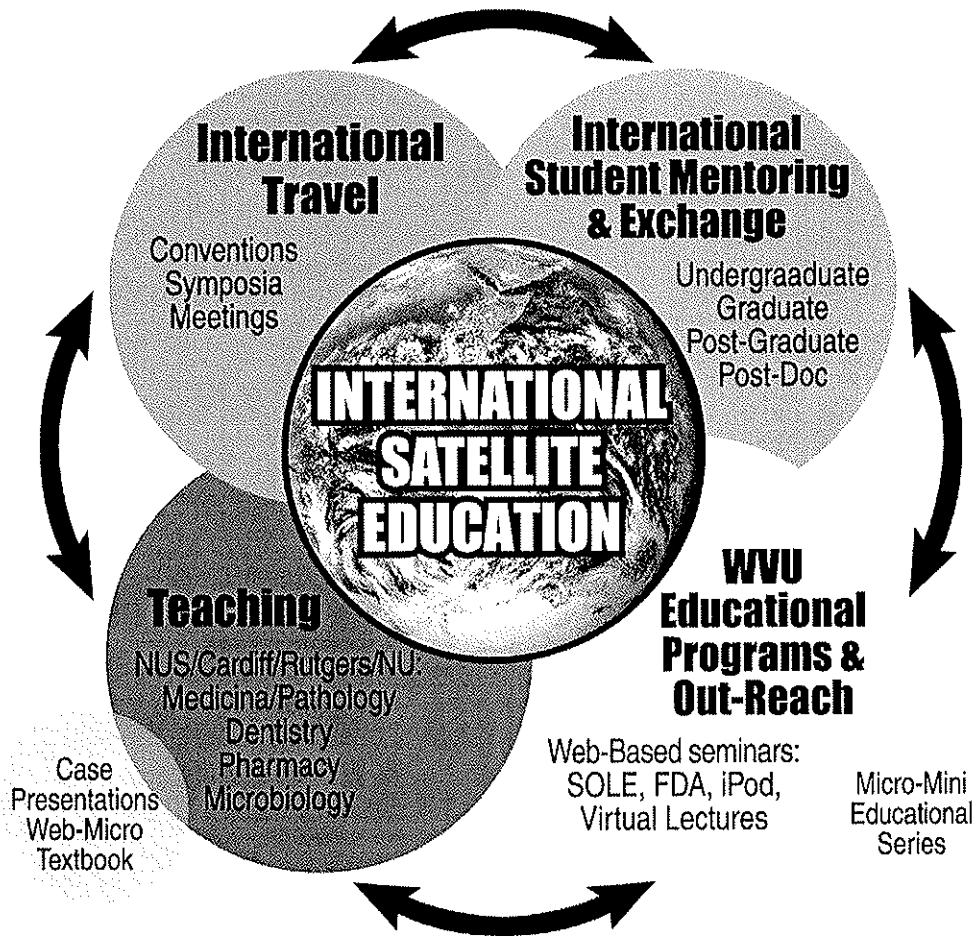
WEST VIRGINIA UNIVERSITY
ROBERT C. BYRD HEALTH SCIENCES CENTER
DEPARTMENT OF PATHOLOGY

Specific Topics within the Four Branches of Research for 2010

<p style="text-align: center;">Lung/VEL</p> <ol style="list-style-type: none">1. Adult VEL2. Infant VEL	<p style="text-align: center;">Oral</p> <ol style="list-style-type: none">1. COHRA2. SAGE
<p style="text-align: center;">Chronic Wounds</p> <ol style="list-style-type: none">1. TriPhasic PLUS Wound Model2. Silver Alginate	<p style="text-align: center;">Pathology/Clinical Microbiology</p> <ol style="list-style-type: none">1. Cancer-Tumor Studies: Linking Prokaryotic and Eucaryotic Tumors

HELPING EDUCATORS MAKE A WORLD OF DIFFERENEC: Linking National & International Microbiologic— Public Health Educational Resources

“Mountaineer Roots: Global Branches”



Prof. John G. Thomas, Director

www.hsc.wvu.edu/som/pathology/thomas/



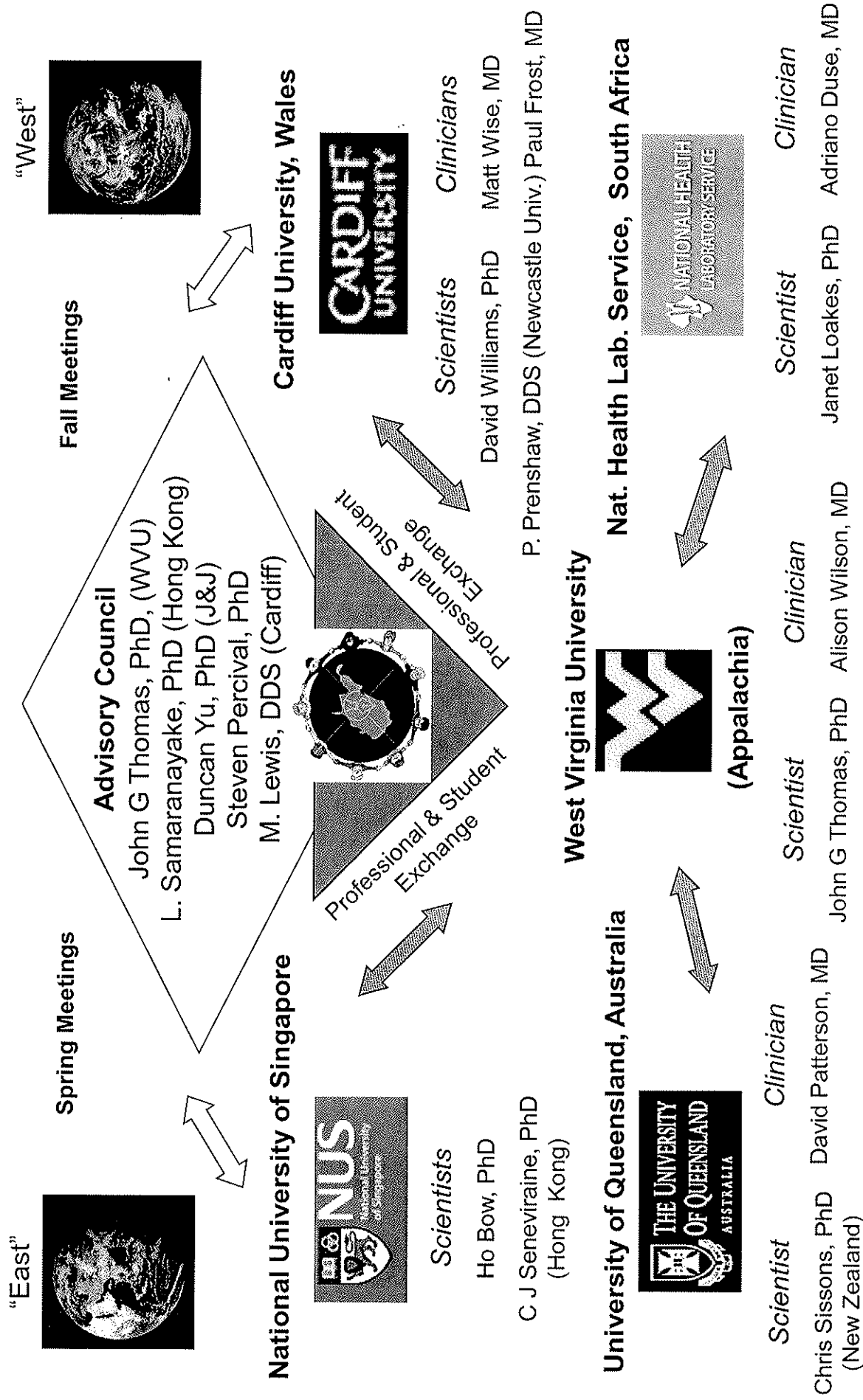
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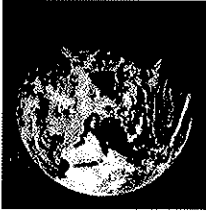
The International University Biofilm Research Consortium

IUBRC



The International University Biofilm Research Consortium Tracking International Antibiotic Resistance/Isolates

"East"



"West"



NARMS

www.cdc.gov/narms

National Antibiotic Resistance Monitoring System

WHO, FDA, CDC, & USDA



National University of Singapore

John G. Thomas, PhD

Visiting Professor



(Malaysia)

Malaysian National Nosocomial
Infection Surveillance System
(NNISS) (WHO Mission Report)

<http://www.wpro.who.int/sites/hds/docume>
nts/Malaysian+Nosocomial+Infect
ion+Surveillance+System.htm

University of Queensland, Australia



(Australia)

Cardiff University, Wales

John G. Thomas, PhD

Visiting Professor



(United Kingdom)

National Public Health Service
for Wales

<http://www.nphs.wales.nhs.uk/>

European Antimicrobial Resistance
Surveillance System (EARSS)

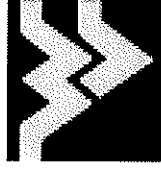
<http://www.rivm.nl/earss/>

Nat. Health Lab. Service, South Africa



(South Africa)

West Virginia University

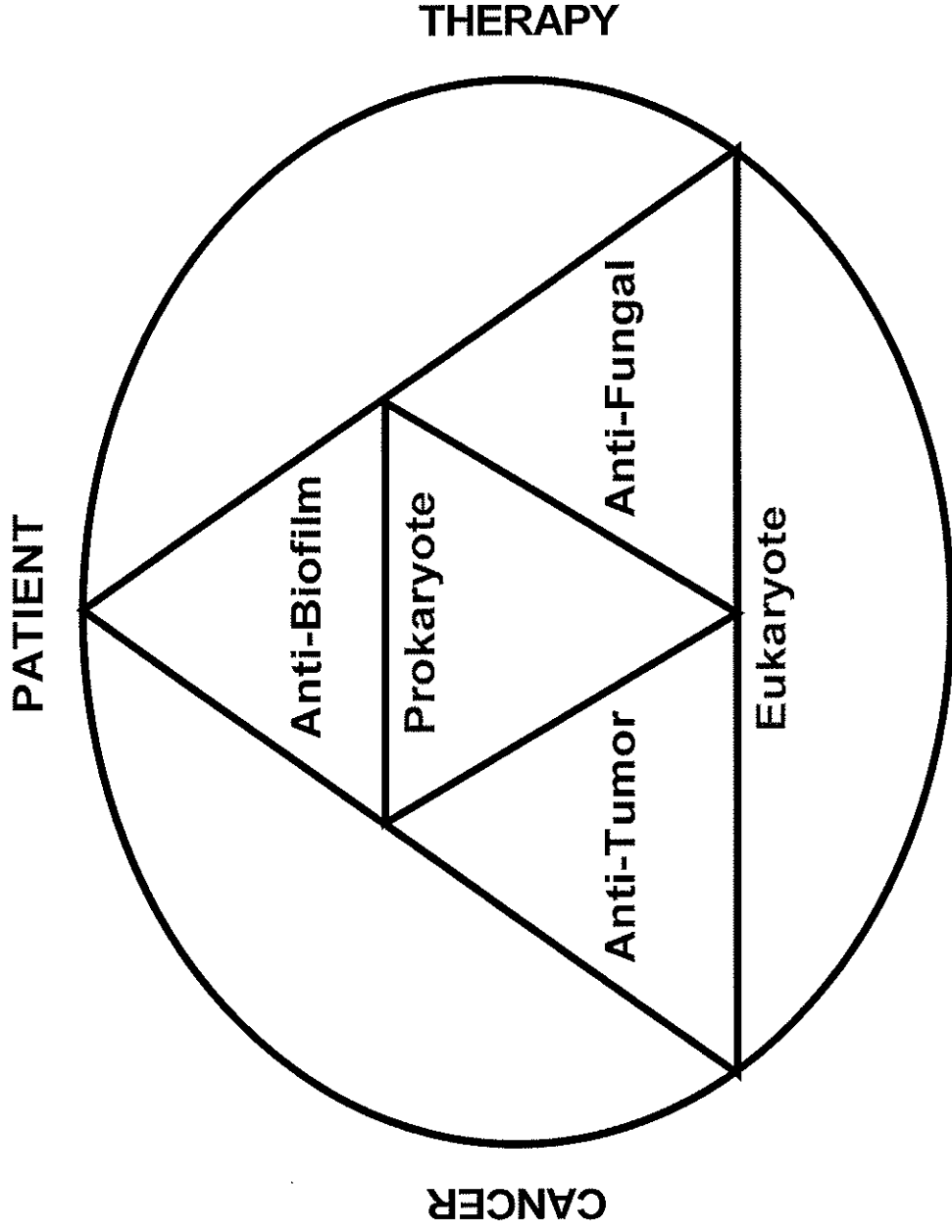


(Appalachia)

The Surveillance Network
TSN Database (USA)

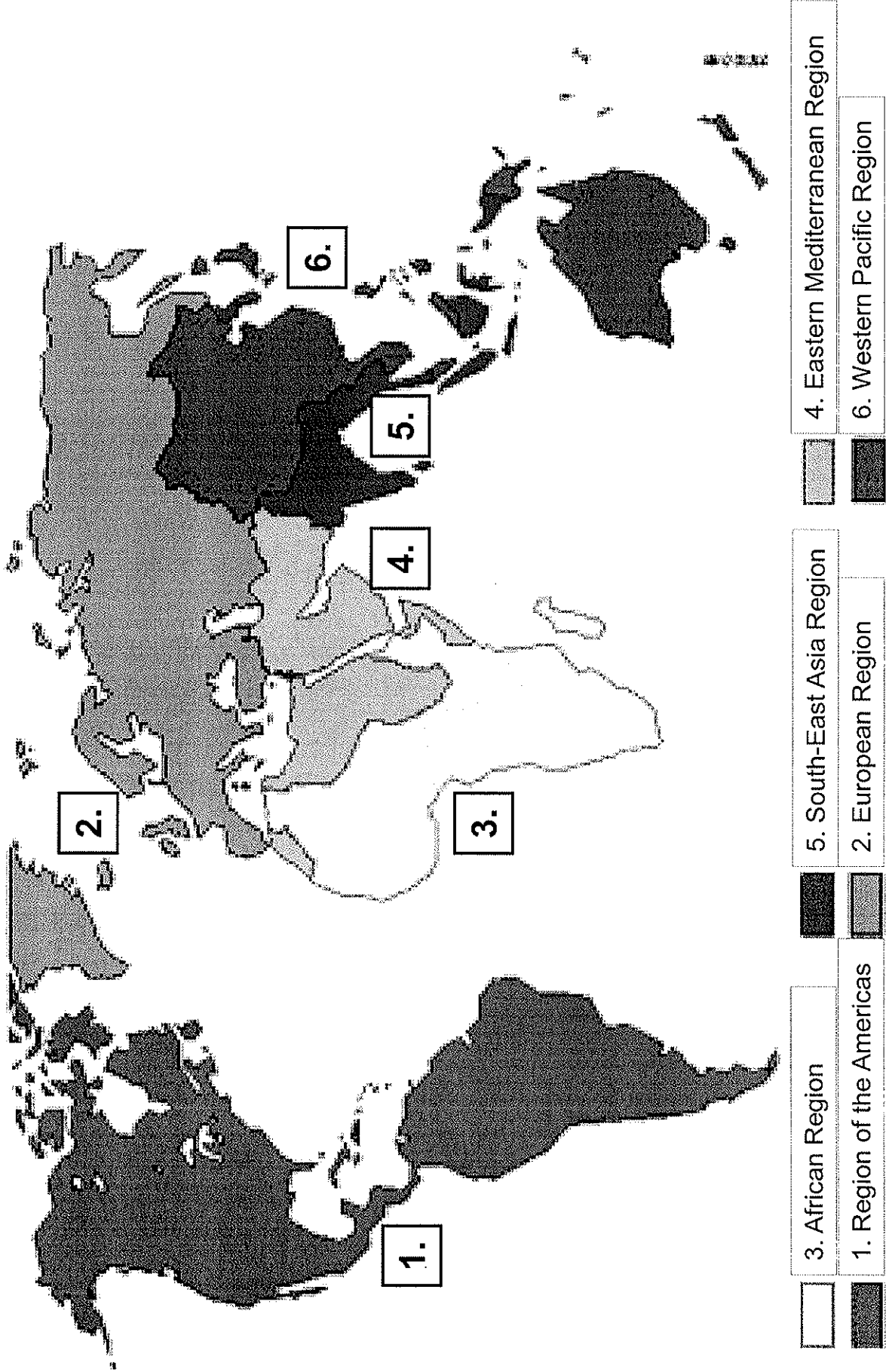
www.mrlworld.com

LINKING PROKARYOTIC AND EUKARYOTIC TREATMENT THERAPY



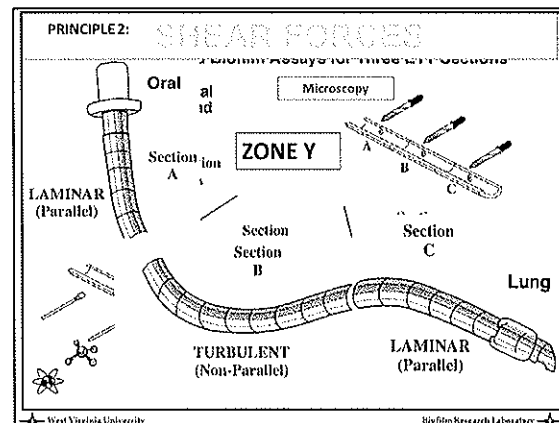
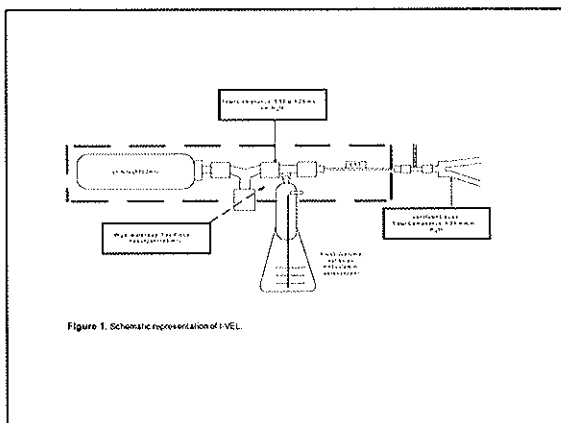
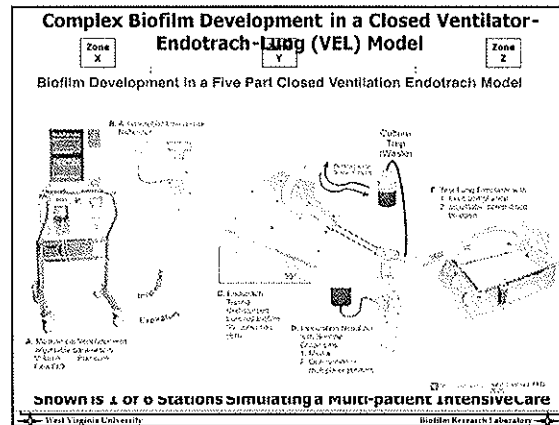
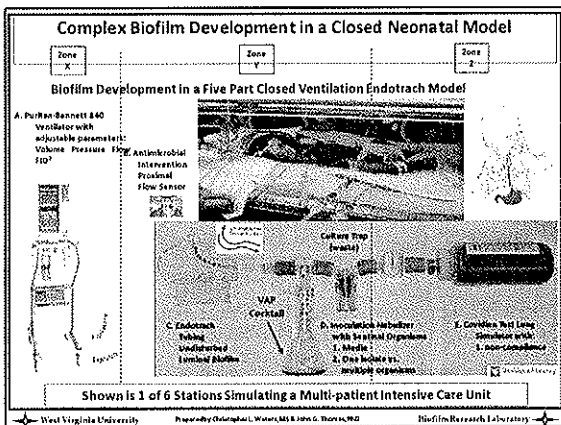
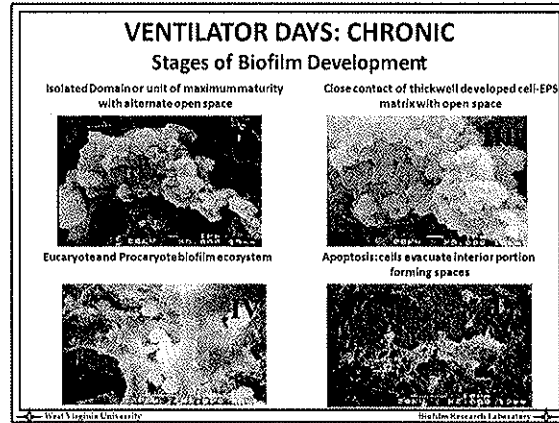
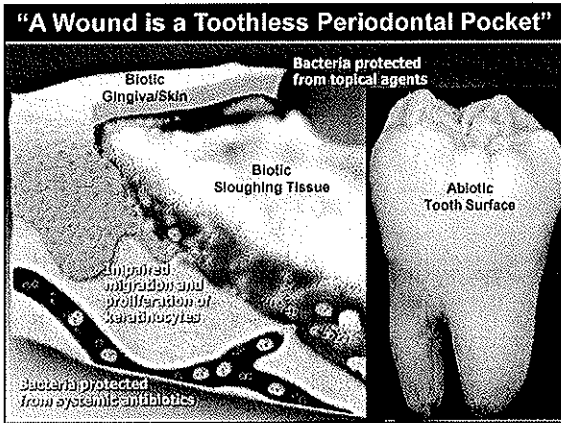
Tumor treatments often originating from anti-fungal design, may manage both eucaryotic and procaryotic tumors (biofilms), the later often engineered through *Candida albicans*, the Universal Co-aggregate or biofilm architect. This logo summarizes our Hypothesis II.

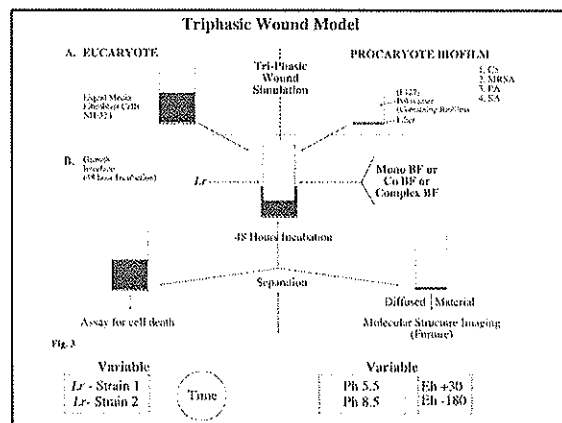
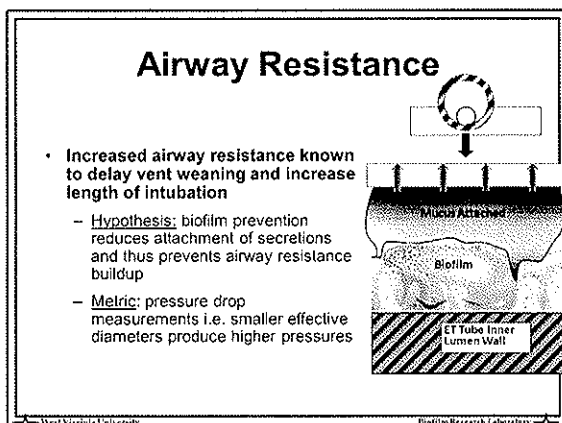
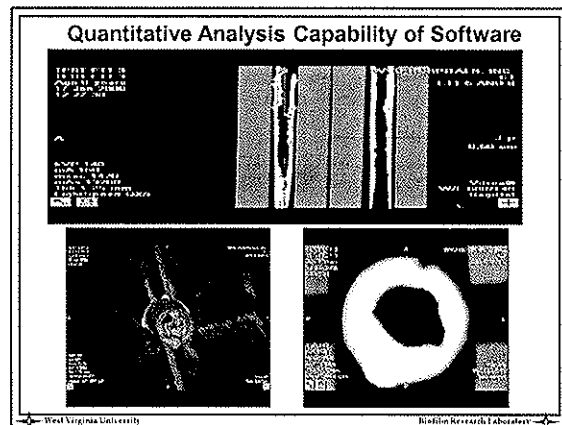
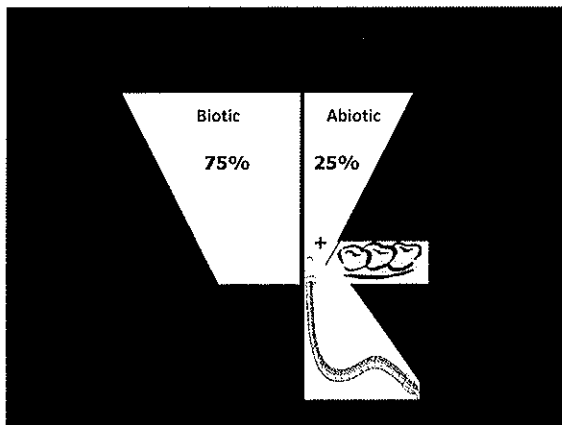
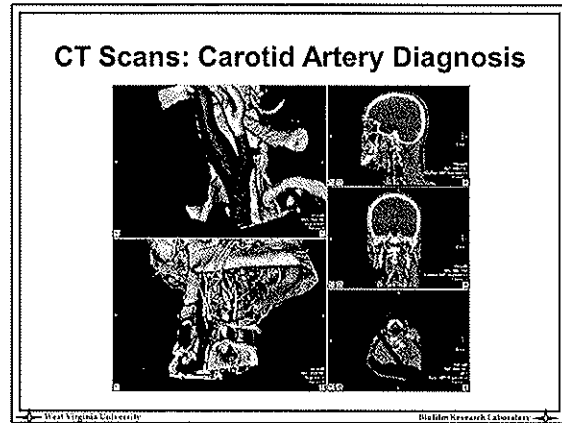
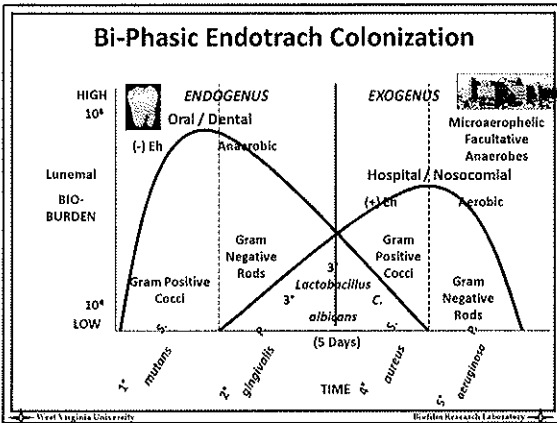
Tracking & Linking Disease by 6 Global Regions: The WHO Gradient

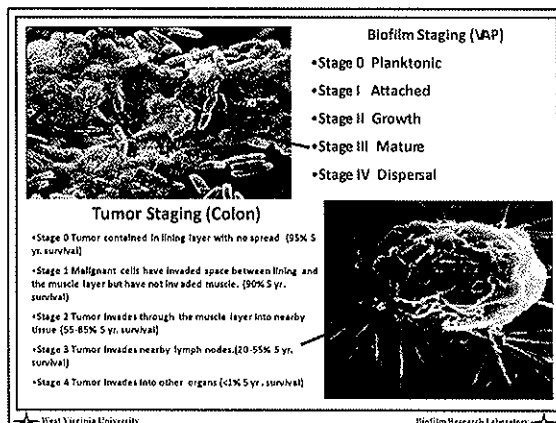
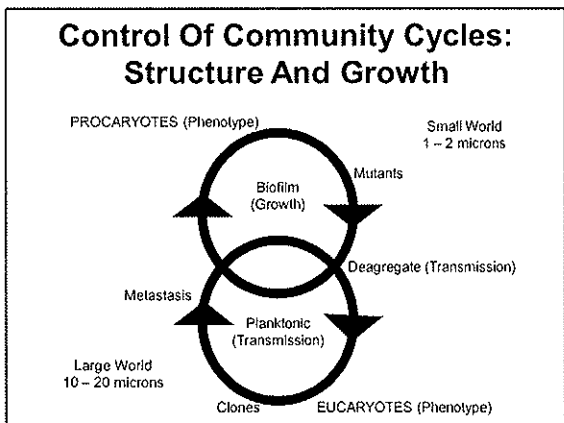
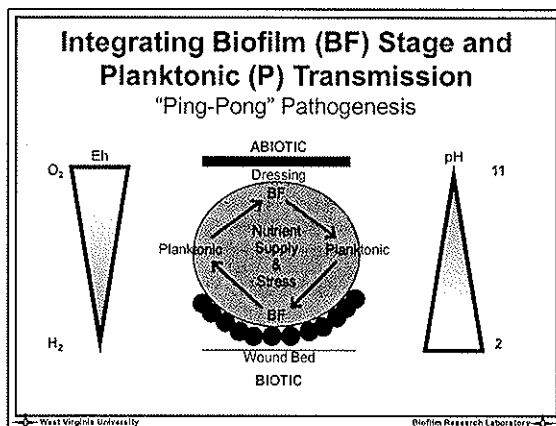
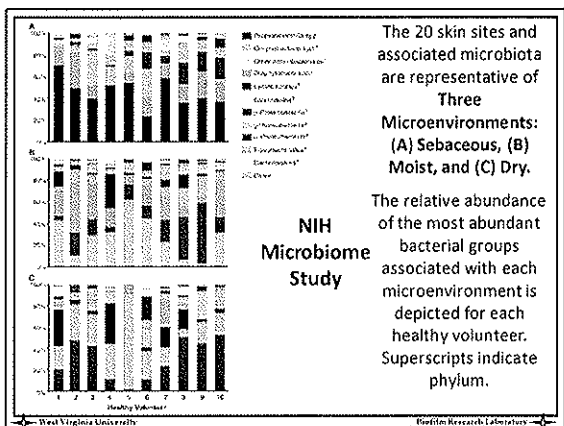
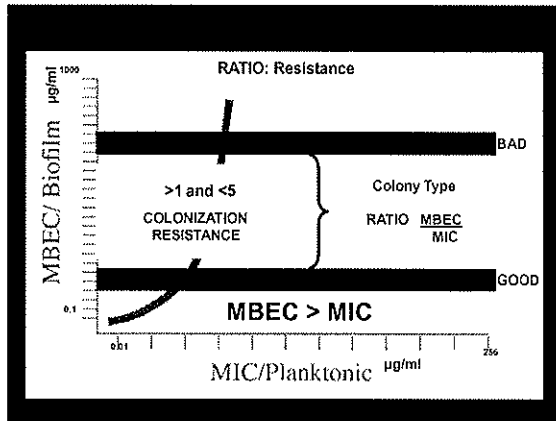
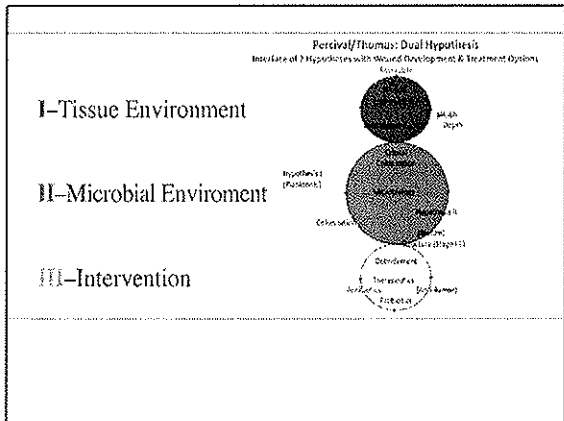


<http://www.who.int/about/regions/en/index.html>

SECTION III. Selected PowerPoint Highlights: “A Picture is worth a Thousand Words”







Structural & Developmental Similarities: Biofilms to Tumors

Gradients of proliferation, cell death and lysis in cancer spheroid foci and biofilm microcolonies

Spheroid model *P. aeruginosa* microcolony

Visualized using LIVE/DEAD staining
(Webb et al., 2003)

West Virginia University Biofilm Research Laboratory

HYPOTHESIS II:

“BIOFILMS ARE PROCARYOTIC TUMORS”

- Communities of Linked Microbes Masquerading as a Biofilm are really Procaryotic Tumors
- **BioTUMOR Classification**

- John G Thomas, Ph.D. (2003)

West Virginia University Biofilm Research Laboratory

Unmasking the Relationship: Biofilms and Tumors

Parameters	Phenotype		
	Procaryote	Eucaryote	
	Planktonic	Biofilm	Tumor
1. Antibiotic Therapy	+		
2. Antibiotic Resistance	+	+	
3. Viscoelastic		+	
4. Hydrated Polymer		+	
5. Loss of Contact Inhibition		+	+
6. Cycling/Stages		+	+
7. Community/3-D Structures		+	+
8. Nutrient Pathways (Angiogenesis)		+	+
9. Anti-Inflammatory/Resistance		+	+
10. Division of Labor (Heterogeneity)		+	+
11. Metastasis		+	+
12. Genotyping			

West Virginia University Biofilm Research Laboratory

BioTumor Classification Scheme

	BioType - Target of Action					
	Anti-Tumor					
	Purine Synthesis	Nucleotides	DNA	RNA → Proteins	Signal Transduction	Microtubules
Antibiotics/Anti-Infectives	A	B	C	D	E	F
Cell Wall	I	IA	IB	IC	ID	IF
Cell Membrane	II	IIA	IIB	IIC	IID	IIF
30/50S Ribosome	III	IIIA	IIIB	IIIC	IID	IIF
Folates	IV	IVA	IVB	IVC	IVD	IVF
DNA Gyrase	V	VA	VB	VC	VD	VF

West Virginia University Biofilm Research Laboratory

HYPOTHESIS I

Candida albicans = THE UNIVERSAL CO-AGGREGATE

- In the diversity of procaryotic biofilms, a biphasic eucaryote, *Candida albicans*, is the key biofilm building block and potential treatment target.

West Virginia University Biofilm Research Laboratory

TEMPLATE A - TESTING BATTERY

1. 3-Organism 2. Anti-

Ag+ 7.5% wt/vol. Periostat LDD (20mg)

30% F127 Poloxamer (BF) or (M-H) [Planktonic] 24/48 hour growth Muller-Hinton

ZOI @ M-H 24/48 hrs.

West Virginia University Biofilm Research Laboratory

The Relationships between Bacteria and Tumors

1. Around (Associated)
 2. Within (Inside)
 3. Stimulating (Etiology/Cause) (Proliferate)
 4. Reducing (Therapy/Treatment)

West Virginia University Biofilm Research Laboratory

IV injection of 10^7 *Salmonella* and photon collection for 1 min; C6 glioma

Day 1 (1 hr)
 Day 2

West Virginia University Biofilm Research Laboratory

IVIS PROSTATE CANCER

Fluorescence Overlay: Reporter and Tag Model

Full reconstruction of implanted P328 cells expressing TG-Tomato (d). P328 cells were tagged with an Avicidin-230 probe (e). An overlay of TG-Tomato expression and fluorescent target can be visualized (f).

West Virginia University Biofilm Research Laboratory

Patients with *G. agnovis* (after clearing)

Treatment with GUM PerioBalance chewing gum

Presentation After 2 weeks

West Virginia University Biofilm Research Laboratory

In vivo Imaging Utilizing IVIS

Caliper Life Sciences has developed a rapid, continuous method for *in vivo* real time monitoring of biofilms through noninvasive imaging of bioluminescent bacteria; these luciferase-engineered strains continuously produce light without need for stimulation.

Mean RLU/Caliper

Day

<http://www.caliperlife.com/assets/010/6116.pdf>

WVU Animal Models and Imaging Facility

West Virginia University Biofilm Research Laboratory

A BIOTIC SUBSTRATUM

In vitro analyses of gram positive organism *B* using poloxamer under increased stress, Stage III-IV: Late/ Apoptosis or Necrosis

Quantification

Biofilm Thickness

Biovolume

Substratum Coverage

Microcolony

Organization pattern

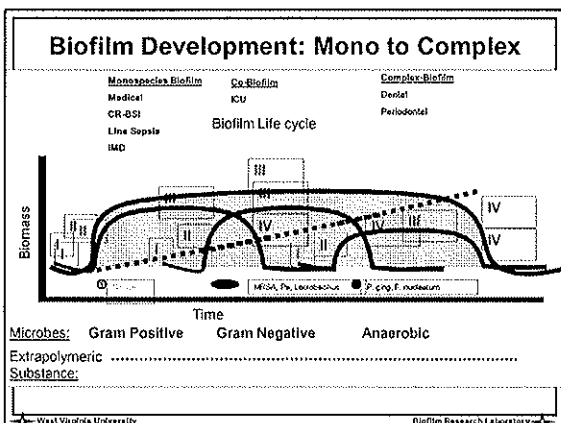
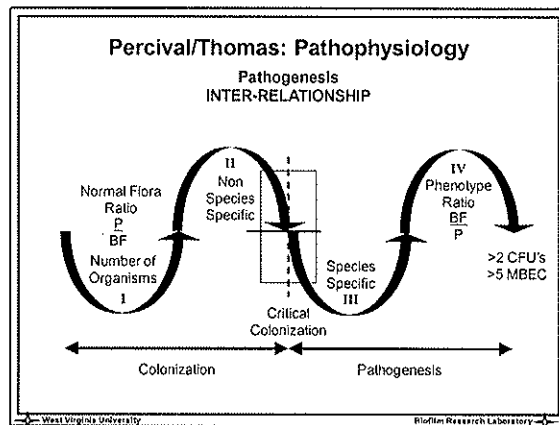
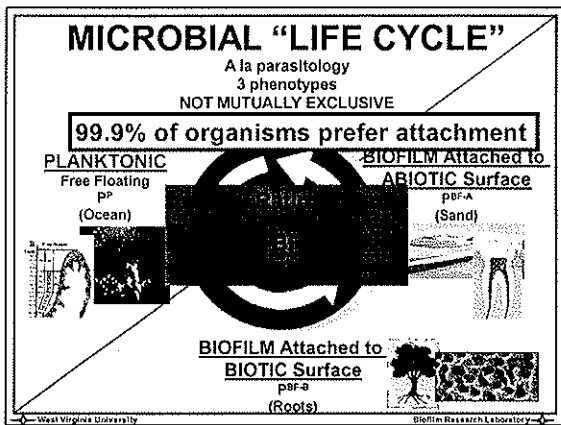
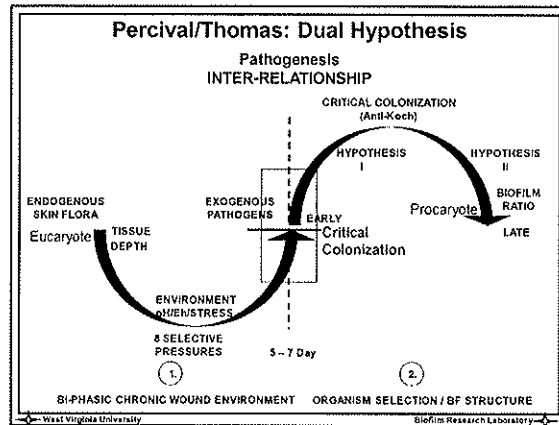
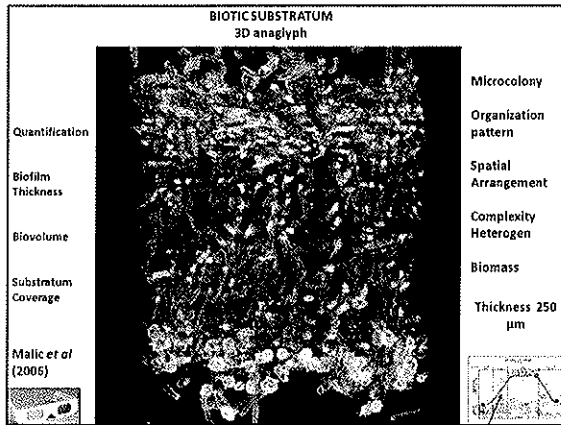
Spatial Arrangement

Complexity Heterogen

Biomass

Thickness 250 μ m

West Virginia University Biofilm Research Laboratory



Peter Kropotkin

- Viewed the theory of "survival of the fittest" as supporting co-operation rather than competition.
- In his book *Mutual Aid: A Factor of Evolution* he set out his analysis leading to the conclusion that the fittest was not necessarily the best at competing individually, but often the community made up of those best at working together.
 - "The animal species, in which individual struggle has been reduced to its narrowest limits, and the practice of mutual aid has attained the greatest development, are invariably the most numerous, the most prosperous, and the most open to further progress."

The "We" View = Biofilm Phenotype

Elie Metchnikoff

- Born in the Ukraine in 1845
- Was a Biologist/Immunologist and Nobel Prize winner
- Developed a theory that lactic acid in the GI tract, could, by preventing putrefaction, prolong life.
- Later restated that "Probiotics are viable bacteria that beneficially affect the host by improving its intestinal microbial balance."

SECTION IV. Abstracts & Posters by Topic

A. Endotrach “Functionality”: I-VEL

INFANT VENTILATOR ENDOTRACH LUNG MODEL (I-VEL)

- It was quite logical that the adult ventilator endotrachs lung model/simulator (A-VEL) would act as a model for infant studies.
- There is an incredible lack of information about ventilator associated pneumonia in infants, its impact in the NICU, and the organisms associated or their consequences.
- Focus of research here would include indwelling medical devices such as endotrachs coated with peptide antimicrobials as well as recognition that the consequence 100% of the time was some form of occlusion associated with excretion buildup due to “biofilm bridging” or “Functionality” (Note to John: need 3 part PPT)
- Here, also, the focus was to use STTR focused NIH Grants, given that the percentage of funding and successful award is considerably higher than the R-21/RO1 classic route. Further, our associations with a number of vendors in biotechnical companies made this a favorable collaborative translational study.

Poster Numbers/Website:

17

19

B. Chronic Wounds and Silver Dressings/Triphasic PLUS Wound Model

Anti-Koch

- Our transition and expansion of biofilms in chronic wounds was quite natural given the link with silver coated dressings and our understandings of silver coated endotrachs from previous studies with Covidien.
- Further, there was a much larger connection given that the pathophysiology of a periodontal pocket and that of a chronic wound share many items, we coined the term “a chronic wound as a periodontal without a tooth.” MMPs, collagenase, tetracycline, CMTs, and other “words” recognize the similarity and common options about pathophysiology and ultimate treatment when comparing periodontal disease, Endodontics, and chronic wounds (or the Perio-Pocket is an Oral Wound).
- This was also a unique time to expand the associations with Dr. Steven Percival and initially Convatec, subsequently Advanced Medical Solutions and the undertaking of unique approaches to resolve, study and ultimately manage biofilms associated with chronic wounds and the use of silver.
- This was a major focus for the year 2010 with multiple studies utilizing a number of silver dressings and the integration of newer molecular methods (DGGE) flow cytometry (FC), IVIS, and Poloxamer to more closely resemble the *in vivo* environment of a biofilm infected wound.
- It also heralded the use of our TriPhasic PLUS Wound Model and the potential treatment with probiotics, or as we call them “Colonization via Restorative Microbiology.” We utilized two oral derived lactobacillus strains to alter the impact of a trivalent biofilm community and its action on a monospecies target monolayer.

- Our studies of chronic wounds also highlighted the expanded dual Hypothesis of Thomas and Percival with several publications and an unmasking of our Hypothesis that the most important feature of wounds and their classification is whether or not they had a ratio of biofilm to planktonic phenotypes and initiated its uses as a “BioMarker”.

Poster Numbers:

4
7
8
11
12
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16
21

C. Biofilms and Tumors

“Tumors Are Eucaryotic Biofilms”

- This is our most adventuresome Study Group and evolved from thoughts germinated at WVUH, fed while in Cardiff at the School of Dental Medicine, and enhanced with discussions with professors at Loma Linda University, particularly Shahrokh Shabahang, DDS, PhD.
- The overall concept is described in the “Clinical Tool Section of the JGT Website, and emphasizes an evolutionary concept with Hypothesis, Aims, And Objectives. A major focus is the significance of *Candida albicans*, its biphasic nature and the fact that it transitions the prokaryotic world as a biphasic eukaryote and in biofilm construction is probably the “university co-aggregate” in biofilms.
- These studies also emphasize the growing University desire to collaborate with multi-disciplines and to integrate multiple visions within the University, nationally recognized centers and/or global interface. For us, the primary interface is with the Cancer Center at WVU Health Sciences Center, but collaborations with Dr. Jeremy Webb at Manchester University, UK, and Loma Linda University, California, demonstrated the universal appeal of this concept.

Posters:

1
2
3
20
25

D. Probiotics

“Restorative Microbiology”

- We initially studied oral probiotics focusing on caries, periodontal and gum diseases. The commercial name is PerioBalance.
- Subsequently, with isolates and support from 1) AMS, Cheshire, England (Dr. Stephen Percival), and 2) Biogaia, Denmark (Dr. _____), we expanded our studies to focus on wound recovery and the use of our TriPhasic PLUS Wound Model (TWM).
- In the TWM, we assayed the impact of a mixed probiotic at different pH and integrated Flow Cytometry (FC) to better evaluate the altered cellular arrangement.

Probiotics are Eco-Friendly (aka Green)

- If Normal Flora is an “Agar System,” then Probiotics are an “Organ Transplant”

Poster Numbers:

7
4

E. Global Tracking: Antibiotic Resistance

“Antibiotic Resistance focusing on MDR and MRSA”

- Since our initial involvement with the TSN-USA (The Surveillance Network), National Antibiotic Registry in 1993, over 500 US hospitals have uploaded antibiotic profiles on a daily basis; TSN allows 27 search parameters.
- This year we focused particularly on wounds and the environmental pressures associated with wound infections; this was drawn by our interest in chronic wounds as outlined in Section C.
- Most recently, we have “Globalized” and via recertification of a CDC Certificate for MRSA and MSSA, we have continued toward our goal of receiving samples from the 6 WHO Regions of the Globe.
- Most recent global samples were burn isolates and MRSA/MSSA from the National Reference Lab, South Africa, Dr. A. Duse, Director. We hope to expand this with our Tour VI (April 2011) to South East Asia, including Australia and New Zealand.

Poster Numbers:

6
9
10
18
23
24

F. Oral Care & Airway Management

As the significance of plaque and oral flora became obvious in pathophysiology of VAP, both adult and infant, we integrated studies from two disparate but complementary research foci: Sage and COHRA, as highlighted below.

- Sage: We initiated a pilot study of 10 patients with a potential of a NIH supported 100 patient study at multiple locations.
- COHRA: We continued the evolution of our OMS (Oral Microbial Signature) and the predictive value of microbial colonization.

Poster Numbers:

24
26
27
28

G. Education: eWeb, Conferences and Tools

Mission:

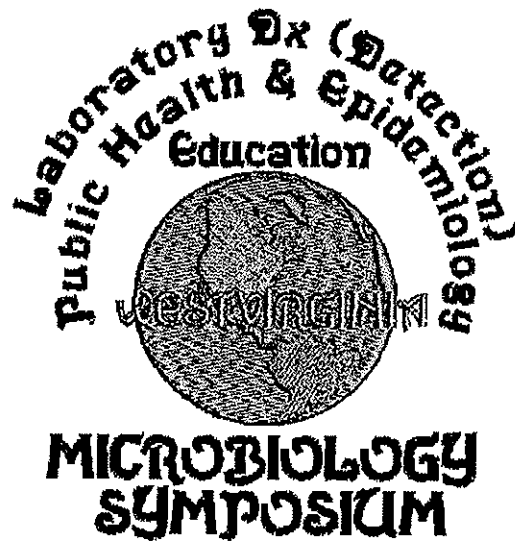
- Education, from WV to the World, has always been our goal

Global:

- This year we advanced our mission more than ever; Google Tracking indicated users from 47 countries visited our website

National:

- Rutgers University and the University of Medicine and Dentistry of New Jersey, Dept of Clinical Sciences, integrated our Biofilm Teaching Material into their course for Graduate Education.



THE ANNUAL MICROBIOLOGY SYMPOSIUM: THE HISTORY

by John G. Thomas, Ph.D.

PHASE 1: 1990 to 1997 – It all began as a mission to increase awareness of microbiology in clinical laboratories within the state of West Virginia. The goals and objectives were clearly outlined as assist in continuing education, improve laboratory performance, monitor antibiotic resistance, and improve cooperation/communication among West Virginia and surrounding laboratories. It was also meant to become a repository for WV unusual microorganisms, recognizing the unique population of Northern Appalachia. Upon his arrival Dr. Thomas initiated the program and by the Fall of 1990 had created enough interest in diagnostic virology to have the first Fall Symposium. The initial symposium was held in the WVU Health Sciences Center with a modest turnout and created the catalyst for more intimate conversations within WV microbiology laboratories.

A “tour” of West Virginia Microbiology Laboratories and friendly evaluations/inspections followed. It was an opportunity for Dr. Thomas to talk to the staff at distant sites, updating not only clinical microbiology but corresponding resistance problems in newer antimicrobials. To further catalyze this interaction, Dr. Thomas created a “Monthly Conference Call” with Laboratories within the state, lasting one hour with topics seasonal and germane to the diseases and the prevalence at that time. Participation expanded to Pennsylvania, a little of Ohio and some distant parts of Washington, DC and Maryland.

PHASE 2: 1998-2002: Interest continued to grow until the Fall meeting reached 100 participants, it became apparent that a Workshop which focused on “bench-top” training would be beneficial. Particular topics included fungal isolates, rapid identification, and mycobacterium. The Spring Meeting was created for two and a half days with a limit of 30 people. Thus the start of the famous “evening social programs” at Coopers Rock.

During this time, it was also apparent that West Virginia and the annual programs could benefit by interfacing with a Regional Microbiology Association. West Virginia joined SCACM in

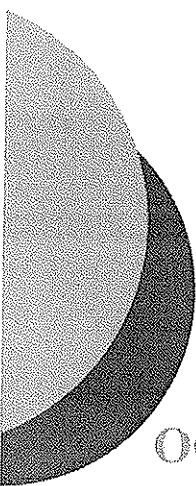
2002 with Dr. Kevin Tu, Charleston Area Medical Center, a major force in assisting Dr. Thomas and the interfacing with the then 6-state SCACM organization; it was of benefit to all.

PHASE 3: 9/11 to Present – SCACM suggested a meeting held in Charleston, WV, which was a benchmark for microbiologists within the state of West Virginia. Our organization also linked with the Anna Arundel group out of Maryland, which again provided collaboration amongst institutions, people and philosophies from the East Coast. Of course, there were the meetings and collaboration of Dr. Malcolm Slifkin, PhD and Herb Layman who initiated a Pennsylvania Microbiology Interest Group and was a giant in clinical microbiology in Pittsburgh, PA.

Surprisingly, our largest attendance was the day after 9/11/2001, and heralded the last phase of our Spring and Fall meetings, which became difficult for attendees. The focus returned to a single meeting in the Fall, expanded to a one and a half day program with a single topic or “Boot Camp” on Thursday with the traditional 50 minute programs on Friday. It also heralded moves from Morgantown to Stonewall Jackson, a more central WV location, and then ultimately to Oglebay Park, a proximity which enticed attendees from Pittsburgh and Cleveland, with a driving force of collaboration and exchange of ideas. This also heralded a name change to recognize the input of the WVU Staff and the Department of Pathology and its continued support.

Phase 4: 2010 and Beyond – Web-based learning and the internet – Now we too have entered the electronic, global interface and incorporated several additional features; 1) the ability to recapture previous Fall presentations, both audio and video (<http://wvuecommerce.wvu.edu>) and 2) presentations from around the globe. It is a new goal to make the Fall Symposium truly International, linking with simulcast clinical microbiologist participation from the UK, Asia-Pacific, Singapore, India, and beyond.

In Summary – Our twenty Fall and four Spring Symposiums have provided education for over 2,000 participants representing 10 states and 15 vendors, featuring 80 speakers covering 30 different topics in clinical microbiology. It has been quite a ride... so far!



John G. Thomas'
21st Annual West Virginia
Fall Microbiology Symposium

OGLEBAY RESORT AND CONFERENCE CENTER
WHEELING, WEST VIRGINIA

THURSDAY, SEPTEMBER 23, 2010
BOOT CAMP—HALF DAY
PREPARING FOR INSPECTION
KNOWING YOUR CPT CODES:
UPDATE

FRIDAY, SEPTEMBER 24, 2010
COMING OF AGE

- Yeast/Fungal Susceptibility Testing: Yes or No?
- Stools and a Bad Bug
- Antibiograms: Getting the most out of them for your customer
- “To be or Not to be? That is the question.”
- Case Studies: Audience Participation
- Problem Gram Positive Cocci using rapid Identification
- Urine town – On time arrival

Contact: ltomago@hsc.wvu.edu

DINNER AND
DISCUSSION

MICROBIOLOGY
EXHIBITORS

LEARN AT
LUNCH

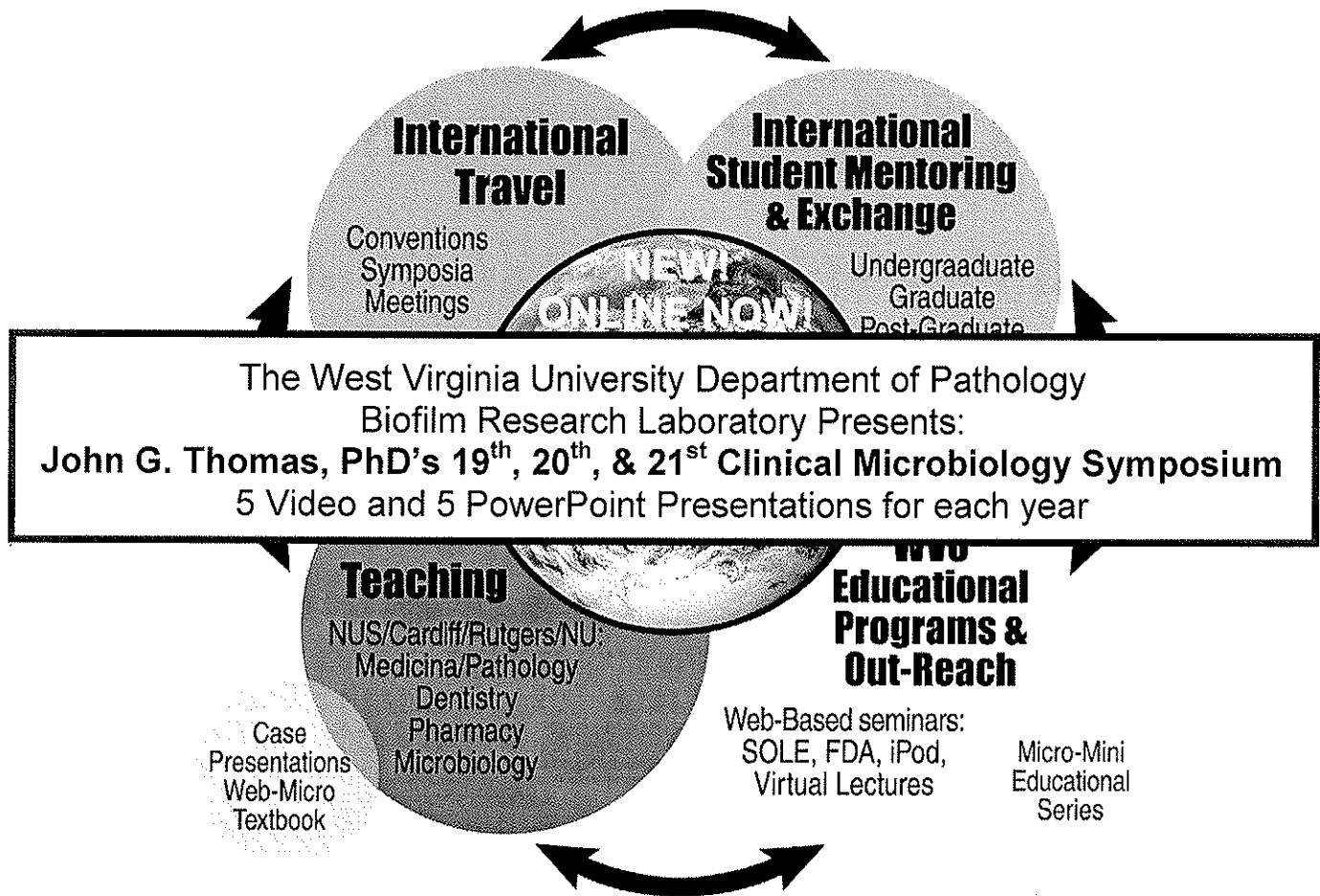
ANTI-
BIOGRAMS

YEAST
MICS

CASES

INSPECTION
READY

HELPING EDUCATORS MAKE A WORLD OF DIFFERENCE: "Mountaineer Roots: Global Branches"



Available at: <http://wvuecommerce.wvu.edu>
Click on Clinical Microbiology Seminars



The 21st Annual Microbiology Symposium
September 23 – 24, 2010
Oglebay Resort and Conference Center
Wheeling, WV
www.hsc.wvu.edu/som/pathology/thomas

NEW!
Online Now

John G. Thomas, PhD's
19th and 20th Clinical Microbiology Symposium
Video and PowerPoint Presentations

21st Annual, Sept 23 & 24, 2010
Oglebay Park, Wheeling, WV

<u>Speaker</u>	<u>Topic</u>
Dr. Vickie Baselski	Preparing for Inspectors Knowing Your CPT Codes: An Update
Dr. Paul Granato	Shiga Toxin Producing <i>Escherichichia Coli</i> : The Emergence of a Pathogen and CDC Guidelines for Its Diagnosis
Dr. Dennis Wegner	Gram Stain Directed Management of Bacterial Pneumonia
Beverly Orr, MT	Confused about those Aerobic Gram Positives? You are Not Alone!
Dr. George Goedesky	Applicability of MALDI TOF Mass Spectrometry and Rapid Bacterial Characterization

20th Annual, Sept 24 & 25, 2009
Oglebay Park, Wheeling, WV

<u>Speaker</u>	<u>Topic</u>
Dr. J. Michael Miller	Specimen Management and Clinical Relevance in Microbiology
Dr. Richard (Tom) Thompson	Susceptibility Testing Challenges: Battling the Resistance Pandemic
Dr. Paul Schreckenberger	Detection and Reporting of Beta-Lactam Resistance in Terobacteriaceae
Dr. Rocco LaSala	<i>Staphylococcus aureus</i> : VISA, VRSA, HA/CA-MRSA, PVL, PCR, etc.
Dr. John G. Thomas	Hot Topics

19th Annual, Sept 25 & 26, 2008
Oglebay Park, Wheeling, WV

<u>Speaker</u>	<u>Topic</u>
Dr. David Williams	Candidosis and Other Fungal Infections of Humans: A Current Perspective
Dr. Gary Procop	Mycology for the Bacteriologist: Basic Mycology for Weekend Coverage
Dr. Joseph Campos	Microbial Jeopardy: Case Presentations
Dr. John G. Thomas	Alphabet Soup of Pneumonia: CAP, HAP, VAP
Dr. John G. Thomas	Hot Topics
Dr. David Williams	Molecular Microbiology and its Current Role in Diagnosis of Fungal Infections
Dr. William Pasculle	<i>Clostridium difficile</i> : Unmasking its Frequency in Disease

Dr. Milton R Hales Lecture in Pathology

June 11, 2010 Speaker:

J. Paul Robinson, Ph.D.

Friend, Research Colleague, Renowned International Scientist and Educator, and Mountaineer Extreme.

Credentials:

SVM Professor of Cytomics

Professor of Immunopharmacology

Professor of Biomedical Engineering

Professor & Director, Purdue University Cytometry Laboratories

Deputy Director, Bindley Biosciences Center, Imaging & Cytomics

Dept. of Basic Medical Sciences, School of Veterinary Medicine

Weldon School of Biomedical Engineering, College of Engineering

Purdue University

1203 W. State, Bindley Bioscience Center

West Lafayette, Indiana 47907

Telephone: (765) 494-0757 Fax: (765) 494-0517

Email: jpr@flowcyt.cyto.purdue.edu Mobile: +1-765-491-3848

Accomplishments:

Awards and Honors:

- President, International Society for Analytical Cytology, 2006-2008
- 2007- Focus Award in recognition of outstanding contribution to the furthering of Purdue University's commitment to disability accessibility and diversity (Access Scope Team)
- 2006 -Team Award, Purdue College of Agriculture, for Biosensor Detection Team, with additional participation from Electrical and Computer Engineering, Basic Medical Sciences, Veterinary Medicine, & Food Science.
- President-elect, International Society for Analytical Cytology, 2004-2006
- Elected to the College of Fellows, American Institute for Medical and Biological Engineering, 2004
- Pfizer Award for Innovative Research, 2004

Scholarly:

- Editorial Board, Int. Journal of Functional Informatics & Personalized Medicine, 2008-
- Editor-in-Chief, Current Protocols in Cytometry, 1995- (Founding Editor)
- Associate Editor, Histochemica et Cytobiologica, 1998-
- Associate Editor, Cytometry Part A, 2007-
- Editorial Board, Cytometry Part B, 1994-2006

Peer Reviewed Publications: 135

Book Chapters: 28

Books Published: 8

Published Multimedia Productions: 15

Editor of Journals:

- Editor-in-Chief: Current Protocols in Cytometry, J. Paul Robinson, Eds. Zbigniew Darzynkiewicz, Alberto Orfao, Jurek Dobrucki, Peter Rabinovitch, Robert Hoffman, John Nolan, Simon Watkins Publisher: John Wiley and Sons, New York, NY. 1997- (this is updated 4 times a year).
- Associate Editor: Folia Histochem Cytobiol, Warsaw, Poland
- Associate Editor: Cytometry (A), 2006-
- Editorial Board: Int. Journal of Functional Informatics & Personalized Medicine, 2008
- Editorial Board: Communications in Clinical Cytometry (Cytometry Part B), 1994-2007

Personal Achievements:

- Summit Mt. Rainier, Washington State, 14,360 ft, May 30, 2008
- Summit Mt. McKinley (Denali), Alaska, 20,160 ft, July 1, 2008 (highest peak, North America)
- Summit Mt. Manaslu, Himalayas, Nepal, 8,163m (27,600ft), Oct 3, 2008 (8th highest mountain in the world)
- Summit Mt. Everest, May 23, 2009, 29,035ft (<http://www.cyto.purdue.edu/trackpaul>)

Note:

Dr. Robinson is probably the most acclaimed cytometrist in the world, either at sea level or 23,035 above it. His research interests are focused on the immune system during stress, and includes thermal injury, diabetes, and surgery; tissue engineering, multi-variant analysis, and when combined, detection of bacteria using laser light scatter. His boundless energy has also addressed global education and heightened awareness of diseases that alter the immune system; he has produced over 15 CDs and videos that enhance the methods of detection.

His three presentations at WVU Dept of Pathology will be complemented by diseases of mountaineering (mountain climbing) and pathologic consequences from scaling the world's tallest peaks.

West Virginia University Medical School

J. Paul Robinson

8 AM: Pathology Residents

Title: "Cytometry in Clinical Pathology: Will it ever be truly diagnostic"

Objectives of the presentation

1. Define current state of cytometry and how it works
2. Establish criteria for determination of a diagnostic utility
3. Outline how future evaluation of clinical specimens might occur
4. Show how future systems may be cost effective and more efficient
5. Show some interesting effects of high altitude on the body from personal experience

Noon: WVU Staff , primarily path , micro/immuno etc, graduate school etc

Title: "The future for cytometry as a research technology"

Objectives of the presentation

1. Provide a historical background to the development of the field
2. Show how new developments have resulted in discovery
3. Discuss new research initiative – high content screening for drug discovery
4. Link current capabilities to next generation ideas

4Pm: State wide assoc for Pathologist

Title: "New approaches to rapid analysis: bacterial colony identification, approaches for predictive medicine and how determination of functional properties of cells in almost real time"

Objectives of the presentation

1. Outline a new technology for bacterial colony identification and discuss how it works
2. Show how we propose to do automated classification of patient data
3. Define how we can study functional properties of cells systems by flow cytometry
4. Show how the field has advanced and where it will be in the next decade

CLINICAL TOOLS WEBSITES

Prof. John G. Thomas Home Page

www.hsc.wvu.edu/som/pathology/thomas/clinicaltools

1. Fungemia Detection using MDRA (Multi-Disease Risk Assessment)

- A computerized algorithm that uses 34 pre-selected, weighted clinical and laboratory values (5,3,1) to optimize laboratory microbiology blood cultures for detecting Fungemia
- Multi-Disease Risk Assessment (MDRA) Fungal Infection Action Form [[Excel Version](#) | [PDF Version](#)]

2. Antibiotic Selection using Pharmaco-Economics Form: Linking X, Y, and Z

- A check sheet based on selected MICs that integrates pharmaco-kinetics (X), pharmaco-dynamics (Y) and pharmaco-economics (Z) for anti-infective comparison based on E-Test (AB Biodisc)
- Includes cost comparison of biofilms
- Pharmacoeconomics Form [[PDF Version](#)]

3. Blood Culture Signature: Time to Detection

- Distribution of frequent Bloodstream Isolates by Time to Detection, segregated by 6-Hospital Service Areas (corresponding to 6 Antibiograms), which allows for comparison of 50%, 75%, and 90% detection time or time organism will not be recovered. [[Click Here](#)]

4. Biofilm Susceptibility, Minimal Biofilm Eradication Concentration (MBEC) by Calgary Biofilm Device

- Susceptibility method utilizing Calgary Biofilm Device (CBD) to determine effective inhibition of biofilms, mono or multiple species aerobic or anaerobic.
- These clinical profiles with predetermined anti-infective dilutions are established for organisms associated with 3 symptoms: respiratory, urinary, and sepsis.
- Also includes an Anti-Fungal MBEC and Dental MBEC using hydroxyapatite to duplicate the tooth enamel surface. The Ratio of MBEC:MIC, may be a clinical indicator or outcomes. [[Click Here](#)]

5. Biofilm Susceptibility by Biofilm Elimination Concentration (BEC) using 30% F-127 Poloxamer (reverse-gel) and E-Strips with interpretation for clinical significance

- The 30% F-127 Poloxamer (liquid at 4°C) converts planktonic to biofilm phenotype formation at 37°C (solid) and allows for the utilization of E-

Strips to measure anti-biofilm phenotype susceptibility simulating a Kirby Bauer Technique.

- By measuring both the planktonic (MH) and biofilm (Poloxamer) MIC and calculating a ratio (BEC/MIC), clinical significance can be evaluated.
- Four antibiotic templates (A-D) list potential antibiotics to evaluate by isolate site of infection (Respiratory, Urinary, Wound, Blood) [link]

6. Selected Antibiograms

- Unique selection of antibiotic profiles, sorted by 1) mechanism of resistance, 2) relative resistance, and 3) clinical diseases: respiratory, wound, and indwelling medical devices (IMDs). Uses the TSN USA electronic database with daily data contributions by >500 hospitals (www.mrlworld.com).
[[Blood](#)] [[Urine](#)] [[All Specimens for All Locations](#)]

7. BioTumor: Tumors are Eucaryotic Biofilms Tumor-Biofilm Studies

- Therapeutic pharmacology discovery (Drug Discovery) (Variable = Drug and Constant = 3 organism mixed biofilm matrix)
- Point of Care Testing (Variable is patient isolate and Constant is 17 drug battery template)
- A list of 18 PowerPoints with text outlines the present studies. (Please see Posters and Abstracts from recent Presentations)

For More Information:

Contact Dr. Thomas at jthomas@hsc.wvu.edu or 304-293-3204 or Sara Posey at sbposey@hsc.wvu.edu

SECTION V. SUMMARY AND CONCLUSION

- **TRANSITION:** Exciting times as we transition from Adult VAP to the NICU and to wounds and tumors. But the Oral-Systemic connections are still critical.
- **GLOBALIZATION:** We want to sample the world recognizing the importance of bacterial clones and the unique pressures of regions of the Globe. MRSA and Fungi (Hong Kong) have become our focus.
- **EDUCATION:** This also allows us to expand our eWEB and webinars.
- **NEW METHODS:** We see the end of traditional microbial methods, both in the laboratory and in translational research. In the former, we are going to investigate BARDOT and MALDI-TOF, in the later, Flow cytometry and IVIS with an emphasis on animal models.

SECTION VI. ABSTRACTS/POSTERS

- West Virginia University School of Pharmacy, Pharmaceutical and Pharmacological Sciences, Health Science Center. Research Day, October 1st 2010.

1. Biofilm (Prokaryote) Communities Predicting Tumor (Eukaryote) Therapy. Posey S., Motlagh, H., Waters, C., Bragg, S., and Thomas, J.G.
2. A Model and Strategy to Compare Antibiotics-Tumor and Anti-Biofilm Efficacy via Unique Biotyping Classification System. Thomas J.G., Bragg, S., Waters, C., and Posey, S.
3. Effects of Anti-Cancer Drugs on Biofilms: Benign Prokaryotic Tumors. Posey, S., Waters, C., Bragg, S., and Thomas, J.G.

Regional and Local:

4. WV IBRE Poster Day, July 31, 2010. Efficacy of Probiotic Strains *Lactobacillus Casei* and *L. Plantarum* in Altering an Infected Wound Biofilm Versus Planktonic Phenotypes. Authors: Thomas, R., Motlagh, H., and Thomas, J.

National:

5. The Society of Acute Wound Care. Orlando Florida. April 17th thru 20th 2010. Are Wound Specimens Colonized With the Most Resistant Isolates and Associated With Antibiotic Pressures. Authors: Thomas, J., Motlagh, H., Percival, S.

6. Tracking MIC Distribution by Different Wound Specimens: Is There An Optimal Source to Measure Changes in Resistant Patterns.

Authors: Thomas, J., Motlagh, H., and Percival, S.

7. Reducing Wound Colonization by “Replacement Therapy”

(Probiotics) Using *Lactobacillus reuteri* in a TriPhasic Wound Model.

Authors: Thomas, J., Motlagh, H., and Percival, S.

8. Establishing “The Critical Ratio” as a Microbial Biomarker for

Chronic Wound Classification and Status. Authors: Thomas, J.,

Motlagh, H., and Percival, S.

- **AMERICAN SOCIETY FOR MICROBIOLOGY ANNUAL MEETING**

Los Angeles, California, May 23rd to 28th of 2010.

9. Poster I – Classifying MRSA Wound Isolates By MIC Distribution

Integrating Geographic Site and Institution Type. Authors: Thomas,

J., Motlagh, H., and Percival, S.

10. Poster II – Creating and Instituting a Global MRSA Tracking

Network. Authors: Thomas, J., Motlagh, H., and Percival, S.

AWARDED 1ST PLACE FOR LABORATORY RESEARCH.

- **SOCIETY OF ACUTE WOUND CARE (SAWC). Fall Meeting 2010.**

SanDiego California. September 23rd, 24th, and 25th, 2010.

11. Poster I – Biofilms in Chronic Wounds Anti-Koch.

12. Poster II – Confirming the Climax Ratio (CR) as a Microbial Biomarker for Wound Organism Classification and Therapy Selection.

Authors: Motlagh, H., Percival, S., and Thomas, J.

13. Poster III – Evaluation of a Probiotic Dressing Matrix to Inhibit

Wound Pathogens, A Feasibility Study. Authors: Motlagh, H.,

Percival, S., and Thomas, J.

14. Poster IV – The Efficacy of Silver Alginate: Corrected Zone of

Inhibition (C-ZOI) For One Hundred Clinical Isolates. Authors:

Thomas, J., Korum, L., Linton, S., Ocle, T., and Sloan, W.

15. Poster V – Use of Flow Cytometry (FC) in Vital Stains to

Compare Antimicrobial Efficacy of Silver Containing Dressings

Against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Authors: Linton, S., Korum, L., Sloan, W., Thomas, J., and Brundage,

K.

16. Poster VI – Use of Flow Cytometry (FC) in Vital Stains to

Compare Antimicrobial Efficacy of MAXORB™ ExtraAG and

AquaCel™ AG Dressings against *Staphylococcus aureus* and

Pseudomonas aeruginosa. Authors: Thomas, J., Linton, S., Korum,

L., Brundage, K., and Percival, S.

- **EASTERN ASSOCIATION FOR THE SURGERY OF TRAUMA (EAST) ANNUAL SCIENTIFIC ASSEMBLY, January 25 to 29, 2011, Naples Florida.**

17. Poster I – Advanced Endotracheal Tube Biofilm Stage, Not Duration, Of Intubation as Related to Pneumonia.

International:

• **ASM SPONSORED 5th INTERNATIONAL BIOFILM SYMPOSIUM**

Cancun Mexico, November 5th thru 9th, 2009.

18. Poster I – Integrating and Interpreting Biofilm Susceptibility Protocol For Clinical Microbiology. Authors: Thomas, J., and Motlagh, H.

19. Poster II - Effects of Mechanical Ventilation and Aerosolized Tobramycin Within an Endotracheal Tube Biofilm. Authors: Thomas, J., Boas, S., and O'Malley, L.

20. Poster III – A Model and Strategy to Compare Antibiotics-Tumor and Anti-Biofilm Efficacy via Unique Biotyping Classification System. Authors: Thomas, J., Bragg, S., Waters, C., and Posey, S.

21. Poster IV – Unmasking the Benefit of a Probiotic *Lactobacillus reuteri* in a TriPhasic Biofilm Wound Model. Authors: Motlagh, H., Hearn, C., and Thomas, J.

• **EUROPEAN WOUND MANAGEMENT ASSOCIATION (EWMA), ANNUAL MEETING, Berne, Switzerland, May 2010.**

22. Poster I – Are Wound Specimens Colonized With More Resistant Isolates and Associated With Antibiotic Pressure. Authors: Thomas J., Motlagh, H., and Percival, S.

23. Poster II – Tracking MIC Distributions By Different Wound Specimens, Is There an Optimal Source to Measure Changes in Resistant Patterns? Authors: Thomas, J., Motlagh, H., and Percival, S.

- **UNIVERSITY OF MICHIGAN, ORAL MEETING ??**

24. Poster 1 - Does Oral Bacterial Diversity Predetermine Progression to Caries in Children? Results of a Pilot Study. Authors: Usha,S., Wen, A., Zhang, L., et al.

- **BIOFILMS 4 – INTERNATIONAL CONFERENCE, September 1 thru 3, 2010, Winchester, England. University of South Hampton.**

25. Poster I - Biofilms (Prokaryote) Communities Predicting Tumor (Eukaryote) Therapy. Authors: Thomas, J., Posey, S., Motlagh, H., and Waters, C.

- **AMERICAN DENTAL EDUCATION ASSOCIATION (ADEA) 4TH ADEA INTERNATIONAL WOMEN’S LEADERSHIP CONFERENCE,**

September 5 thru 8, 2010. Salvador Brazil.

26. Poster I – A Unique Oral Microbial Signature For Women in Appalachia. Authors: Motlagh, H., Thomas, J., and Crout, R.

- **INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH (IADR), IADR/AADR/CADR 89TH GENERAL SESSION AND EXHIBITION**, March 16 to 19, 2011, San Diego California.

27. Poster I – The Effect of Oral Microbes on Biofilm Formation by Respiratory Pathogens. Authors: Motlagh, H., Thomas, J., and Crout, R.

28. Poster II – A Unique Oral Microbial Signature For Women in Appalachia: Update. Authors: Motlagh, H., Thomas, J., and Crout, R.