Public Health & Food Safety in the Landscape of Changing Climate: Short Course, Microbiology Testing Laboratory Development, and Process Authority Consultation in Georgia

USAID Assignment on May 14 to June 4, Zugdidi/Tbilisi, Georgia

Dr. Aliyar Cyrus Fouladkhah, PhD, MS, MPH, CFS, CPH
Founding Director, Public Health Microbiology Foundation
Associate Professor, Tennessee State University
Yale School of Public Health Alumnus

Climate Change, Transboundary Diseases Epidemiology & FSMA Preventive Control Qualified Individual Workshop
Sole/Lead Instructor: Dr. Aliyar Cyrus Fouladkhah
(May 2022, Zugdidi, Georgia)

It was a great pleasure and lifetime honor for me to come to the culturally-rich and beautiful country of Georgia for a USAID assignment. First and foremost I would like to thank great colleagues in the Washington and Tbilisi USAID programs for all they have done for harmonizing the events of the assignment. A special thank is necessary to Ms. Magda Menabde for her leadership in the Tbilisi office for harmonizing the event of this program. Additionally, I would like to thank Mr. George Gegeshidze, the USAID field officer who assisted in all aspects of this assignment, from travel and accommodation to translation and sharing great information about the culture of Georgia. Additionally, I would need to thank Ms. Lana Chvamania, Ms. Diana Shipman, and Ms. Camille Paulston who were also instrumental in harmonizing the event of this assignment. Finally, a special thank is needed to the Public Health Microbiology Foundation™, which co-sponsored this event.

During the course of this program, 9 individuals were able to receive training and certification. A very similar workshop is scheduled in Alexandria VA USA on September 12, 2022, which costs each attendee $895 for obtaining the certification. So conservatively estimating, the cost-saving for the host is (9*$850=) $7,650. Additionally, conservatively estimating the host is receiving >20 hours of process authority consultation from the Public Health Microbiology Foundation™ that for the US-based entrepreneurs costs $165 per hour so this additional cost-saving will be $3,300 i.e. total cost saving for the host, funded by the Public Health Microbiology Foundation™, is ($7,650+$3,300=) $10,950.

In addition to these activities, the assignment was very fruitful in building capacity and improving the laboratory procedures in the host institution, Association for Agriculture Development in Zugdidi, Georgia. I would like to specially commend Ms. Maia Mikava for her enthusiasm and willingness to learn and implement new and important procedures for ensuring the safety of commodities in her region and further ensuring the public's health.

This project is very timely as we all are going to celebrate World Food Safety Day on June 7 to recognize the great work and collaboration among members in academia, governmental and non-governmental agencies, and private industries to ensure the safety of the public against foodborne diseases. This day is also a great moment to further reflect on identifying needs and opportunities in the private industry to improve their current practices for further mitigating the risk of microbial, chemical, and physical hazards associated with food commodities. The current

unfortunate conflict between the courageous people of Ukraine and unmerited Russian occupiers and the subsequent global food security challenges is another reminder of the importance of global food production and manufacturing infrastructure for international commerce and prosperity as now several nations are bearing the consequences of this conflict with food security challenges.

This assignment was designed to provide food safety training for the Association for Agriculture Development (AFAD) in Zugdidi. In addition to this extensive training for the 6 individuals from the association, one from the USAID Tbilisi office, and two from the leading Food Safety governmental agency of the country, I spend some time in their developing microbiology laboratory for establishing microbiological testing procedures for their operation. This will assist the association to start testing commodities for ensuring the safety of various products.

The city of Zugdidi is in the north-western region of the beautiful country of Georgia and is a culturally-rich region of the country, home to around 42,000 Georgians, and is historically underserved due to its distance from the capital of Georgia, Tbilisi. These capacity buildings in microbiological testing and regulatory compliance could assist the region in further ensuring the safety and exportation of the regional commodities that are much needed in the world today. During the training sessions, I shared critical information about climate change and its effects on both food safety and security and we had vivid discussions on how Georgia could play an even more pronounced role in global food production under the landscape of climate change. I additionally hold interactive presentations about chemical, microbiological, and physical hazards in food and how they could be eliminated using risk assessment in form of hazard analyses and preparing and implementing a food safety plan. Additionally provided information about transboundary infectious diseases of importance for Georgian stakeholders and entrepreneurs.

One of the main commodities in the region is hazelnut and other tree nuts that are exported in large quantities to various regions of the world and one of the main challenges associated with the sale of those products is testing for aflatoxins. As such, I provided documentation and testing procedures for developing and implementing this infrastructure in the Zugdidi region. Additionally, I was able to assist in making recently purchased instrumentation operational and provide information on basic culture-dependent microbiological procedures in the association.

Perhaps most importantly, and since the association is administering various antibiotics in their operation, I developed a testing program and antibiotic stewardship procedures for better administration of these life-saving and important medications. In the proposed procedures, within the first 24 hours, the association could determine the best category of antibiotics needed to be utilized based on Gram-staining and within 72 hours they could choose the most effective antibiotic or antibiotic combinations based on Antibiotic Susceptibility Testing. These procedures are further elaborated in the final report of this assignment.

With these being said, I would like to, once again, thank the colleagues in the Washington and Tbilisi offices of USAID and the Public Health Microbiology Foundation™ for all they have done to support this timely and highly productive assignment in Georgia. I commend the host institution for their willingness to learn and implement these new procedures and protect the public's health in their future endeavors.

Best wishes,

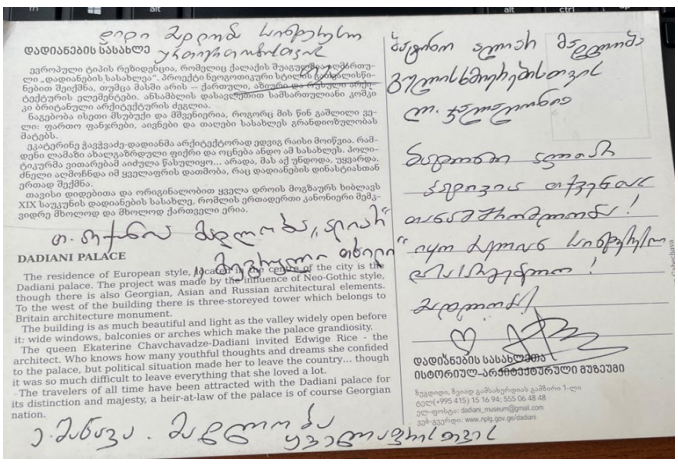
Aliyar Cyrus Fouladkhah, PhD, MS, MPH, MACE, CFS, CPH

Founding Director, Public Health Microbiology Foundation

Associate Professor, Tennessee State University

Faculty Director, Public Health Microbiology Laboratory

Yale School of Public Health Alumnus



LinkedIn Post May 21, 2021

USAID| Public Health Microbiology Foundation

Zugdidi/Tbilisi Georgia Short Course and Technical Assistance

Aliyar Cyrus Fouladkhah • You

Faculty Director, Public Health Microbiology Foundation| Process Authority| ...

4d • Edited •

I am here in the city of Zugdidi in the north-western region of the beautiful country of Georgia for a USAID short course and consultation program. Zugdidi is a culturally-rich and beautiful region of the country, home to around 42,000 Georgians, and is historically underserved due to its distance from the capital of Georgia, Tbilisi.

This week, finished a food safety, climate change, and public health short course and certification program and started consultation with a regional laboratory for building capacity in microbiological testing and regulatory compliance. So that great commodities in this region, that are much needed in the world today, could be tested locally for safety and be prepared for further domestic and global commerce. I also had a chance for a brief and productive meeting with representatives of the United Nation who are one of the funding agencies for this developing testing laboratory.

At the end of the workshop, I met a local artist who was kind enough to perform an informal song and share this video with me. I would like to cordially invite you all to listen to their recording entitled "We Are the World". Their music is beautiful and thoughtful, and I think well illustrates the enormous talent in Zugdidi region; I think these young artists could perform successfully in the best concert halls in the world.

#Public Health #Zugdidi #Food Safety

The Certificates:

Certificate of Participation for FSMA PC QI Workshop

Additional Information about the Public Health Microbiology Foundation Could be Accessed at:

https://publichealthmicrobiology.education/

FSPCA®

FOOD SAFETY PREVENTIVE CONTROLS ALLIANCE

CERTIFICATE OF TRAINING

is awarded to

Eduard Shanava

in recognition for having successfully completed
the Food Safety Preventive Controls Alliance course:

FSPCA Preventive Controls for Human Food

delivered by Lead Instructor

Dr. Aliyar Cyrus Fouladkhah

completed on

05/19/2022

Jason Wan, Interim Director
Institute for Food Safety and Health

IFSH INSTITUTE FOR FOOD SAFETY AND HEALTH
ILLINOIS INSTITUTE OF TECHNOLOGY

Gerald Wojtala, Executive Director
International Food Protection Training Institute

ifpti

Steve Mandernach, Executive Director
Association of Food and Drug Officials

AFDO

Certificate # 9a8c9946

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Dr. Aliyar Cyrus Fouladkhah

completed on

05/19/2022

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Institute for Food Safety and Health

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ILLINOIS INSTITUTE OF TECHNOLOGY

Gerald Wojtala, Executive Director
International Food Protection Training Institute

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Steve Mandernach, Executive Director
Association of Food and Drug Officials

AFDO

Certificate # 3ab190bf

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Ketevan Mania

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the Food Safety Preventive Controls Alliance course:

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Dr. Aliyar Cyrus Fouladkhah

completed on

05/19/2022

Jason Wan, Interim Director
Institute for Food Safety and Health

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ILLINOIS INSTITUTE OF TECHNOLOGY

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Levan Jalaghonia

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Dr. Aliyar Cyrus Fouladkhah

completed on

05/19/2022

Jason Wan, Interim Director
Institute for Food Safety and Health

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ILLINOIS INSTITUTE OF TECHNOLOGY

Gerald Wojtala, Executive Director
International Food Protection Training Institute

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Steve Mandernach, Executive Director
Association of Food and Drug Officials

AFDO

Certificate # ab8e9602

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is awarded to

Maia Gogilashvili

in recognition for having successfully completed
the Food Safety Preventive Controls Alliance course:

FSPCA Preventive Controls for Human Food

delivered by Lead Instructor

Dr. Aliyar Cyrus Fouladkhah

completed on

05/19/2022

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Institute for Food Safety and Health

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ILLINOIS INSTITUTE OF TECHNOLOGY

Gerald Wojtala, Executive Director
International Food Protection Training Institute

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Steve Mandernach, Executive Director
Association of Food and Drug Officials

AFDO

Certificate # 13360a79

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FOOD SAFETY PREVENTIVE CONTROLS ALLIANCE

CERTIFICATE OF TRAINING

is awarded to

Maia Mikava

in recognition for having successfully completed
the Food Safety Preventive Controls Alliance course:

FSPCA Preventive Controls for Human Food

delivered by Lead Instructor

Dr. Aliyar Cyrus Fouladkhah

completed on

05/19/2022

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Institute for Food Safety and Health

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ILLINOIS INSTITUTE OF TECHNOLOGY

Gerald Wojtala, Executive Director
International Food Protection Training Institute

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Steve Mandernach, Executive Director
Association of Food and Drug Officials

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Mako Kvrivishvili

in recognition for having successfully completed
the Food Safety Preventive Controls Alliance course:

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Dr. Aliyar Cyrus Fouladkhah

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Gerald Wojtala, Executive Director
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Steve Mandernach, Executive Director
Association of Food and Drug Officials

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Certificate # 6ce86f9e

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FOOD SAFETY PREVENTIVE CONTROLS ALLIANCE

CERTIFICATE OF TRAINING

is awarded to

Shorena Zarandia

in recognition for having successfully completed
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FSPCA Preventive Controls for Human Food

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Dr. Aliyar Cyrus Fouladkhah

completed on

05/19/2022

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Gerald Wojtala, Executive Director
International Food Protection Training Institute

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is awarded to

Tea Anchabadze

in recognition for having successfully completed
the Food Safety Preventive Controls Alliance course:

FSPCA Preventive Controls for Human Food

delivered by Lead Instructor

Dr. Aliyar Cyrus Fouladkhah

completed on

05/19/2022

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ILLINOIS INSTITUTE OF TECHNOLOGY

Gerald Wojtala, Executive Director
International Food Protection Training Institute

ifpti

Steve Mandernach, Executive Director
Association of Food and Drug Officials

AFDO

Certificate # ddda0f48

Excerpts of Teaching Material

Additional Information about the Public Health Microbiology Foundation Could be Accessed at:

https://publichealthmicrobiology.education/

Public Health Microbiology in Georgia:
Food Safety Under the Landscape of Climate Change

Aliyar Cyrus Fouladkhah, PhD, MPH, MACE, CFS, CPH
Associate Professor, Tennessee State University
Faculty Director, Public Health Microbiology Laboratory
Founding Director, Public Health Microbiology Foundation
Tennessee State University

F2F USAID Program
May 14 to June 3, 2022
Ubilisi and Zugdidi, Georgia

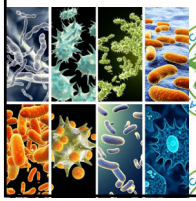

Photo Courtesy: Adobe Stock, graphic purchased/standard licensed by public health microbiology laboratory

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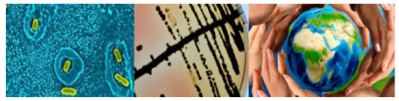


Brief Introduction to my Program

2

- Microbiology and Food Safety, PhD (CSU Animal Science Dept.)
- Applied Statistics and Data Analysis, Graduate Certificate (CSU Statistics Dept.)
- Food Science & Human Nutrition, MS (CSU Food Science Dept.)

Yale SCHOOL OF PUBLIC HEALTH

- Biostatistics and Epidemiology, Advanced Professional MPH
- Food and Drug Regulatory Affairs, Graduate Certificate
- Climate Change and Health, Graduate Certificate

CPH Certified in Public Health

SAS Certified Base Programmer for SAS'9

Colorado State University

Microbac

BOULDER BRANDS established 1906 RODELLE established 1976

Advanced Professional MPH Program

Website: https://publichealth.yale.edu/education/degrees/mph/advanced/
Video: https://www.youtube.com/watch?v=IGVN9JfoIt8

3

Public Health Microbiology Laboratory
Tennessee State University

MPH Curriculum Food Safety and Applied Epidemiology (now under CEPH certification)

- Secured extramural support >$3.4M as PD or Co-PD since 2015
- **T&P** applications both approved, will be effective July 31, 2021.
- **Funding sources**

(1) Dean's Office: $7,000/year and a Research Technician

(2) Association of Food and Drug Officials (AFDO) Process Authority: $15-50K per year depending on the projects

(3) Extramural Funding: >$3.4M since 2015

- National Institute of Health: $**33,680** (PD of Sub-award, 2020-21)*
- Pressure BioScience Inc.: **$35,000** (Role: PD, 2019-2024)
- USDA-NIFA CBG: **$350,000** (Role: PD, 2018-2022)
- USDA-NIFA HEC: **$50,000** (Role: PD, 2018-2021)
- USDA-NIFA FSOP: **$165,000** (Role: PD, 2018-2021)
- Pressure BioScience Inc.: **$23,500** (Role: PD, 2017-2019)
- USDA-NIFA FSOP: **$59,750** (Role: PD, 2016-2019)
- Pressure BioScience Inc.: **$9,400** (Role: PD, 2017-2019)
- NIFA FSOP.: **$880,000** (Role: CO-PD, 2019-2023)**
- USDA-NIFA FSOP.: **$1,197,751** (Role: CO-PD, 2015-2020)**
- NIFA CBG.: **$300,000** (Role: CO-PD, 2018-2022)

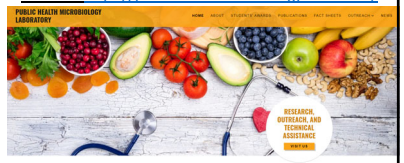
*Pending account setting and internal administrative approval.
** Sub-awardee of Southern Center Main Awards

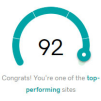
USDA NIFA United States Department of Agriculture National Institute of Food and Agriculture

NIH National Institutes of Health

PBI Pressure BioSciences Inc.

Website: https://publichealthmicrobiology.education/





Congrats! You're one of the top-performing sites

Website performance: 4/22/2020

4

Public Health Microbiology Laboratory Current Members

Current Graduate Students (Primary Advisor/Degree Chair: A. Fouladkhah):

- *Sadive Aras (2018-),* Graduate Research Assistant, (PhD candidate, Biological Sciences c. Food Microbiology)
- *Jyothi George (2019-),* Graduate Research Assistant (PhD student, Biological Sciences c. Food Microbiology)
- *Sabrina Wadood (2020-),* Graduate Research Assistant (MS student, c. Food Microbiology)

Current Dean Scholar/Undergraduate Students:

- *Akiliyah Sumlin* (2018-), Dean Scholar/Undergraduate Research Assistant,*
- *Simen Asefaw (2019-), Undergraduate student (adviser for senior project)*
- *Kennedye Miller (2020-), Undergraduate student (adviser for senior project)*

Current Research Technician, Associates, and Interns (Primary Supervisor: A. Fouladkhah):

- *Mr. Shahid Chowdhury*,* Research Technician (2016-present)
- *Dr. Niamul Kabir, PhD.* Post-doctoral Research Associate (2018-2021).
- Ms. Amir Kashipazha, MS. Data Visualization Intern and Web Editor (2018-present).
- Dr. Naraghi, PhD, MSPH. Visiting Scholar (2020-present).

Current Graduate Student Committee:

- *Shreya Singh Hamal, PhD candidate,* Biological Sciences con. Food Microbiology (Committee member).
- *Yun Tian, PhD student,* Biological Sciences con. Genomics & Immunology (Committee member).
- *Zedonia Williams, MS student,* M.S. degree in Food and Animal Sciences (Committee member).

** Supported by office of the Dean*

Recent Graduates

Abimbola Allison, PhD

Tyler Keene, MS

Monica Henry-Smith, MS

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Students Awards Adviser: A. Fouladkhah

> 45 awards and Scholarships (2017-2020)

Students Success Available at: https://publichealthmicrobiology.education/students-awards

Public Health Microbiology Laboratory

A. Allison, Outstanding PhD Student in College of Agriculture, Receiving an Award from Dean Reddy.

2020 Tennessee Academy of Science
Virtual Health and Medical Sciences Division
1st (S. Wadood);2nd (S. Aras); 3rd (N. Kabir): Adviser: A. Fouladkhah

M. Henry (2nd from left), Outstanding MS Student in College of Agriculture, Received an Award from Dean Reddy.

2019 Tennessee Academy of Science Health and Medical Sciences Division
1st (A. Allison);2nd (S. Aras); 3rd (M. Henry)
Adviser: A. Fouladkhah

2018 Tennessee Academy of Science Health and Medical Sciences Division
1st (M. Henry);2nd (A. Allison); 3rd (J. Adhikari
Adviser: A. Fouladkhah

6

Available at: https://publichealthmicrobiology.education/annual-competitions

Annual State-Wide Competitions For Food Safety Modernization Act, Food Safety and Infectious Disease Students

- **Networking and Stakeholder Engagement**
- **Competition**
- **Regulatory Compliance Booth**
- **Tour of HPP Facilities**
- **Categories:**
- *Graduate Poster and Oral*
- *Undergraduate Poster*
- *Emerging Leader (Post Doc and recent graduates) [Added in 2020]*
- **2017-2020: 31 funded award (extramural grants of A. Fouladkhah)**
- **Current AFRI Application Pending**

Nuss LI, PhD student at UT, presenting her research poster during the Poster Competition session at the IFT Volunteer Section Nashville Meeting

3rd Annual State-Wide Competition for Food Safety Modernization Act, Food Safety, and Food Science Students Winners	
Poster Division	**Oral Division**
1st: Kaleb Karim (TSU) (tied), Jennifer Vaia-Riser (UT) (tied)	1st: Monica Henry (TSU) (tied), Trent Kerley (UT) (tied)
2nd: Abimbola Allison (TSU)	3rd: Abimbola Allison (TSU)
3rd: Shreya Singh Hamal (TSU) (tied), Purni Wickramasinghe (UT) (tied)	3rd: Shan Hong (UT)
3rd: Jayashan Adhikari (TSU)	
Co-Chairs and Judges	
Dr. Alizer Fouladkhah	
Dr. Niamul Kabir	
Mr. Shahid Chowdhury	
Dr. Monique McCallister	

A. Fouladkhah: Competition Founder and Director

4th (2020) Annual State-Wide Competition for Food Safety Modernization Act, Food Safety, and Food Science Students

Emerging Leaders Category:
First Place (tied): Dr. M. Naraghi, Vanderbilt University of Medical Sciences (**Award: $350**)
First Place (tied): Dr. Tyler-Bowden, Tennessee State University (**Award: $350**)
Second Place: Dr. Md Niamul Kabir, Tennessee State University (**Award: $200**)
Third Place: Not awarded this year.

Finalists in Oral Competitions for Students:
First Place: Sabrina Wadood, Tennessee State University (**Award: $400**)
Second Place: Jyothi George, Tennessee State University (**Award: $300**)
Third Place: Sadiye Aras, Tennessee State University (**Award: $200**)

Finalists in Poster Competitions for Students:
First Place: Andrea Neto Veloza, University of Tennessee (**Award: $350**)
Second Place: Anika Chowdhury, Middle Tennessee State University (**Award: $250**)
Third Place (tied): Shreya Hamal, Tennessee State University (**Award: $150**)
Third Place (tied): Akiliyah Sumlin, Tennessee State University (**Award: $150**)

3rd (2019) Annual State-Wide Competition for Food Safety Modernization Act, Food Safety, and Food Science Students

2019 Graduate Oral Competition Winners
1st Place (tied): Monica Henry, TSU
1st Place (tied): Trent Kerley, UT
2nd Place: Abimbola Allison, TSU
3rd Place: Shan Hong, UT

2019 Graduate Oral Competition Winners
1st Place (tied): Jennifer Vaia-Riser, UT
1st Place (tied): Kaleb Karim, TSU
2nd Place (tied): Shreya Singh Hamal, TSU
2nd Place (tied): Purni Wickramasinghe, UT
3rd: Place Jayashan Adhikari, TSU

2nd (2018) Annual State-Wide Competition for Food Safety Modernization Act, Food Safety, and Food Science Students

2018 Graduate Competition Winners
1st Place (tied): Monica Henry: Graduate Competition, TSU
1st Place (tied): Grace Skupe: Graduate Competition, UT
2nd Place: Devendra Bhandari: Graduate Competition, TSU
3rd Place Michelle Heatherly: Graduate Competition, UT

Public Health Microbiology Laboratory

Also Available at: https://www.ift.org/events/event-listing/2020/sep/4th-annual-statewide-competition-for-food-safety-modernization-act-food-safety-and-food-science-stud

7

Teaching in Tennessee and Internationally

International Travel Reports Available at:
https://publichealthmicrobiology.education/international-programs

2021, 2022 Jamaica
November 2021

Graduate Course in Food Policy and Regulations

2020 Student Evaluation:

- *"…Dr. Fouladkhah is easily the nicest professor I have ever had the pleasure of meeting. He seriously cares about you and how you're doing."*
- *""I loved this class it was so interactive and different from any other class I have taken here at TSU!"*

2019 Student Evaluation:

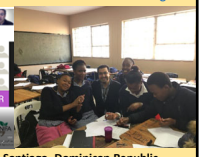
- *"Dr. Fouladkhah is an excellent professor. He does the absolute best job of making students feel comfortable making discussion in class and is exceptionally knowledgeable in the area of food sciences. The in class exercises are definitely helpful to make sure the lectures are being retained and assists in requiring little to no studying outside of the class meetings."*
- *"This course is top notch, one of the best courses I have ever taken, Much gratitude to the lead instructor Dr. Fouladkhah. I learned so much in the class and my knowledge on food policies and regulation has increased a thousandfold."*
- *"Everything was well organised, I think it is perfect. Nothing else is needed."*

2018 Student Evaluation:

- *"This man is so amazing. Learned so much in his class thank you Dr. Fouladkhah."*
- *"He is very helpful and always very encouraging. He helped me planned my studies and even future goals."*

2020, 2022 Haiti (Distant Appointment)
Haiti Government, Fortification with iron, vitamin b12, and zinc

2019, Philippi Township, Cape Town, South Africa:
HIV Prevention Training



2018 & 2020, 2200 Guatemala
Food Safety Training for Food Industry Leadership

2017 Santiago, Dominican Republic
USAID Public Health and Microbiology Training Faculty and Staff of ISA University

8

Process Authority, Variance Committee, PC QI Certifications

(1) **Process Authority**, for state of Tennessee

- List of current 63 process authorities: https://www.afdo.org/directories/fpa/

(2) Additionally, Serve on State **Variance**

"Variance" *means a written document issued by the REGU AUTHORITY that authorizes a modification or waiver of on requirements of this Code if, in the opinion of the REGULA AUTHORITY, a health HAZARD or nuisance will not result modification or waiver.*

Serving as FSPCA Lead Instructor since 2016:

(3) **Preventive Control Qualified Indivi QI) Certifications**. Meeting requirements drug administration.

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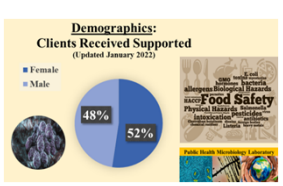

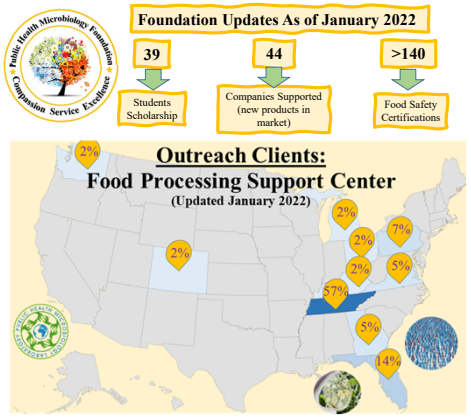

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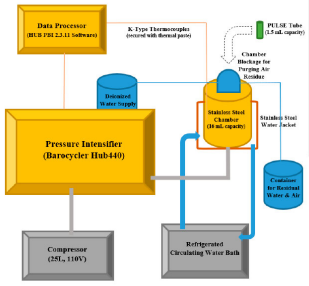
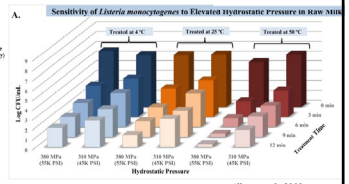
Research Responsibility:
1. Elevated Hydrostatic Pressure
2. Bacterial Biofilm
3. Effects of Climate Change on infectious disease

PBI Pressure BioSciences Inc.

- Elevated Hydrostatics Pressure:
- Hub880, up to **650 MPa**
- Deepest part of Oceans (Mariana Trench): c. **110 MPa**
- Programable unit Hub 440, 380 MPa
- Controlling the temperature
- Synergism with bacteriocin and bactericidal compounds

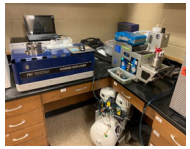




Allison et al., 2018

High Pressure Processing, Public Health Microbiology Laboratory

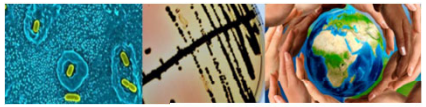
Information about the units: https://ir.pressurebiosciences.com/press-releases/detail/284/pressure-biosciences-announces-commercial-release-of-the



High Pressure Processing, Public Health Microbiology Laboratory

11

12



Epidemiology of Foodborne Diseases

13

Emerging pathogens

Vertical and Horizontal Gene Transfer and Emerging Pathogens

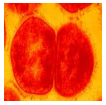
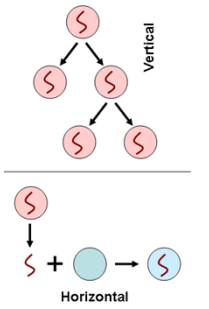
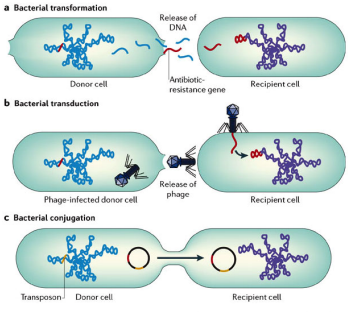


Photo Courtesy: http://www.daviddarling.info/encyclopedia/B/binary_fission.html



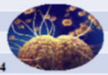


14

Bacterial Multiplication

Binary Fission: 20 minutes or less when intrinsic and extrinsic factors are optimal.

Time	# of Bacteria
0 minutes	1
20 minutes	2
40 minutes	4
1 hour	8
2 hours	64
4 hours	4,096
6 hours	262,144
8 hours	16,777,216
12 hours	68,719,476,736



Bacteria	Estimated Infective Dose*
Salmonella serovars	<10 cells
Shiga toxin-producing *E. coli*	10 to 100 cells
Cronobacter sakazakii	10 to 100 cells
Listeria monocytogenes	<1000 cells
Campylobacter spp.	5000 to 10,000 cells
Staphylococcus aureus	>100,000 cells
Vibrio cholerae	1,000,000 cells

Information and photos are modified and adapted from FDA of Food and Drug Administration, Public Health Reservoir, of Centers for Disease Control and Prevention. Photo Courtesy: Adobe Stock (standard license of photos purchased by the Public Health Microbiology laboratory).

* Calculated for oral ingestion based on epidemiological data from outbreaks and human feeding trials of volunteers. Data obtained from FDA of Food and Drug Administration (2nd edition).

Public Health Microbiology Laboratory: Education, Research, Outreach, and Technical Assistance: https://publichealthmicrobiology.education/

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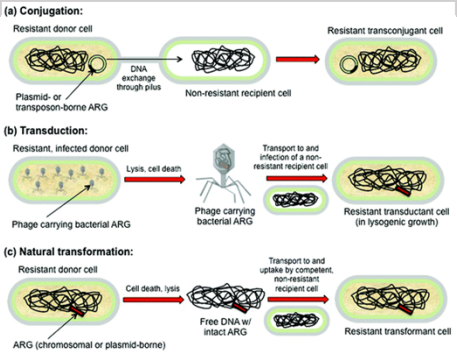
Horizontal Gene Transfer



Donn, 2012

16

Planktonic cells and Biofilm Communities

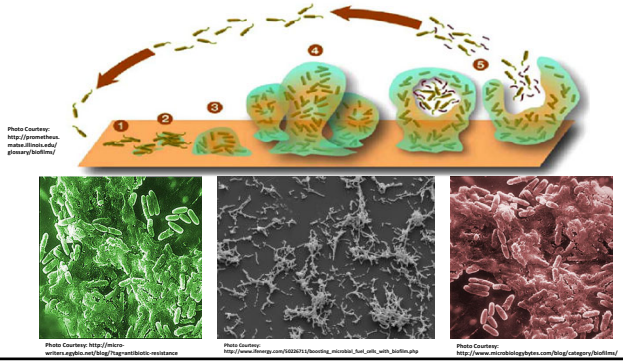


Photo Courtesy: http://prometheus.matse.illinois.edu/glossary/biofilms/

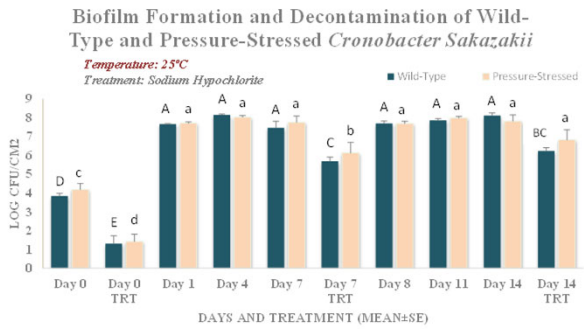
Photo Courtesy: http://micro-writers.egybio.net/blog/?tag=antibiotic-resistance

Photo Courtesy: http://www.ifenergy.com/50226711/boosting_microbial_fuel_cells_with_biofilm.php

Photo Courtesy: http://www.microbiologybytes.com/blog/category/biofilms/

17

Cronobacter sakazakii
Two outbreaks in Tennessee (1998, Memphis; 2001 Knoxville)



Allison et al., 2020

18

Quorum Sensing and Biofilm formation

Shiga toxin producing *E. coli*, not antibiotic treatment due to Quorum Sensing Concerns

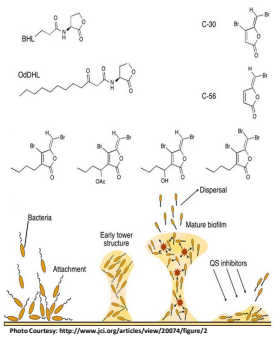


Photo Courtesy: http://www.jci.org/articles/view/20074/figure/2

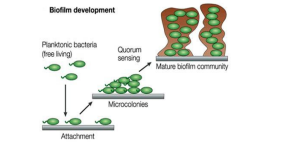


Photo Courtesy: http://labrat.fieldofscience.com/2010/07/quorum-sensing-and-biofilms.html

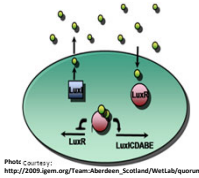


Photo Courtesy: http://2009.igem.org/Team:Aberdeen_Scotland/WetLab/quorumsensing

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Infectious Diseases is a Moving Target…

- It is estimated only 1% of microbial community has been identified.
- Currently etiological agent of 80.3% of foodborne illnesses, 56.2% of hospitalization, and 55.5% of deaths remain unknown.

"Emerging" Pathogens:

- Vertical and horizontal gene transfer spores and biofilm formation
- Quorum sensing and cell to cell communication

"It is the microbes who will have the last word."
-Louis Pasteur

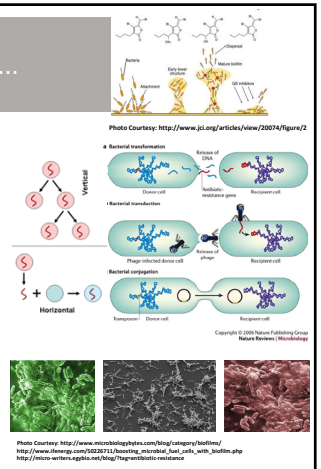

Photo Courtesy: http://www.jci.org/articles/view/20074/figure/2

Photo Courtesy: http://www.microbiologybytes.com/blog/category/biofilms/
http://www.ifenergy.com/50226711/boosting_microbial_fuel_cells_with_biofilm.php
http://micro-writers.egybio.net/blog/?tag=antibiotic-resistance

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Epidemiology of Foodborne Diseases

- **Based on data from 1990s:** *(Mead et al., 1999)*

76 million illnesses, 323,000 hospitalizations, 5,200 deaths in the United States.

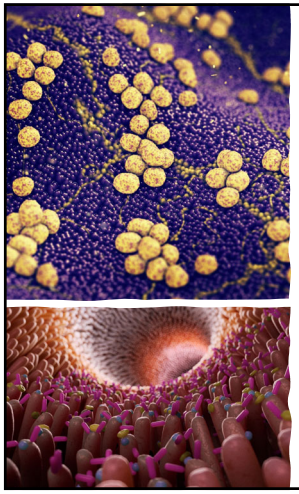
- **More recent estimates show:** *(Scallan et al., 2011)*
- 47.8 million illnesses, 127,839 hospitalizations, and more than **3,037** deaths in the United States.
- 9.4 million illnesses, 55,961 hospitalizations, and 1,351 deaths are cause by 31 known foodborne agents.
- In addition to consumer insecurity, foodborne diseases cause around $**77.7 billion** for losses in productivity and economical losses.
- Approximately 30% of population are especially "at risk" for foodborne diseases (The YOPI's: The young, the old, Pregnant, and Immunocompromised)

21

Significant foodborne pathogens… *based on Mead et al., 1999 and Scallan et al., 2011 studies*

- **Leading etiological agents for illnesses**: *Norovirus* (58%), Nontyphoidal *Salmonella* serovars (11%), *Clostridium perfringens* (10%), and *Campylobacter* spp (9%).
- **Leading etiological agents for hospitalization:** Nontyphoidal *Salmonella* serovars (35%), *Norovirus (26%), Campylobacter* spp (15%), and *Toxoplasma gondii* (8%).
- **Leading etiological agents for death:** Nontyphoidal *Salmonella* serovars (28%), *T. gondii* (24*%), Listeria monocytogenes* (19%), and *Norovirus (11%).*

22



Signs and Symptoms of Foodborne Diseases

- Mild illness (no medical care sought)
- **Guillain–Barré syndrome** (*Campylobacter* and *Salmonella*)
- **Post-infectious irritable bowel syndrome** (*Campylobacter* and *Salmonella*)
- **Reactive arthritis** (*Campylobacter* and *Salmonella*)
- **Haemolytic uraemic syndrome** (*E. coli* O157)
- **End-stage renal disease** (*E. coli* O157)
- Death

23

Significant foodborne pathogens… *based on Scallan et al., 2015 study*

- **Disability adjusted life year** (DALY). *DALY: Loss of life and health due to illness*
- Non-typhoidal *Salmonella* (329000)
- Toxoplasma (32700)
- *Campylobacter* (22500)
- Norovirus (9900)
- *Listeria monocytogenes* (8800)
- *Clostridium perfringens* (4000)
- *Escherichia coli* O157 (1200)

One DALY can be thought of as one **lost year of "healthy" life**.

DALY= YLL+YLD

YLL: Years of Life Lost (YLL) due to **premature mortality** in the population
YLD: Years Lost due to Disability (YLD) for **people living with the health condition**

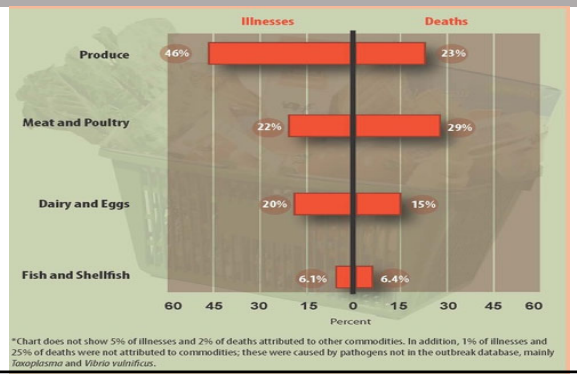
Source: WHO, 2019

62% bacterial agents; 29% parasitic agents; 9% viral agents

24

CDC Estimates of Food Safety Burden

http://www.cdc.gov/foodborneburden/attribution-image.html#foodborne-illnesses



25

Are these outbreaks associated with corporates and lager manufactures?

26

Prevalence of Pathogens in Medium-sized Poultry Operations

- 200–300 ft houses, 3000 to 5000 birds, conventional operation

(Alali et al., 2010)

	Salmonella serovars
Fecal samples (n=420)	38.8%
Feed (n=140)	27.5%

- Total of 135 sample from commercial free-range chicken producers

(Bailey et al., 2005)

	Salmonella serovars
Chicken Carcasses in Operation 1	64%
Chicken Carcasses in Operation 2	31%

Alali et al., 2010, J Foodborne Pathogens and Diseases; Bailey et al., 2005, J Food Protection

27

Prevalence of Pathogens in Small Poultry Farms

- Study of 60 Small poultry slaughterhouses (fewer than 200 birds slaughtered per day)

Sampling sites	*Salmonella* serovars (Albany, Hadar, Indiana, and Enteritidis sub-species)
Carcasses after slaughter	42%
Utensils	23.1%
Storage freezers and refrigerators	71.4%

- The Study concluded "*The widespread occurrence of Salmonella in small slaughterhouses reinforces the need for implementation of effective control measures…*"

Terumi et al., 2000, Journal of Food Protection

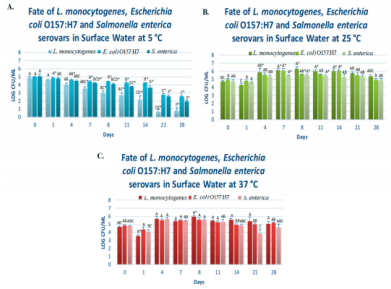
28

Water Safety Study

Public Health Burden of Waterborne Disease

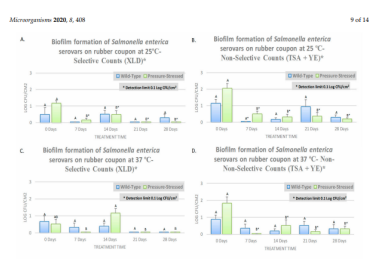
17 waterborne pathogens cause estimated: (Collier et al., 2021)

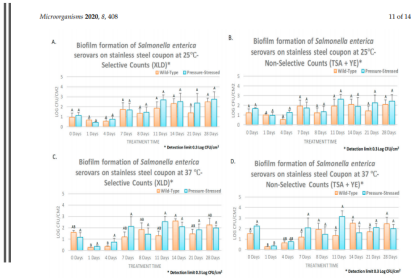
601,000 **illness**; 118,000 **hospitalization**; 6,630 **deaths**, and cost the economy up to $ 8.77 **billions.**



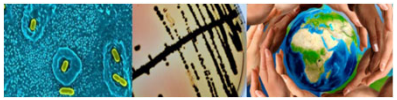
29

Water Safety Study- Biofilm Formation on Abiotic Surfaces





30



Impact of Climate Change on Foodborne and Waterborne Infectious Diseases

31

Salmonella serovars (Non-typhoidal)

- **Annual illness (death): 1,027,561 (378) in humans**
- **Infection** causes nausea, vomiting, diarrhea, fever, headache
- **Primary sources**: Intestinal tract of people and animals
- **Transmitted by** meat, poultry, eggs, raw milk, unpasteurized juice, many other foods (nuts, spices, produce, chocolate, flour)
- **Contributing factors**: cross-contamination, undercooked food, poor agricultural practices

Growth parameters	Minimum	Optimum	Maximum
Temperature	41°F (5.2°C)	95-109°F (35-43°C)	115°F (46.2°C)
pH	3.7	7-7.5	9.5
a_w	0.94	0.99	>0.99
Other	Non-spore former		
Atmosphere	Facultative - grows with or without oxygen		

Sources: ICMSF 1995 and Bad Bug Book 2nd edition, Scallan et al., 2011, and FSPCA

32

Climate Change and Public Health Microbiology

Non-typhoidal *Salmonella enterica* serovars

- **Global death:** 50,000 global death in 2010 (WHO, 2020)
- **Public Health Burden in the U.S.:** >1 million annual cases in 2011 (CDC, 2011)

Climate Change:

- **1 °C increase : 5 to 10% increases in Salmonellosis (WHO, 2010)**
- 2500 to 5000 additional global death
- 50,000 to 100,000 U.S. morbidity

At our current rate (2021 IPCC report)

- >1.5 °C by 2040
- >4.8 °C by 2100


Changing climate
A "threat multiplier" for foodborne and waterborne infectious diseases and antibiotic resistance


microorganisms
Safety of Food and Water Supplies in the Landscape of Changing Climate

33

Vibrio spp.

Currently 760,000 global illness/24,000 death per year.

- Causing about **80,000 illness and 100 death** annually in the United States.
- **Infection symptoms** vary depending on strain, ranging from diarrhea to high fever
- Vibrio is a **halophilic bacterium** and is a major concern in aquaculture industry
- **Primary sources**: Salt water environments and seafood
- Requires salt to reproduce (halophile)

Growth parameters	Minimum	Optimum	Maximum
Temperature	41°F (5°C)	99°F (37°C)	114°F (45.3°C)
pH	4.8	7.8-8.6	11
a_W	0.94	0.98	0.996 (10% NaCl)
Other	Non-sporeformer, requires salt		
Atmosphere	Facultative - grows with or without oxygen		

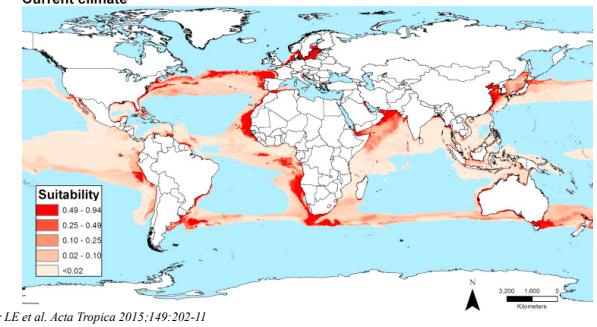
Sources: Seafood Hazards Guide 2011, ICMSF 1995 and Bad Bug Book 2nd edition

34

Vibrio cholerae proliferation in sea water: Current Climate

***Vibrio Cholerae*: currently 760,000 global illness/24,000 death per year**

Current climate

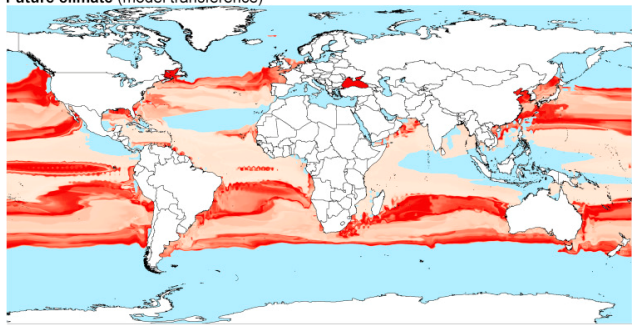


Escobar LE et al. Acta Tropica 2015;149:202-11

35

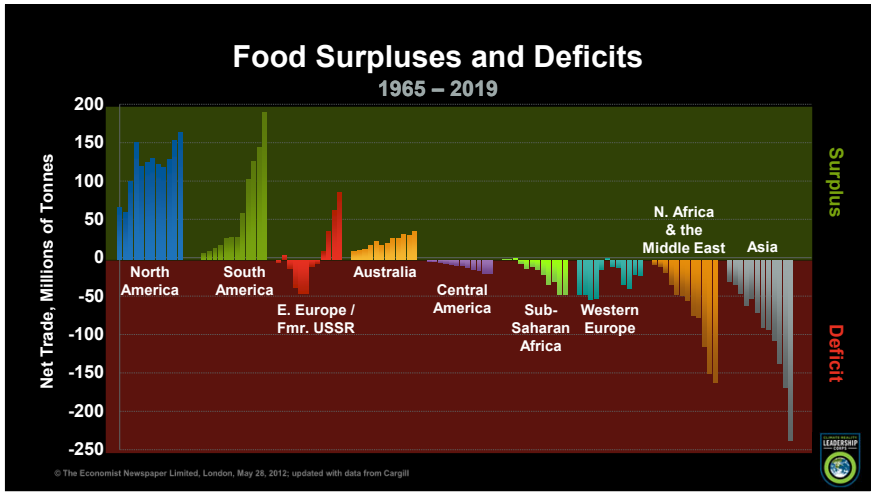
Vibrio cholerae proliferation in sea water: Business-as-Usual Projection in 2100

Future climate (model transference)



Escobar LE et al. Acta Tropica 2015;149:202-11

36


Food Surpluses and Deficits
1965 – 2019
Net Trade, Millions of Tonnes
200
150
100
50
0
-50
-100
-150
-200
-250
North America
South America
E. Europe / Fmr. USSR
Australia
Central America
Sub-Saharan Africa
Western Europe
N. Africa & the Middle East
Asia
Surplus
Deficit
© The Economist Newspaper Limited, London, May 28, 2012; updated with data from Cargill

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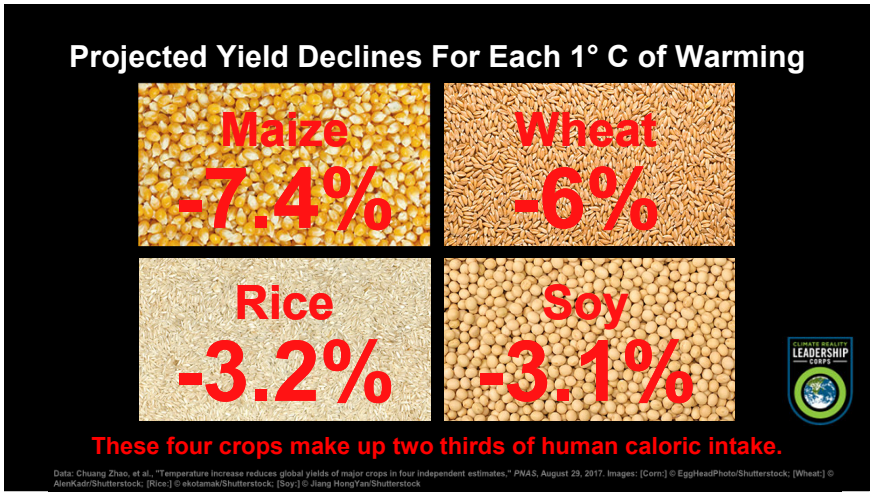
Projected Yield Declines For Each 1° C of Warming
Maize
-7.4%
Wheat
-6%
Rice
-3.2%
Soy
-3.1%
These four crops make up two thirds of human caloric intake.
Data: Chuang Zhao, et al., "Temperature increase reduces global yields of major crops in four independent estimates," PNAS, August 29, 2017. Images: [Corn:] © EggHeadPhoto/Shutterstock; [Wheat:] © AlexKadr/Shutterstock; [Rice:] © ekotamak/Shutterstock; [Soy:] © Jiang HongYan/Shutterstock

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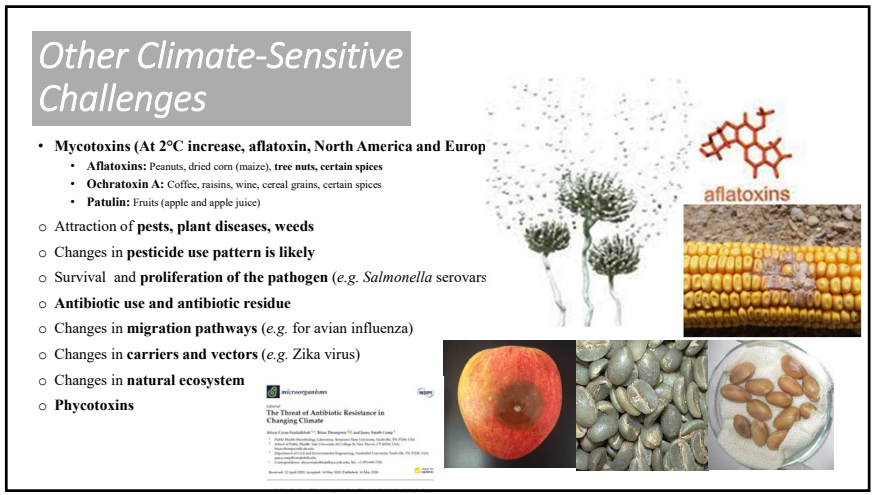
Other Climate-Sensitive Challenges
• Mycotoxins (At 2°C increase, aflatoxin, North America and Europ
  • Aflatoxins: Peanuts, dried corn (maize), tree nuts, certain spices
  • Ochratoxin A: Coffee, raisins, wine, cereal grains, certain spices
  • Patulin: Fruits (apple and apple juice)
○ Attraction of pests, plant diseases, weeds
○ Changes in pesticide use pattern is likely
○ Survival and proliferation of the pathogen (e.g. Salmonella serovars
○ Antibiotic use and antibiotic residue
○ Changes in migration pathways (e.g. for avian influenza)
○ Changes in carriers and vectors (e.g. Zika virus)
○ Changes in natural ecosystem
○ Phycotoxins
aflatoxins
The Threat of Antibiotic Resistance in Changing Climate

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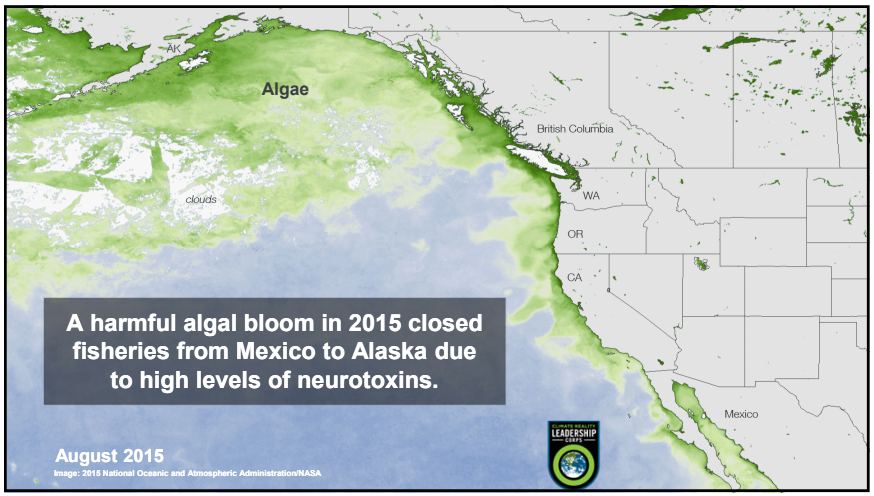
AK
Algae
British Columbia
clouds
WA
OR
CA
Mexico
A harmful algal bloom in 2015 closed fisheries from Mexico to Alaska due to high levels of neurotoxins.
August 2015
Image: 2015 National Oceanic and Atmospheric Administration/NASA

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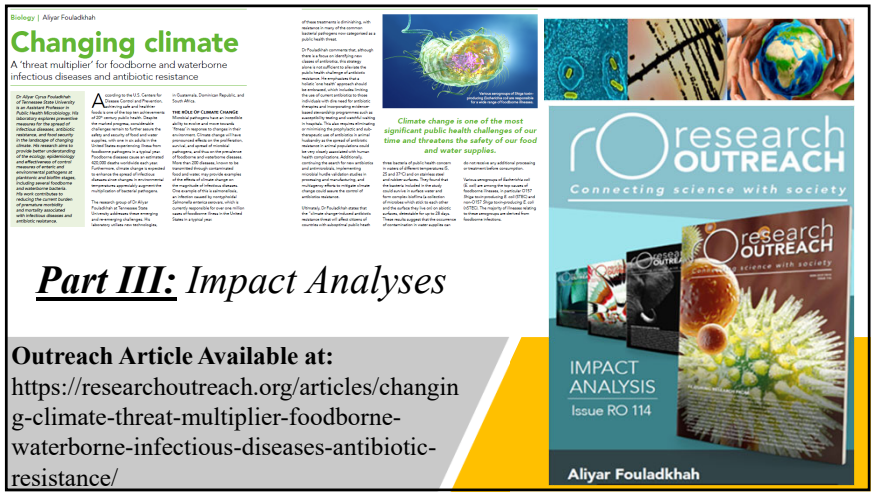
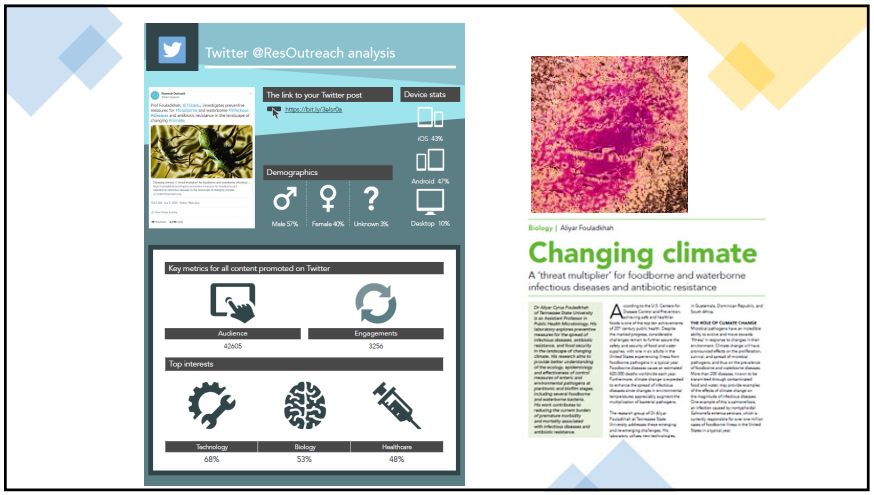

Biology | Aliyar Fouladkhah
Changing climate
A 'threat multiplier' for foodborne and waterborne infectious diseases and antibiotic resistance
Climate change is one of the most significant public health challenges of our time and threatens the safety of our food and water supplies.
research OUTREACH
Connecting science with society
IMPACT ANALYSIS
Issue RO 114
Aliyar Fouladkhah
Part III: Impact Analyses
Outreach Article Available at:
https://researchoutreach.org/articles/changing-climate-threat-multiplier-foodborne-waterborne-infectious-diseases-antibiotic-resistance/

41


Twitter @ResOutreach analysis
The link to your Twitter post
Device stats
Demographics
Key metrics for all content promoted on Twitter
Audience
Engagements
Top interests
Technology
Biology
Healthcare
Changing climate
A 'threat multiplier' for foodborne and waterborne infectious diseases and antibiotic resistance

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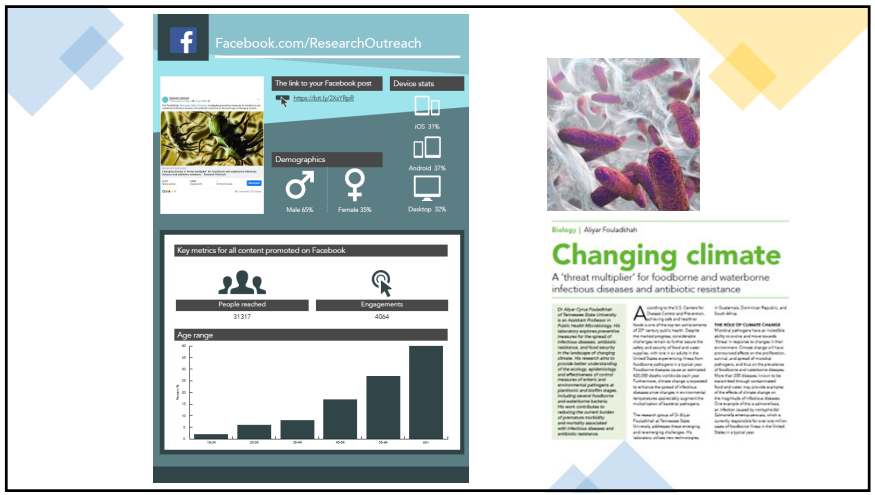
Facebook.com/ResearchOutreach
The link to your Facebook post
Device stats
Demographics
Key metrics for all content promoted on Facebook
People reached
Engagements
Age range
Changing climate
A 'threat multiplier' for foodborne and waterborne infectious diseases and antibiotic resistance

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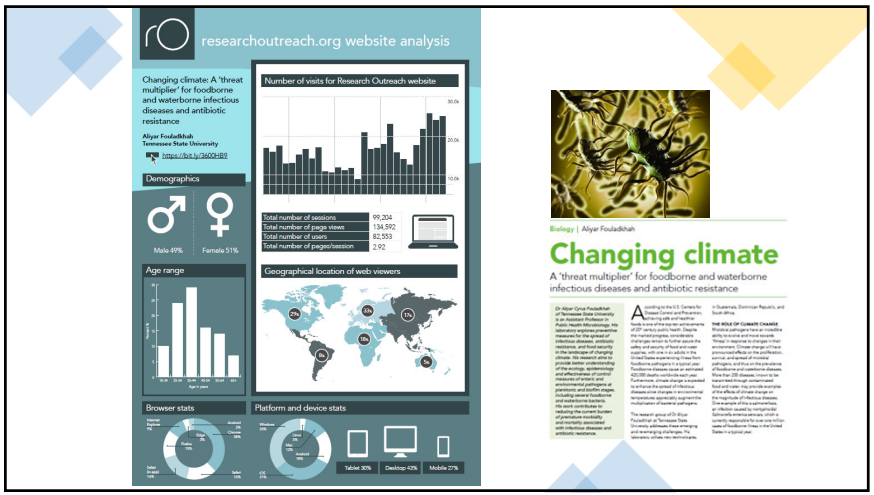
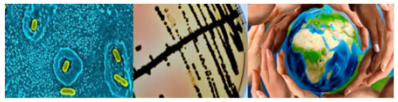

researchoutreach.org website analysis
Changing climate: A 'threat multiplier' for foodborne and waterborne infectious diseases and antibiotic resistance
Number of visits for Research Outreach website
Demographics
Age range
Geographical location of web viewers
Browser stats
Platform and device stats
Changing climate
A 'threat multiplier' for foodborne and waterborne infectious diseases and antibiotic resistance

44



Response of the Government: Food Safety Modernization Act

45

Food Safety Modernization Act (FSMA)

- Signed to law in January of 2011, FSMA is the largest expansion of U.S. food safety authorities since the 1930s.
- Many sectors of agriculture and manufacturing will undergo strict regulations for the **first time in the history of the country**.
- Shifting responses from food safety problems to **proactively prevent** the episodes
- FSMA, a large and comprehensive legislation **broaden FDA's ability** to:
  - **Mandatory recall** of contaminated food products
  - **Enhanced surveillance** to investigate foodborne illness outbreaks
  - Established **new preventive controls** and food safety plans at some food processing facilities and farms
  - Enhanced FDA's **traceability capacity**
  - **Increased inspection** frequencies of high-risk food facilities (both domestic and foreign facilities)
  - Expanded authority and oversight capabilities with regard to **foreign companies**

46

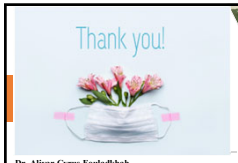
Mandated by FSMA
PC QI Certifications

- Food manufacturing (processors)
- Farmers and growers (producers)
- Transportation, retailers
- Imported foods
- Third party laboratories
- Local, state, and federal agencies
- Foreign governments

Not mandated by FSMA

- FSMA does not directly address sectors under **pre-existing** jurisdictions. HACCP will remain the dominant regulation for:
- Meat, poultry, and egg products (USDA-FSIS)
- Juices, seafood, and shell eggs (DHHA-FDA)
- Very small producers and processors could receive exception from FSMA requirements (**cottage industry**).
- FSMA does not mandate **GM products, antibiotic resistant organisms, organic production, and pesticide and fertilizer** use.

47



Dr. Aliyar Cyrus Fouladkhah,
Faculty Director, Public Health Microbiology Laboratory, Associate Professor, Tennessee State University
Email: afouladk@tnstate.edu or
aliyar.Fouladkhah@ava.yale.edu (life-time alumni account)
Phone: (970) 690-7392
Website:
https://publichealthmicrobiology.education/

Contributions of members of the Public Health Microbiology laboratory is greatly acknowledged. Finding supports of the program funders are additionally and gratefully acknowledged.

Photos Courtesy: Adobe Stock, royalty purchased (standard license) by public health microbiology laboratory

48

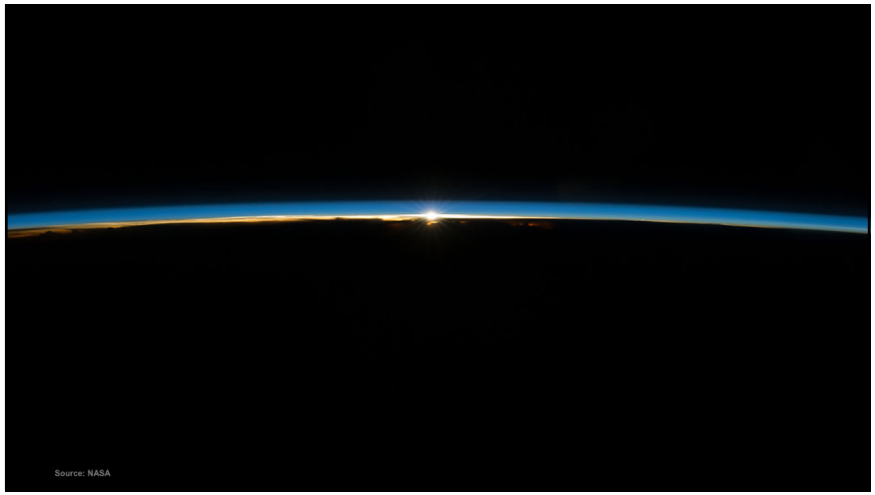
Public Health Microbiology Laboratory

Global Climate Change in Picture

Public Health Microbiology Laboratory
Tennessee State University, Nashville, TN
A. Fouladkhah: Director, Public Health Microbiology Laboratory
5-14 to 6-3-2022 Tbilisi and Zugdidi, Georgia

1

2



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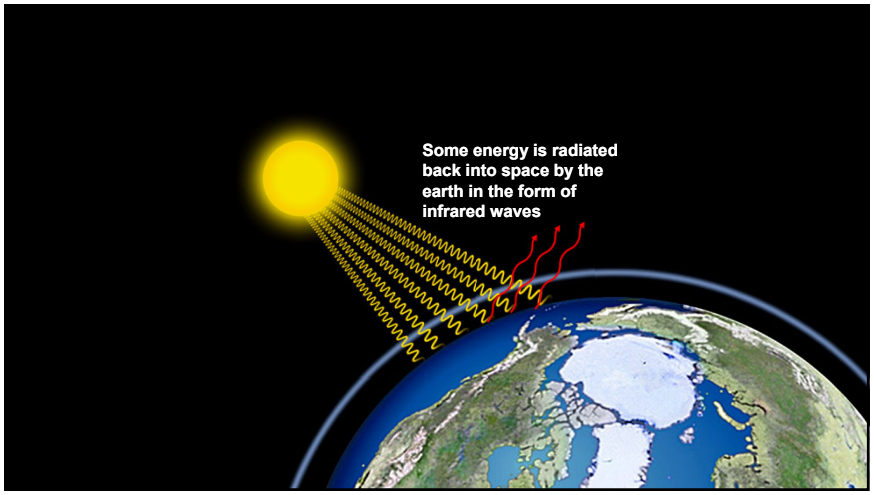

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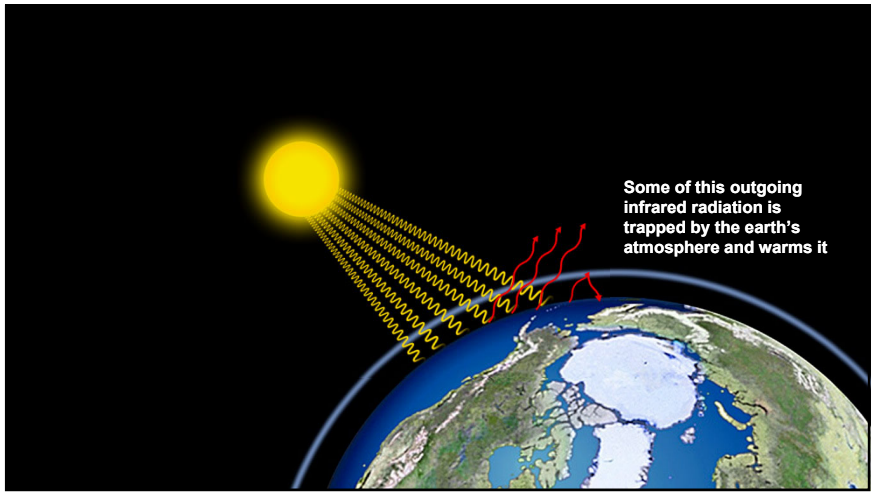
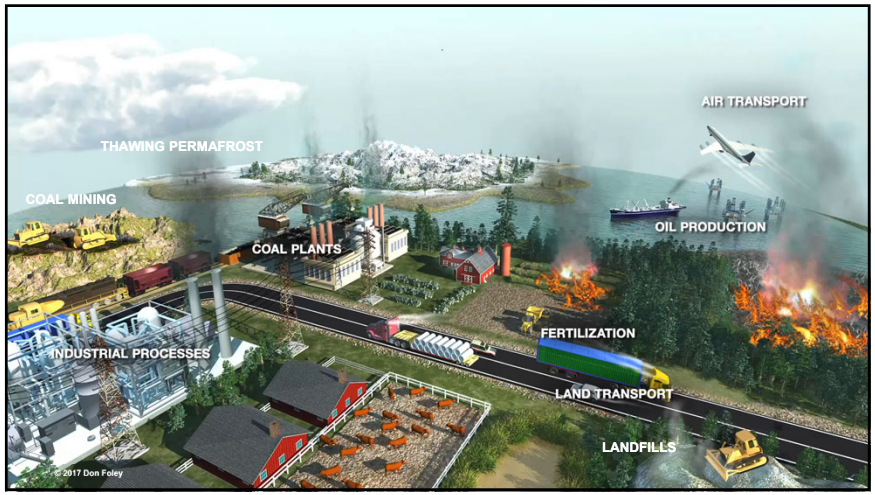

Some of this outgoing infrared radiation is trapped by the earth's atmosphere and warms it

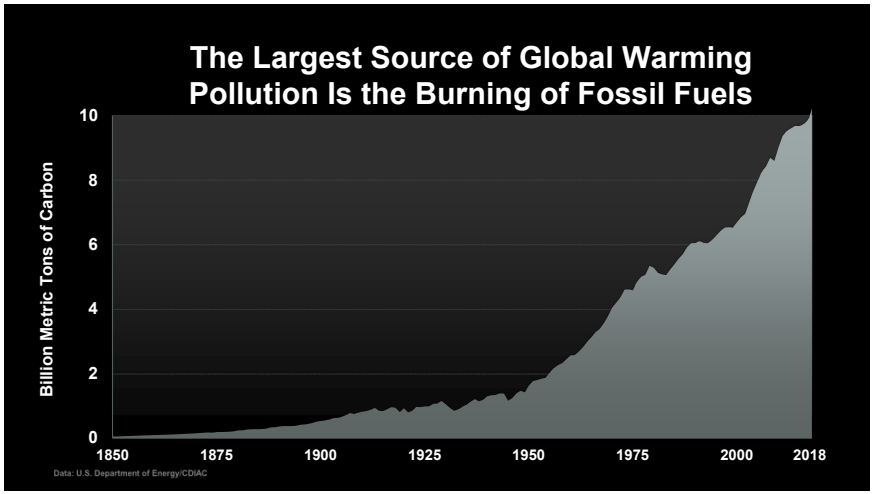
5


We are now spewing 110 million tons of manmade global warming pollution into the thin shell of our atmosphere every 24 hours, as if it were an open sewer.
© Peter Weber/Shutterstock

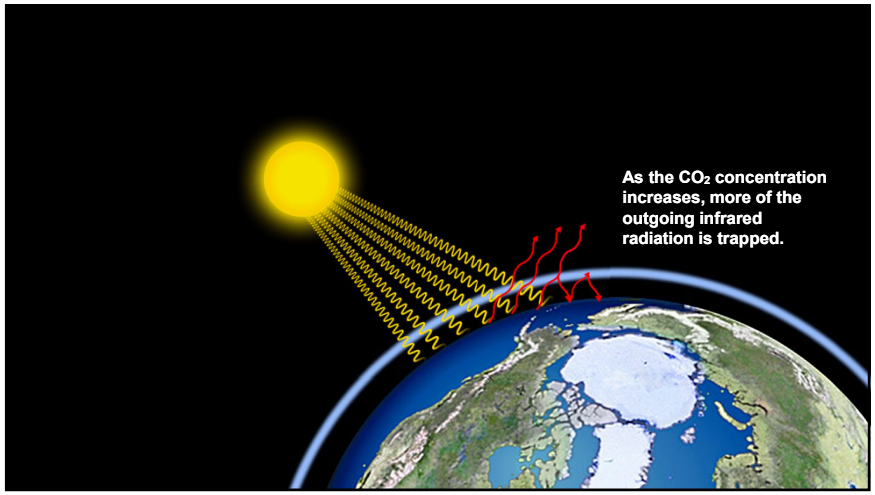
6


AIR TRANSPORT
THAWING PERMAFROST
COAL MINING
COAL PLANTS
OIL PRODUCTION
FERTILIZATION
INDUSTRIAL PROCESSES
LAND TRANSPORT
LANDFILLS
© 2017 Don Foley

7


The Largest Source of Global Warming Pollution Is the Burning of Fossil Fuels
Billion Metric Tons of Carbon
10
8
6
4
2
0
1850
1875
1900
1925
1950
1975
2000
2018
Data: U.S. Department of Energy/CDIAC

8



9

10

The energy trapped by man-made global warming pollution is now "…equivalent to exploding

600,000

First-generation atomic bombs per day 365 days per year."

James Hansen
Former Director, NASA Goddard Institute for Space Studies

11

At least 224 locations around the world set all-time heat records in 2018.

12

The Hottest of All Have Been the Last Seven Years
19 of the 20 Hottest Years on Record Have
Occurred Since the Year 2002

2020 2016 2019 2017 2015 2018 2014 2010 2013 2005

2007 2009 2012 2006 2002 2003 2011 1998 2008 2004

Data: NASA/GISS

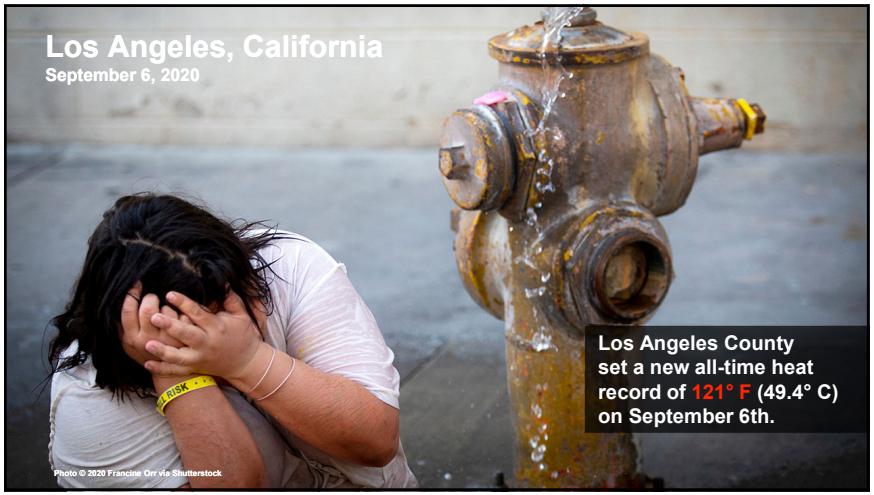
13

The U.S. Southeast is projected to warm up to 8 °F this century.

14

Of the 100 U.S. counties projected to suffer the worst impacts of the climate crisis, 97 are located in the U.S. South.

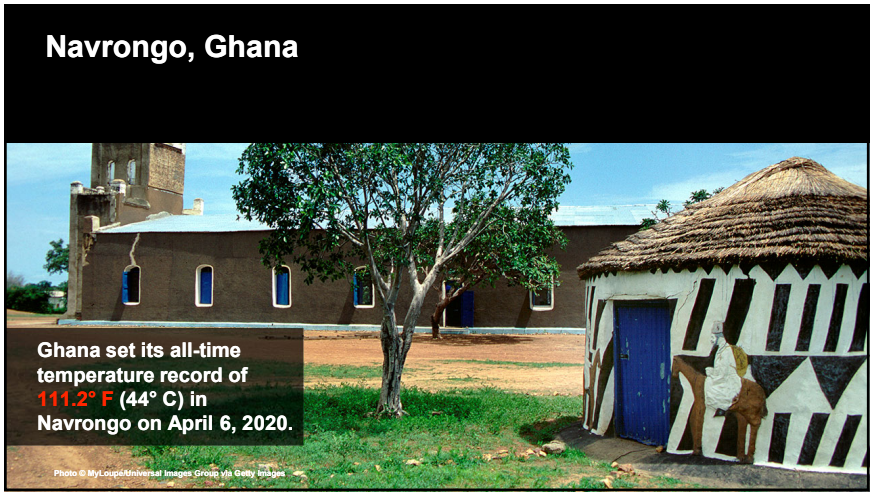
15



16


Paris, France
August 11, 2020
2020 was Europe's hottest year ever, breaking the record set in 2019.
Photo © 2020 AP Photo/Kamil Zihnioglu

17


Navrongo, Ghana
Ghana set its all-time temperature record of 111.2° F (44° C) in Navrongo on April 6, 2020.
Photo © MyLoupe/Universal Images Group via Getty Images

18


Montreal, Canada
July 5, 2018
At least 90 people died in Quebec during the July 2018 heat wave as ambulance services were overwhelmed.
Data: Global News Canada
Photo © 2018 Graham Hughes/The Canadian Press via AP

19


Seoul, South Korea
August 1, 2018
August 1st was the hottest day in Korean history, with temperatures up to 105.8°F (41.0°C).
© 2018 AP Photo/Ahn Young-joon

20

21

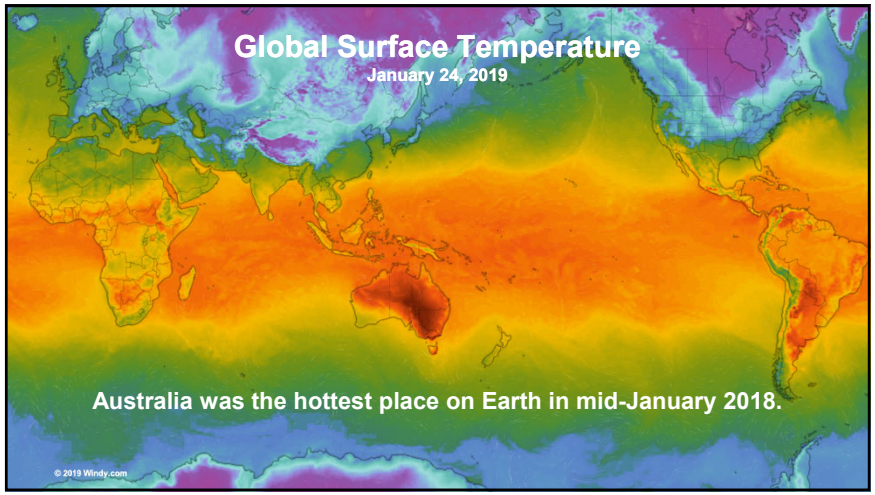
22

On May 19, 2016
India set a new all-time
high temperature record of

124° F (51° C)

23

24


Global Surface Temperature
January 24, 2019
Australia was the hottest place on Earth in mid-January 2018.
© 2019 Windy.com

25


© 2016 Mikael Erekiassen

26


Macon County, Georgia
October 11, 2018
Hurricane Michael caused at least $2.5 billion in damages to Georgia's agricultural sector.
© 2018 Jason Vorhees/The Macon Telegraph via AP

27


Corozal, Puerto Rico
September 24, 2017
An estimated 2,975 people died in Puerto Rico as a result of Hurricane Maria.
© 2017 Ricardo Arduengo/AFP/Getty Images

28

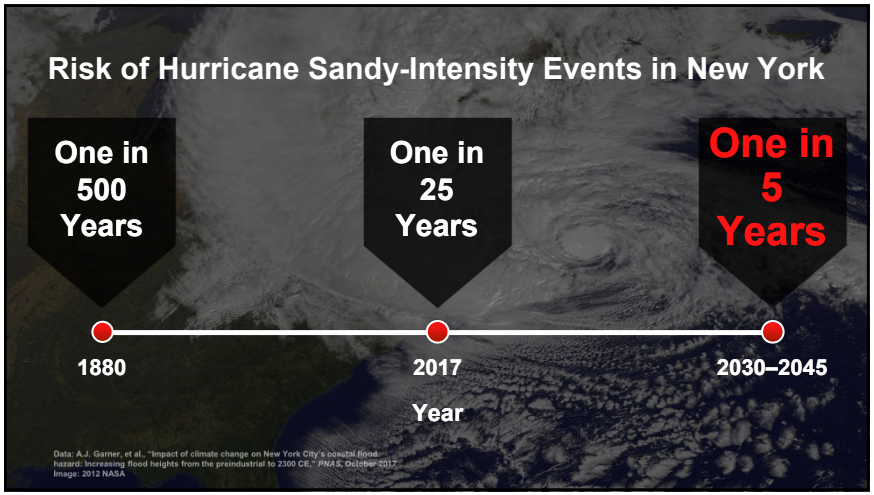
29

30

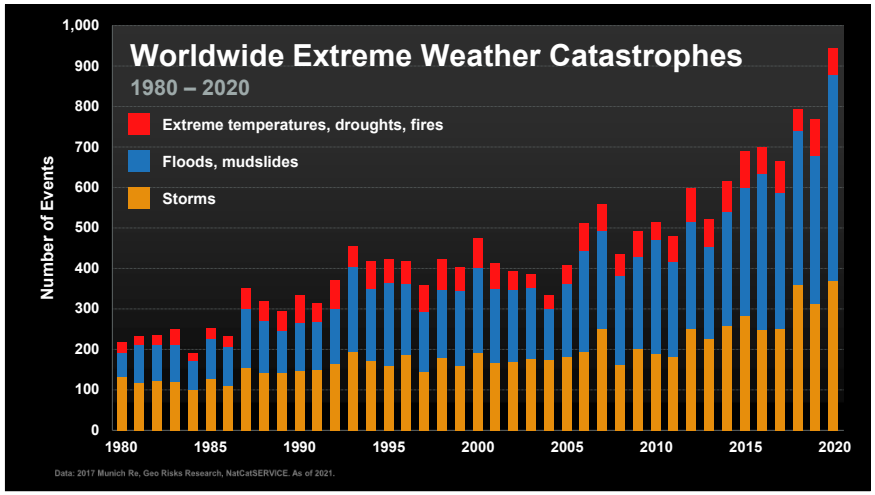
"Unrestrained climate change means we will see many more Harveys in the future."

Michael Mann
Director, Earth System Science Center, Penn State
August 2017

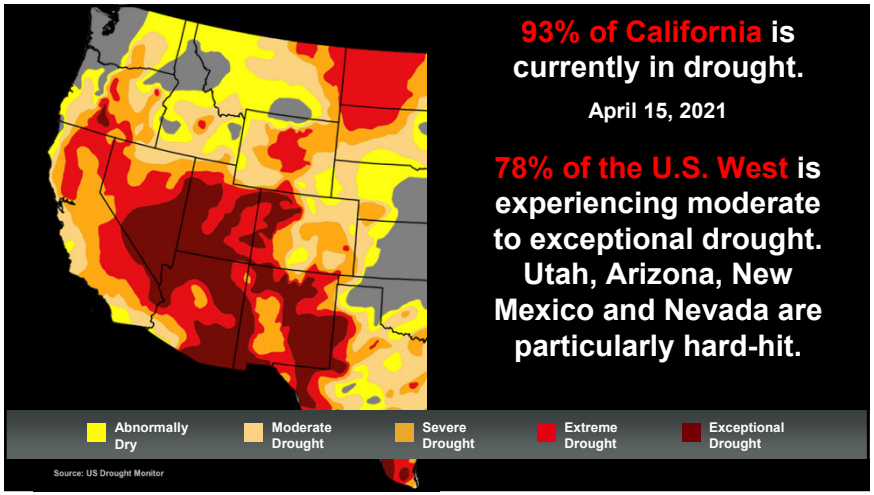
31



32


Worldwide Extreme Weather Catastrophes
1980 – 2020
Extreme temperatures, droughts, fires
Floods, mudslides
Storms
Number of Events
1,000
900
800
700
600
500
400
300
200
100
0
1980
1985
1990
1995
2000
2005
2010
2015
2020
Data: 2017 Munich Re, Geo Risks Research, NatCatSERVICE. As of 2021.

33


93% of California is currently in drought.
April 15, 2021
78% of the U.S. West is experiencing moderate to exceptional drought. Utah, Arizona, New Mexico and Nevada are particularly hard-hit.
Abnormally Dry
Moderate Drought
Severe Drought
Extreme Drought
Exceptional Drought
Source: US Drought Monitor

34


Nearly Dry Well, Gokwe, Zimbabwe
Southern Africa experienced one of its worst droughts in years.
© 2015 Reuters/Philimon Bulawayo

35


Harbin, China
June 1, 2018

36


Yangtze River, Chongqing, China
May 8, 2020
2.4 million people in the lower Yangtze river basin were still affected by drought in early 2021.
Photo © 2020 Imaginechina via AP Images

37


Chennai, India
May 17, 2017
Chennai's state of Tamil Nadu experienced its worst drought in 140 years.

38


Pocone, Brazil
September 26, 2020
Brazil's drought caused $3 billion in damages in 2020.
Photo © 2020 Buda Mendes/Getty Images

39


Schwüblingsen, Germany
August 22, 2018
2018's droughts in Europe resulted in $3.9 billion in economic losses.
© 2018 Julian Stratenschulte/picture alliance via Getty Images

40


The Swiss Army had to airlift water to thousands of cows affected by drought.
Rossinière, Switzerland
August 7, 2018
© 2018 Reuters/Denis Balibouse

41


Saint-André-de-Corcy, Ain, France
July 23, 2017
© 2017 Konrad K./Sipa AP Images

42


Gila National Forest, New Mexico
June 24, 2018
The Buzzard fire burned over 50,000 acres.
© U.S. Forest Service/Gila National Forest

43


Edwards, Colorado
April 1, 2018
The fire season in the U.S. west is now 105 days longer than in 1970.
Photo: © 2018 Chris Dillmann/Vail Daily via AP Source: Climate Central Western Wildfires—A Fiery Future, 2016

44

45

46

47

The 2006 – 2010 drought turned 60% of Syria's fertile land into desert

…and drove 1.5 million people into Syria's already crowded cities

48

"…the Syrian minister of agriculture
...stated publicly that economic and social fallout
from the drought was
'beyond our capacity as a country to deal with.'"

Cable from the U.S. Embassy in Damascus
to the State Department
November 8, 2008

49

United Nation Building Entrance, NY, USA

"Human beings are members of a whole,
In creation of one essence and soul.
If one member is afflicted with pain,
Other members uneasy will remain.
If you have no sympathy for human pain,
The name of human you cannot retain."

Poem from S. Shirazi 1210-1291

50

"In future, the climate in large parts of
the Middle East and North Africa
could… render some regions

uninhabitable,

which will surely contribute to
the pressure to migrate."

Jos Lelieveld
The Max Planck Institute for Chemistry
May 2016

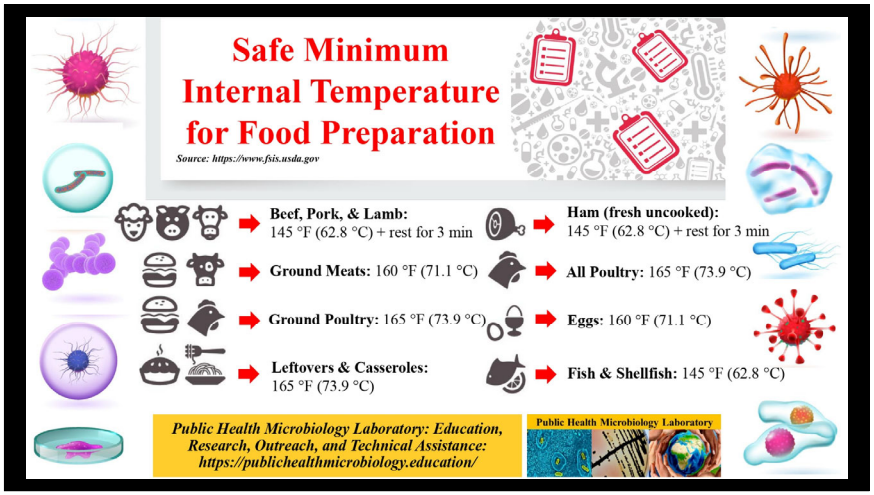
51

The heat index in
Bandar Mahshahr reached

165° F

(74° C) on July 31, 2015

52

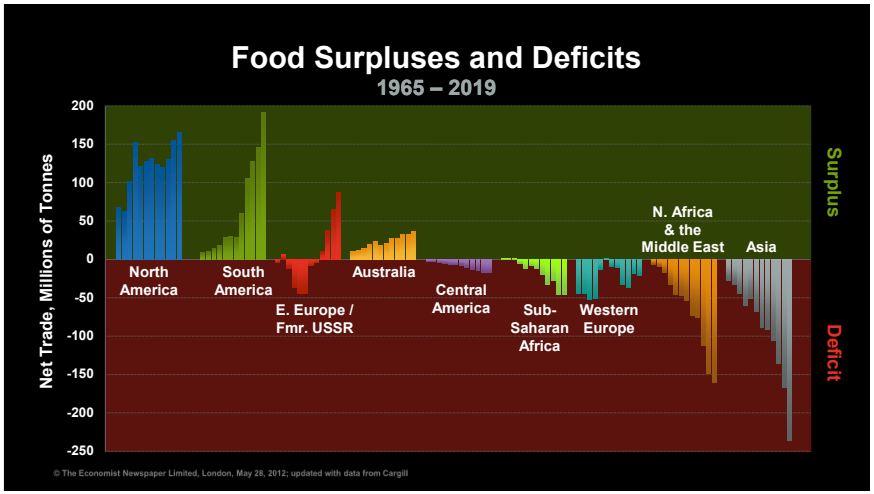


53

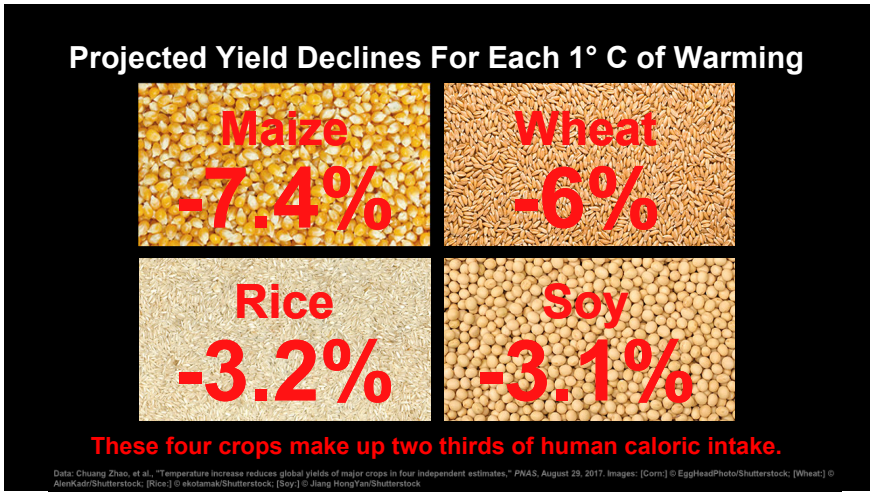
The world could see over
1 billion climate migrants
by the end of this century.

The Lancet Countdown Report
October 2017

54



55



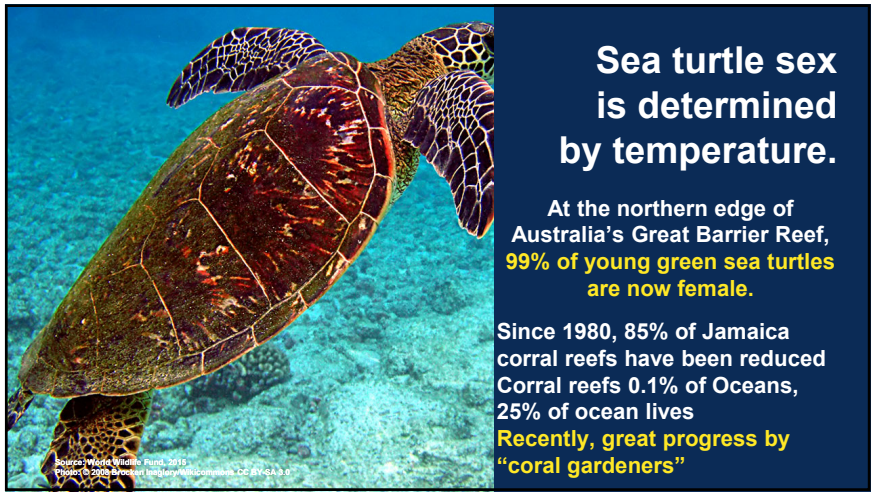
56


Climate change is projected to lower Georgia's soy yields up to 34% and corn yields by as much as 46% by 2050.
Photo © Christopher Boswell via Shutterstock
Soybean Farm, Georgia

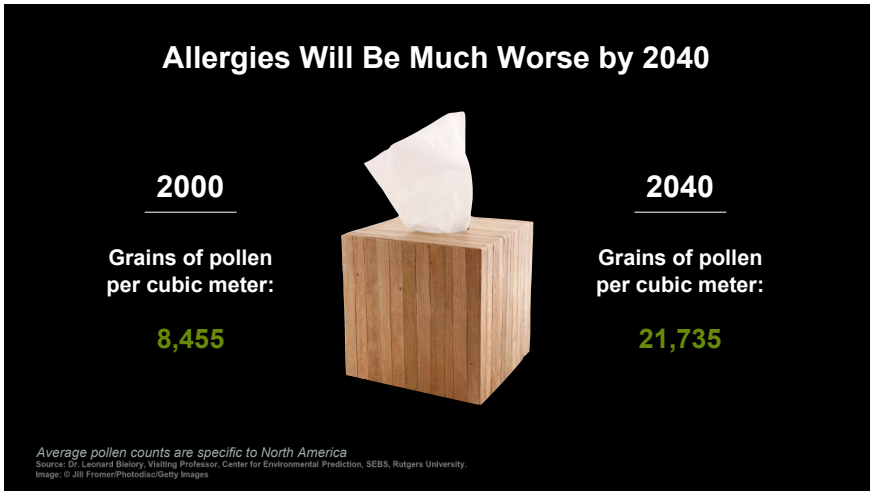
57


Tick-borne diseases affect up to 80% of the world's livestock, with a cost of up to $19 billion per year.
Data: S. Machalaba, WB Karesh, "Vector-borne Diseases: Animals and Patterns," Forum on Microbial Threats, National Academies of Sciences, Engineering and Medicine, 2016
Photo © 2018 Mohssen Assanimoghaddam/picture-alliance/dpa/AP Images
Namibia

58


Sea turtle sex is determined by temperature.
At the northern edge of Australia's Great Barrier Reef, 99% of young green sea turtles are now female.
Since 1980, 85% of Jamaica corral reefs have been reduced
Corral reefs 0.1% of Oceans, 25% of ocean lives
Recently, great progress by "coral gardeners"
Source: World Wildlife Fund, 2015
Photos: © 2009 Brocken Inaglory/Wikicommons, CC BY-SA 3.0

59


Allergies Will Be Much Worse by 2040
2000
Grains of pollen per cubic meter:
8,455
2040
Grains of pollen per cubic meter:
21,735
Average pollen counts are specific to North America
Source: Dr. Leonard Bielory, Visiting Professor, Center for Environmental Prediction, SEBS, Rutgers University.
Image: © Jill Fromer/Photodisc/Getty Images

60

We now risk losing up to **50% of all land-based species** in this century

Most common blood pressure medication (ACE Inhibitor- Captopril) is originally isolated from a snake Venom…

We only have access to non-extinct species.

Source: Nicholas Stern, *The Economics of Climate Change*
Photo: © Dirk Ercken/Shutterstock

61

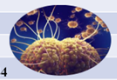
Source: NASA

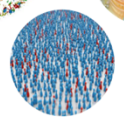
62

Bacterial Multiplication

Binary Fission: 20 minutes or less when intrinsic and extrinsic factors are optimal.

Time	# of Bacteria
0 minutes	1
20 minutes	2
40 minutes	4
1 hour	8
2 hours	64
4 hours	4,096
6 hours	262,144
8 hours	16,777,216
12 hours	68,719,476,736





Bacteria	Estimated Infective Dose*
Salmonella serovars	<10 cells
Shiga toxin-producing *E. coli*	10 to 100 cells
Cronobacter sakazakii	10 to 100 cells
Listeria monocytogenes	<1000 cells
Campylobacter spp.	5000 to 10,000 cells
Staphylococcus aureus	>100,000 cells
Vibrio cholerae	1,000,000 cells

Information and photos are modified and adopted from BBB of Food and Drug Administration, BAM Resources of Centers for Disease Control and Prevention. Photo Courtesy: Adobe Stock (standard license of photos purchased by the Public Health Microbiology laboratory).

** Calculated for oral ingestion based on epidemiological data from outbreaks and human feeding trials of volunteers. Data obtained from BBB of Food and Drug Administration (2nd edition).*

Public Health Microbiology Laboratory: Education, Research, Outreach, and Technical Assistance: https://publichealthmicrobiology.education/

63

Emerging pathogens

Vertical and Horizontal Gene Transfer and Emerging Pathogens

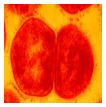
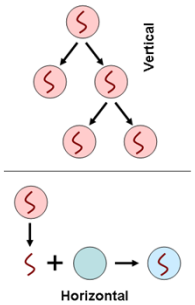
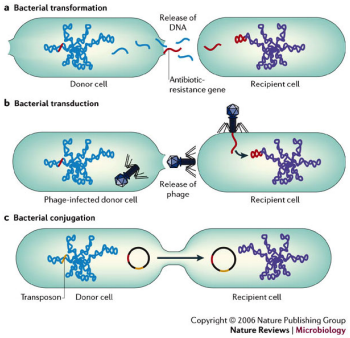


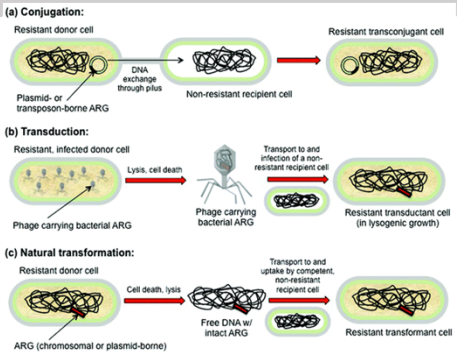
Photo Courtesy:
http://www.davidsarling.info/encyclopedia/B/binary_fission.html





64

Horizontal Gene Transfer



Donn, 2012

65

Planktonic cells and Biofilm Communities

Biofilm formation on **biotic** and **abiotic** surfaces

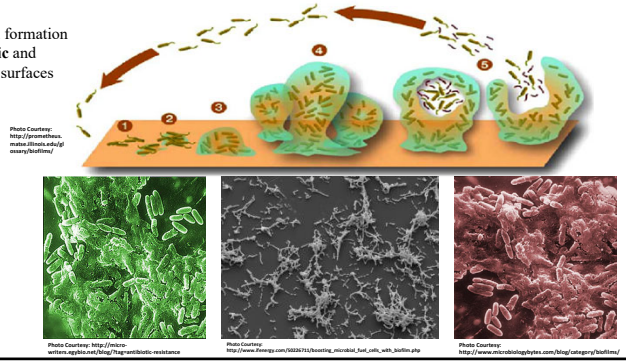


Photo Courtesy: http://prometheus.matse.illinois.edu/glossary/biofilms/

Photo Courtesy: http://micro-writers.agsbio.net/blog/?tag=antibiotic-resistance

Photo Courtesy: http://www.ifenergy.com/50226711/boosting_microbial_fuel_cells_with_biofilm.php

Photo Courtesy: http://www.microbiologybytes.com/blog/category/biofilms/

66

Quorum Sensing and Biofilm formation

Shiga toxin producing *E. coli*, not antibiotic treatment due to Quorum Sensing Concerns

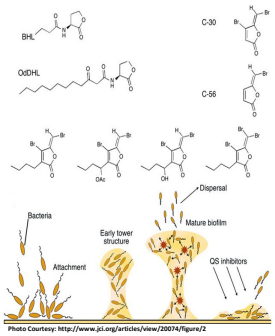


Photo Courtesy: http://www.jci.org/articles/view/20074/figure/2

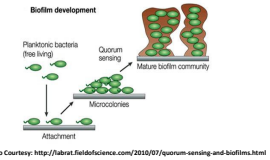


Photo Courtesy: http://labrat.fieldofscience.com/2010/07/quorum-sensing-and-biofilms.html

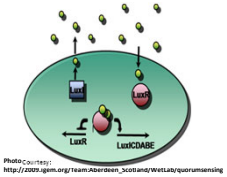


Photo Courtesy: http://2009.igem.org/Team:Aberdeen_Scotland/WetLab/quorumsensing

67

Infectious Diseases is a Moving Target…

- It is estimated only 1% of microbial community has been identified.
- Currently **etiological agent** of 80.3% of foodborne illnesses, **56.2% of hospitalization**, and 55.5% of deaths remain unknown (in a typical year, Scallan et all., 2011).

"Emerging" Pathogens:

- Vertical and horizontal gene transfer spores and biofilm formation
- Quorum sensing and cell to cell communication

"*It is the microbes who will have the last word.*"
-Louis Pasteur

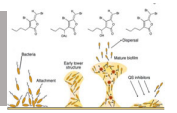
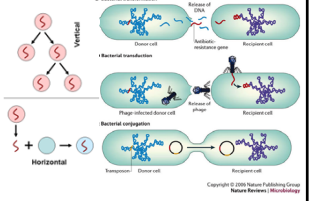


Photo Courtesy: http://www.jci.org/articles/view/20074/figure/2



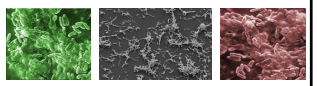


Photo Courtesy: http://www.microbiologybytes.com/blog/category/biofilms/
http://www.ifenergy.com/50226711/boosting_microbial_fuel_cells_with_biofilm.php
http://micro-writers.agsbio.net/blog/?tag=antibiotic-resistance

68

Epidemiology of Foodborne Diseases in the United States

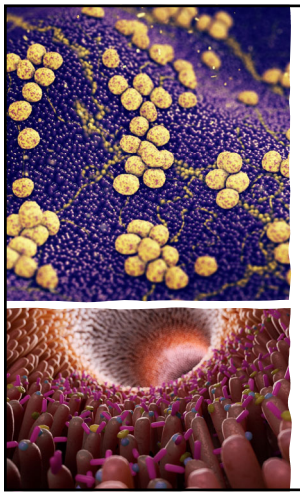
Based on data from 1990s: *(Mead et al., 1999)*

76 million illnesses, 323,000 hospitalizations, 5,200 deaths in the United States.

More recent estimates show: *(Scallan et al., 2011)*

- 47.8 million illnesses, 127,839 hospitalizations, and more than **3,037** deaths in the United States.
- 9.4 million illnesses, 55,961 hospitalizations, and 1,351 deaths are cause by 31 known foodborne agents.
- In addition to consumer insecurity, foodborne diseases cause around **$77.7 billion** for losses in productivity and economical losses.
- Approximately 30% of population are especially "at risk" for foodborne diseases (The YOPI's: The young, the old, Pregnant, and Immunocompromised)

69



Signs and Symptoms of Foodborne Diseases

- Mild illness (no medical care sought)
- **Guillain–Barré syndrome** (*Campylobacter* and *Salmonella*)
- **Post-infectious irritable bowel syndrome** (*Campylobacter* and *Salmonella*)
- **Reactive arthritis** (*Campylobacter* and *Salmonella*)
- **Haemolytic uraemic syndrome** (*E. coli* O157)
- **End-stage renal disease** (*E. coli* O157)
- Death

70

Water Safety Study

microorganisms

MDPI

Article

Fate and Biofilm Formation of Wild-Type and Pressure-Stressed Pathogens of Public Health Concern in Surface Water and on Abiotic Surfaces

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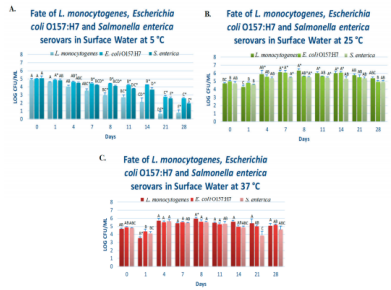
Public Health Burden of Waterborne Disease

17 waterborne pathogens cause estimated: (Collier et al., 2021)

601,000 **illness**; 118,000 **hospitalization**; 6,630 **deaths**, and cost the economy up to $ 8.77 **billions.**



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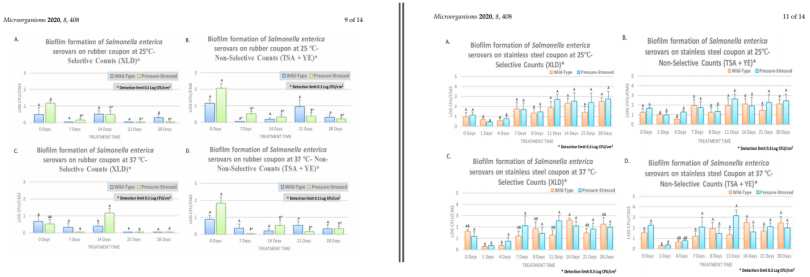
Water Safety Study- Biofilm Formation on Abiotic Surfaces



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Salmonella serovars (Non-typhoidal)

- **Annual illness (death): 1,027,561 (378) in humans**
- **Infection** causes nausea, vomiting, diarrhea, fever, headache
- **Primary sources**: Intestinal tract of people and animals
- **Transmitted by** meat, poultry, eggs, raw milk, unpasteurized juice, many other foods (nuts, spices, produce, chocolate, flour)
- **Contributing factors**: cross-contamination, undercooked food, poor agricultural practices

Growth parameters	Minimum	Optimum	Maximum
Temperature	41°F (5.2°C)	95-109°F (35-43°C)	115°F (46.2°C)
pH	3.7	7-7.5	9.5
a_w	0.94	0.99	>0.99
Other	Non-spore former		
Atmosphere	Facultative - grows with or without oxygen		

Sources: ICMSF 1995 and Bad Bug Book 2nd edition, Scallan et al., 2011, and FSPCA

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Climate Change and Public Health Microbiology

Non-typhoidal *Salmonella enterica* serovars

- **Global death:** 50,000 global death in 2010 (WHO, 2020)
- **Public Health Burden in the U.S.:** >1 million annual cases in 2011 (CDC, 2011)

Climate Change:

- **1 °C increase : 5 to 10% increases in Salmonellosis (WHO, 2010)**
- 2500 to 5000 additional global death
- 50,000 to 100,000 U.S. morbidity

At our current rate (2021 IPCC report)

- >1.5 °C by 2040
- >4.8 °C by 2100


Biology | Aliyar Fouladkhah

Changing climate

A 'threat multiplier' for foodborne and waterborne infectious diseases and antibiotic resistance


microorganisms

Safety of Food and Water Supplies in the Landscape of Changing Climate

74

Vibrio spp.

Currently 760,000 global illness/24,000 death per year.

- Causing about **80,000 illness and 100 death** annually in the United States.
- **Infection symptoms** vary depending on strain, ranging from diarrhea to high fever
- Vibrio is a **halophilic bacterium** and is a major concern in aquaculture industry
- **Primary sources**: Salt water environments and seafood
- Requires salt to reproduce (halophile)

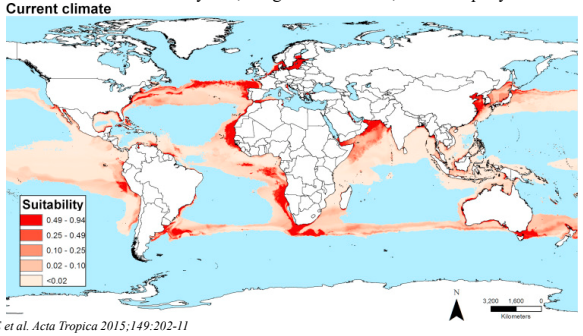
Growth parameters	Minimum	Optimum	Maximum
Temperature	41°F (5°C)	99°F (37°C)	114°F (45.3°C)
pH	4.8	7.8-8.6	11
a_w	0.94	0.98	0.996 (10% NaCl)
Other	Non-sporeformer, requires salt		

75

Vibrio cholerae proliferation in sea water: Current Climate

***Vibrio Cholerae*: currently 760,000 global illness/24,000 death per year**

Current climate



Escobar LE et al. Acta Tropica 2015;149:202-11

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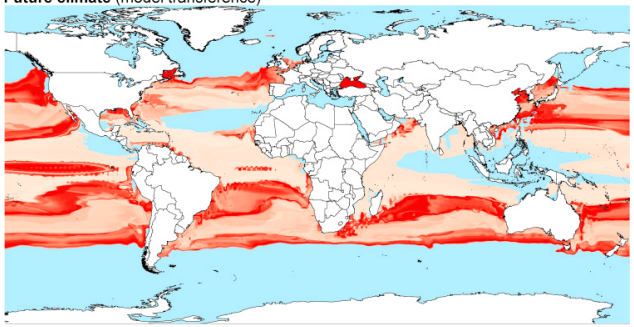
Vibrio cholerae proliferation in sea water: Business-as-Usual Projection in 2100

Future climate (model transference)



Escobar LE et al. Acta Tropica 2015;149:202-11

77

Other Climate-Sensitive Challenges

- **Mycotoxins (At 2°C increase, aflatoxin, North America and Europ**
  - **Aflatoxins:** Peanuts, dried corn (maize), **tree nuts, certain spices**
  - **Ochratoxin A:** Coffee, raisins, wine, cereal grains, certain spices
  - **Patulin:** Fruits (apple and apple juice)
- Attraction of **pests, plant diseases, weeds**
- Changes in **pesticide use pattern is likely**
- Survival and **proliferation of the pathogen** (*e.g. Salmonella* serovars)
- **Antibiotic use and antibiotic residue**
- Changes in **migration pathways** (*e.g.* for avian influenza)
- Changes in **carriers and vectors** (*e.g.* Zika virus)
- Changes in **natural ecosystem**
- **Phycotoxins**

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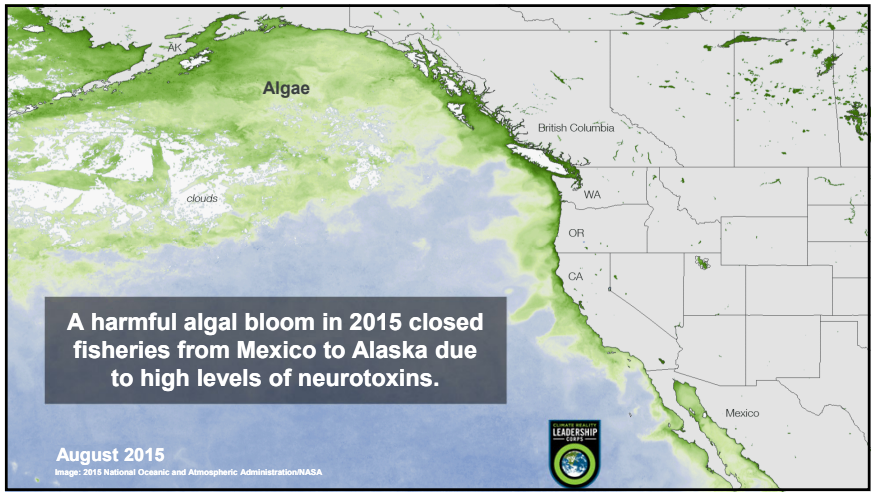

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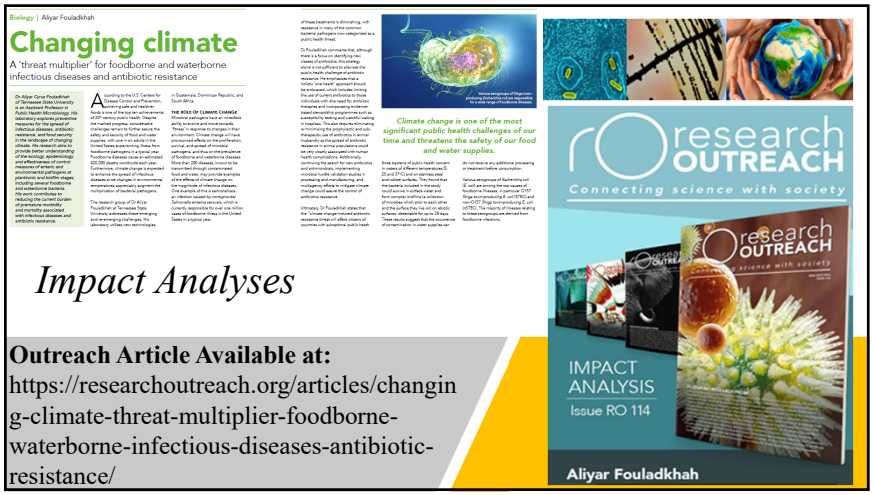

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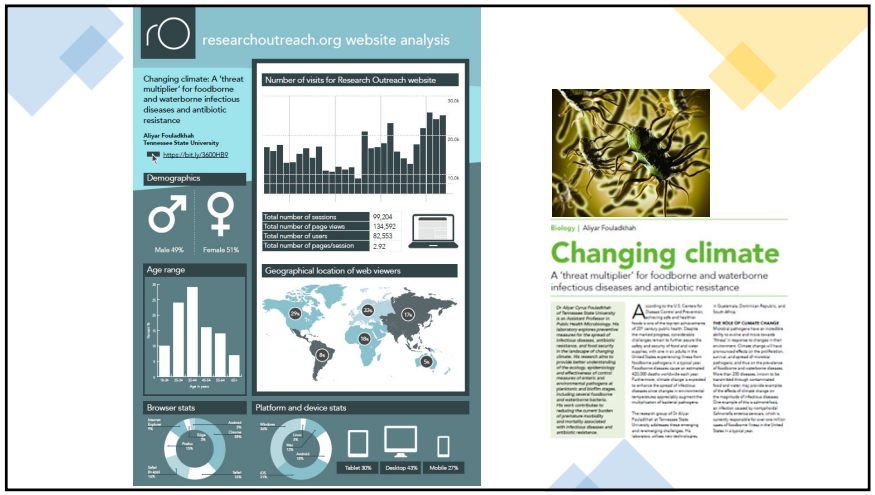

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Exercise one

- In your opinion, in what capacity the climate change will impact the local communities?
- What can an individual do in personal life to minimize the impact of climate change?
- What policies and regulations could be implemented in the food industry to minimize the negative impacts of the climate?

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Foodborne Diseases of Public Health Importance and Transboundary Diseases

Public Health and Food Safety Workshop, Georgia

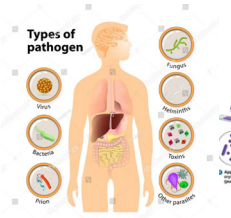
May 14 to June 3, 2022

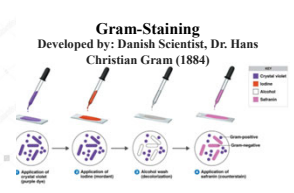
Aliyar Cyrus Fouladkhah, PhD, MS, MPH, CFS, CPH
Faculty Director, Public Health Microbiology Laboratory

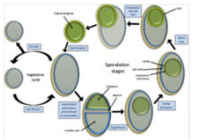
1

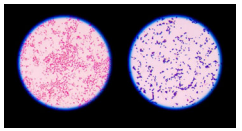
Anthrax

- Causative agent: *Bacillus anthracis*
- A **Gram-positive** and **spore-forming** bacteria
- Can be found as a spore in the **soil worldwide**
- Spores **viable** for **decades in soil**
- **In the US**: Dakotas, northwest Minnesota, Texas, and Nevada
- Common in parts of Africa, Asia, and Middle East
- In Human:
  - Skin
  - Intestine
  - Inhalation
- Animal disease
  - Septicemia and rapid death



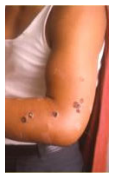


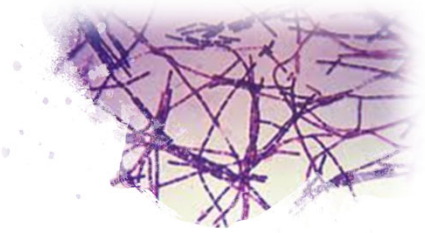




2

Anthrax



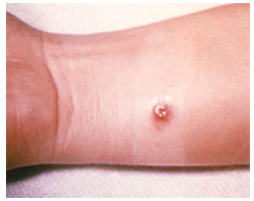


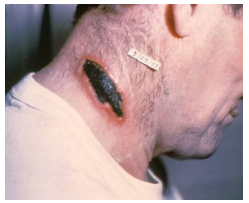
- Spores highly infective
- Remain effective during aerosolization
- Low lethal dose
- High mortality
- Person-to-person transmission rare
- **Symptoms** begin between **one day** and **two months** after the infection

3

Anthrax- Control and Treatment

- **Four types in human:** Cutaneous (skin); Inhalation; Gastrointestinal; Injection anthrax
- Vaccine for livestock annually to prevent
- Personal Protective Equipment
  - When handling sick animals
- Disinfection:
  - **Sporicidal agents:** 5% formaldehyde, 2% glutaraldehyde, 10% sodium hydroxide
  - **Sterilization:** chlorine dioxide, formaldehyde gas, heating to 121°C for at least 30 minutes
- **Antibiotics:** effective for humans when **prescribed early**
- **Zoonotic Disease**





4

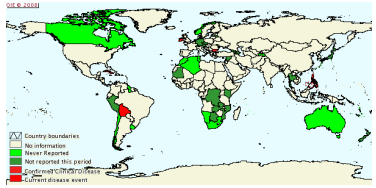
Pseudorabies

- **Contagious** viral diseases from **herpes family**
- Primary concern in domesticated **pigs and feral swine (around 75 million hogs in the United States in 2021)**
- Primarily spread through direct **animal-to-animal** (nose-to-nose)
- **Other mammals**
  - Reproductive
  - Nervous system
- **Humans are not affected**
- Could be a **ubiquitous virus** in some area
- **Eradicated in many countries**
  - Still occurs in parts of world
- Current **USDA Surveillance** to detect any potential case



-Different than rabies that is an important zoonotic diseases.
-Rabies death in the U.S. now < 5 per year
-About 59,000 annually worldwide (>98% from stray dogs)

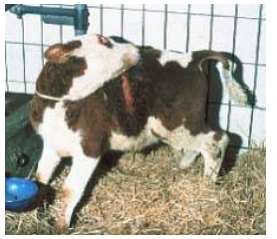
Source: CDC, 2021



5

Pseudorabies

- **Transmission:**
  - Direct contact,
  - Reproductive,
  - Aerosol,
  - Ingestion
- **Incubation period: 2-6 days**
- Common symptoms:
  - Neurological
  - Respiratory issues
  - Itching intensively
  - **Stillbirths and abortion**
- **Morbidity and mortality up to 100%**
- **Neonates are particularly susceptible** to the virus



6

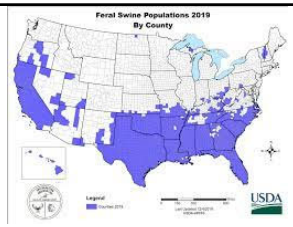
Pseudorabies

- Considered a **reportable disease**
- Could lead to **economic** and **trade restrictions**
- **Treatment** usually **not recommended**
- Current **control practices**:
  - **Depopulation** of the diseased
  - **Test and removal** of carries
  - **Offspring segregation**
- **Vaccine available** in some countries for affected animals

7

Prevention of Pseudorabies

- **Isolation:** new or returning animals before entry into the herd
- **Disinfect** vehicles, equipment, premises, footwear
- Separation of pigs and feral swine
- USDA extensive **surveillance program**
  - All 50 states are current free since **April 2008 (commercially)**
  - Feral swine remain as a reservoir of the pathogen



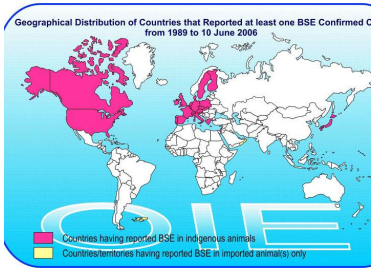
Source: USDA APHIS accessed 2021

8

BSE- Bovine Spongiform Encephalopathy

Commonly known as Mad Cow Disease

- Caused by **prions** (infectious protein particles)
- **Cattle and humans** are susceptible
- A neurological disease that could be fatal
- **Transmitted** by:
  - Consumption of **scrapie-infected feed**
  - **Spontaneous mutation**
- Distribution is worldwide



9

Symptoms of BSE

- In Cattle
  - Incubation period is 2-8 **years**
  - Initial signs are mild and subtle
  - At final stages
    - tremors
    - loss of balance
    - death
- In Humans
  - **Unknown incubation period** (many years to many decades)
  - Neurological signs
  - Depression and schizophrenia-like symptoms
  - Could lead to death

10

BSE Management

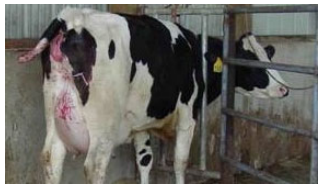
- **Very resistant infectious agent (sanitization very difficult)**
- **Currently no effective treatment or vaccine**
- Prevention:
  - **Surveillance program** and **testing**
  - **Restriction in trade**
  - **Animal feed regulation** (bone meals and mammalian products)
- Outbreak in 2001-2002 in United Kingdom: Cost the industry 3.7 billion Euro

11

Brucellosis

- Caused by bacteria (several species)

(Genus *Brucella* e.g. *B. melitensis, B. abortus, B. suis, and B. canis)*
  - **Highly infectious (N95 or KN95 mask during farm visits?)**
  - Easily **aerosolized**
- **Transmission**:
  - Ingestion
  - Inhalation
  - Direct contact
- **Signs in animal**:
  - Reproductive complications
- **Signs in humans**:
  - Cyclic fever and
  - Flu-like symptoms



12

Brucellosis- Treatment & Prevention

- Treatment: long-term antibiotics (Problem: Diversity of causative agents)
- Prevention:
  - **Vaccination** of calves
  - **Minimizing exposure to wildlife**
  - **Segregation of infected animals**
  - **Disinfection of environment**
- **No vaccine available for human**

Main infection source for human:

- Contaminated milk, cheese, and ice-creams
- Handling farm animals (glove, goggle, secondary outfit +mask?)
- Hunting Activities

13

Equine Encephalitis Viruses

- Three viruses:
  - Eastern (EEE)
  - Western (WEE)
  - Venezuelan (VEE)
- Transmitted by mosquitoes (**vector-borne disease**)
- **Birds** could be **asymptomatic carrier**
- **Clinical signs** in human and Equids (Horses, mules, donkeys)
  - No to mild signs to
  - Flu-like illness
  - Encephalitis in small proportions
  - **Can also infect a wide range of animals including:** mammals, birds, reptiles, and amphibians

14

Equine Encephalitis Viruses

- The viruses are **very unstable** in environment
- **Supportive care** is the only current treatment
- **Vaccine are available** for Equine
- **Vaccine for human very expensive** primarily for:
  - Researchers
  - Public health workers with enhanced exposure
  - **Travel Clinics for International Travel**

15

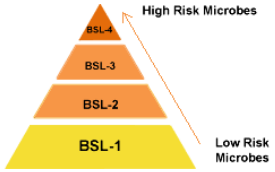
Hendra Virus

- Viral disease **consider as emerging** (**first observed in Australia**)
- Natural infections had been **reported only** in:
  - Horses
  - Humans (first reported in 1994, very rare and under-reported)
- Current transmission by:
  - Fruit bats
  - **Bodily fluids** and **urine** of those infected
- Clinical signs in horses
  - Sudden respiratory signs
  - Nasal discharge
  - Fever
  - Encephalitis
  - Sudden death
- Clinical signs in Humans
  - Flu-like illness
  - respiratory complications
  - **Highly fatal in human, could be as high as 2 in 3 cases**

16

Hendra Virus

- Little is known about pathogen
- **People at risk:**
  - Those occupational or recreational **exposure to horses**
  - Those **living close to "Flying fox" bats** (genus *Pteropus*)
  - **Researchers**
- Highest level of security (**CDC biosafety level 4**) needed for studying the pathogen (around 4 labs in the US and <50 in the words, as of 2021 [US has about 1,500 BSL3)
- Could cause high mortality in humans
- Currently no treatment option is available



17

Main Bacterial Pathogens Associated with Animal and Human Health Diseases

18

Infectious Diseases in Animals and Human is a Moving Target…

- It is estimated only 1% of microbial community has been identified.
- Currently **etiological agent** of **80.3% of foodborne illnesses**, **56.2% of hospitalization**, and **55.5% of deaths** remain unknown.

"Emerging" Pathogens:

- Vertical and horizontal gene transfer spores and biofilm formation
- Quorum sensing and cell to cell communication

"It is the microbes who will have the last word."
-Louis Pasteur

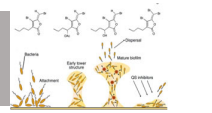
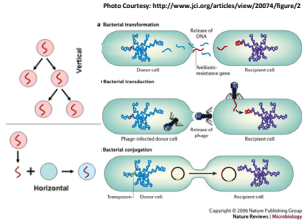


Photo Courtesy: http://www.jci.org/articles/view/20074/figure/2



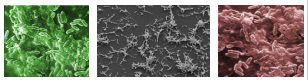


Photo Courtesy: http://www.microbiologybytes.com/blog/category/biofilms/
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http://micro-writers.wpbio.net/blog/?tag=antibiotic-resistance

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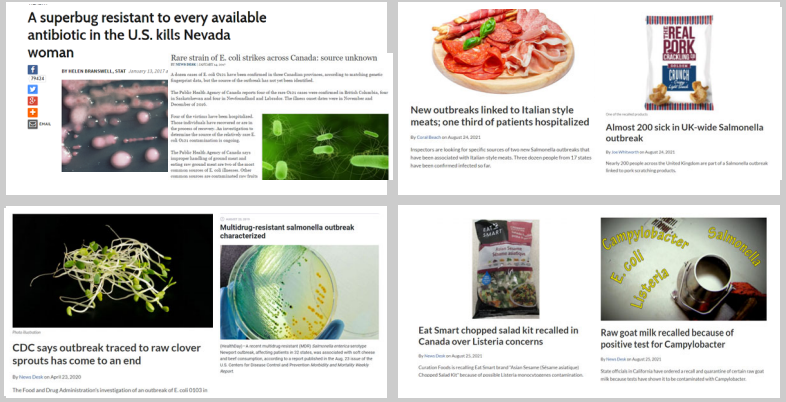

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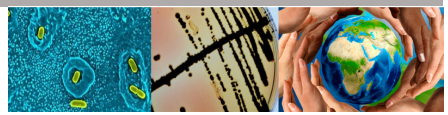
Foodborne Pathogens of Public Health Concerns *>200 foodborne diseases*



- *Salmonella* serovars
- *Staphylococcus aureus*
- *Campylobacter* spp.
- *Bacillus cereus*
- Shiga Toxin-Producing *Escherichia coli* (STEC)
- *Vibrio* spp.
- *Yersinia enterocolitica*
- *Streptococcus* spp.
- *Shigella* spp.
- *Listeria monocytogenes*
- *Mycobacterium bovis*
- *Cronobacter sakazakii*

21

Salmonella serovars

- **Annual illness (death): 1,027,561 (378) in American adults and children**
- **Infection** causes nausea, vomiting, diarrhea, fever, headache
- **Primary sources**: Intestinal tract of people and animals
- **Transmitted by** meat, poultry, eggs, raw milk, unpasteurized juice, many other foods (nuts, spices, produce, chocolate, flour) [Low-moisture environment]
- **Contributing factors**: cross-contamination, undercooked food, poor agricultural practices

Growth parameters	Minimum	Optimum	Maximum
Temperature	41°F (5.2°C)	95-109°F (35-43°C)	115°F (46.2°C)
pH	3.7	7-7.5	9.5
a_w	0.94	0.99	>0.99
Other	Non-spore former		
Atmosphere	Facultative - grows with or without oxygen		

Sources: ICMSF 1995 and Bad Bug Book 2nd edition, Scallan et al., 2011, and FSPCA

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Salmonella serovars

- **Carriers**: **Reptiles** (turtles, lizards, and snakes); **Amphibians** (frogs and toads); **Poultry** (chicks, chickens, ducklings, ducks, geese, and turkeys); **Other birds** (parakeets, parrots, and wild birds); **Rodents** (mice, rats, hamsters, and guinea pigs); Other **small mammals** (hedgehogs); **Farm animals** (goats, calves, cows, sheep, and pigs); **Dogs; Cats; Horses. [Pretty much ubiquitous!]**
- **Dogs and cats** that become ill from *Salmonella* infection generally will have **diarrhea** that may contain blood or mucus
- Some cats do not have diarrhea, but will have a **decreased appetite, fever, and excess salivation**.

Prevention:

- **Minimizing direct contact**, **washing hands**, and **cleaning up after the pets** could minimize the risk of transmission from infected animals to human.

23

Salmonella serovars

CDC Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Salmonella Outbreaks Linked to Backyard Poultry

Investigation Notice

Posted July 23, 2021

One in four sick people is a child younger than 5 years. Don't let young children touch chicks, ducklings, or other backyard poultry.

Fast Facts

- Illnesses: 672 (198 new)
- Hospitalizations: 157 (54 new)
- Deaths: 2 (1 new)
- States: 47 (1 new)
- Investigation status: Active

FDA U.S. FOOD & DRUG ADMINISTRATION

Pet Turtles: Cute But Commonly Contaminated with Salmonella

Turtles commonly carry bacteria on their outer skin and shell surfaces that can make people very ill. Geckos and bearded dragons can also infect people.

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Staphylococcus aureus

Foodborne Diseases
- Infection
- Intoxication
- Toxico-infection

- **Annual illness (death): 241,148 (6) Americans every year**
- Both causes infection and toxico-infection
- Produces **heat stable toxins** after extensive growth
- **Primary sources**: Boils, nasal passages and skin (around 20% positive on nasal passage, >10% hands)
- **Transmitted** by recontaminated **cooked foods**, and foods with high salt or high sugar (**Gram-positive, poor competitor**)
- **Contributing factors**: Recontamination and **time/temperature abuse**

Growth parameters	Minimum		Optimum		Maximum	
	Growth	Toxin	Growth	Toxin	Growth	Toxin
Temperature	45°F (7°C)	50°F (10°C)	99°F (37°C)	104-113°F (40-45°C)	122°F (50°C)	118°F (48°C)
pH	4	4	6-7	7-8	10	9.8
a_w	0.83	0.85	0.98		>0.99	
Other	Poor competitor, non-sporeformer					
Atmosphere	Facultative – grows with or without oxygen, but slower without					

Sources: ICMSF 1995 and Bad Bug Book 2nd edition, Scallan et al. 2011, and FSPCA

25

Campylobacter spp.

- **Annual illness (death): 845,024(76)**
- Infection causes diarrhea, and potential nerve damage
- **Primary sources**: Intestinal tract of animals
- **Transmitted** by raw poultry, raw milk products, contaminated water, poultry (dump tank, nearly 80%). Relatively high infective dose
- **Contributing factor**: cross contamination and undercooking

Growth parameters	Minimum	Optimum	Maximum
Temperature	86°F (30°C)	108-109°F (42-43°C)	113°F (45°C)
pH	4.9	6.5-7.5	9.5
a_w	>0.987	0.997	-
Other	Non-spore former		
Atmosphere	3-5% oxygen optimum		

Sources: ICMSF 1995 and Bad Bug Book 2nd edition and FSPCA

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Bacillus cereus

- **Annual illness (death): 63,400 (0)**
- Produces spores and toxins and extensive growth is required for illness
- **Primary source**: soil and GI track
- **Transmitted** by: rice and starchy foods, meats, vegetables, milk products, sauces
- Contributing factors: **temperature abuse**

Growth parameters	Minimum	Optimum	Maximum
Temperature	39°F (4°C)	82-95° F (28-35°C)	131°F (55°C)
pH	4.3	6.0-7.0	9.3
a_w	0.92	-	-
Other	Spore former; toxin is heat stable		
Atmosphere	Facultative – grows with or without oxygen		

Sources: Seafood Hazards Guide, ICMSF 1995, Bad Bug Book, Scallan et al. 2011, and FSOCA

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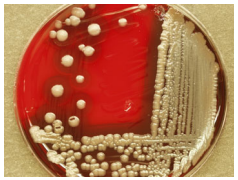
Bacillus cereus

- Some studies indicate the bacterium could behave as an agent of mammary gland **infection in cows** and **goats** thus causing **mastitis.**
- Cases of **food poisoning in dogs and cats** had also been reported, although not very frequent in nature.
- Many agricultural animals carry the **bacterium in their intestinal area** without symptoms.



28

Shiga Toxin-Producing *Escherichia coli* (STEC)

- **Annual illness (death): 176,152 (20)**
- **Notable outbreak:** 1992-1993 outbreak in pacific northwest- Very important regulatory status (adulterant)
- **Infection causes** bloody diarrhea, and sometimes kidney failure and death [HUS in kids]
- **Primary sources**: Intestinal tract of ruminant animals (e.g., cows, sheep)
- **Transmitted** by raw and undercooked beef, poultry, leafy greens, and unpasteurized milk and juices
- **Contributing factors**: poor GAP, inadequate heating, and person-to-person

Growth parameters	Minimum	Optimum	Maximum
Temperature	**44°F (6.5°C)**	95-104°F (35-40°C)	121°F (49.4°C)
pH	4	6-7	10
a_w	0.95	0.995	-
Other	Non-spore forming		
Atmosphere	Facultative - grows with or without oxygen		

Sources: ICMSF 1995 and Bad Bug Book 2nd edition,Scallan et al. 2011, and FSPCA

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Shiga Toxin-Producing *Escherichia coli* (STEC)

- **Animals that can spread *E. coli* O157 to humans include**:
  - -Cattle, especially calves (As high 80% in some herds),
  - *[Concentrated and genetic similarity]*
  - -Goats
  - -Sheep
  - -Deer

- *E. coli* infection very common in **cats and puppies younger than one week**.
- **Colostrum**, plays a pivotal role in protecting a newborn the animal's undeveloped immune system against *E. coli* infection.
- As high as **80% of agricultural animals could carry various serogroups of shiga-toxigenic *E. coli*** without having symptoms

30

Vibrio spp.

- Causing about **80,000 illness and 100 death** annually in the United States.
- **Infection symptoms** vary depending on strain, ranging from diarrhea to high fever
- Vibrio is a **halophilic bacterium** and is a major concern in aquaculture industry
- **Primary sources**: Salt water environments and seafood
- Requires salt to reproduce (halophile)

Growth parameters	Minimum	Optimum	Maximum
Temperature	41°F (5°C)	99°F (37°C)	114°F (45.3°C)
pH	4.8	7.8-8.6	11
a_w	0.94	0.98	0.996 (10% NaCl)
Other	Non-sporeformer, requires salt		
Atmosphere	Facultative - grows with or without oxygen		

Sources: Seafood Hazards Guide 2011, ICMSF 1995 and Bad Bug Book 2nd edition

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Yersinia enterocolitica

- *Not a reportable disease, no statistics available*
- **Infection causes** abdominal pain, fever and diarrhea. May mimic appendicitis.
- **Primary sources**: Raw pork, raw milk
- **Contributing factors**: Cross-contamination between raw pork products and RTE foods

Growth parameters	Minimum	Optimum	Maximum
Temperature	30°F (-1.3°C)	77-99°F (25-37°C)	108°F (42°C)
pH	4.2	7.2	10
a_w	0.945	-	-
Other	Non-spore former, raw milk in fridge?		
Atmosphere	Facultative - grows with or without oxygen		

Sources: Seafood Hazards Guide, ICMSF 1995, and Bad Bug Book

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Listeria monocytogenes

- **Infection causes** severe illness in susceptible people – mortality 15-30%
- **Primary sources**: Occurs widely in agriculture (soil, plants and water) –(Important during pregnancy)
- **Transmitted by**: Refrigerated RTE foods that support growth (South Africa, Largest in History in 2018)
- **Contributing factors**: Environmental pathogen spread by environmental contamination, equipment, people, incoming raw ingredients (ubiquitous in nature)
- **Common in domesticated ruminates particularly** sheep, poultry, and birds.
- **Could cause sporadic and farm outbreaks in ruminants**
- **Could cause: Encephalitis, late abortion, and GI problems in ruminants**.

Growth parameters	Minimum	Optimum	Maximum
Temperature	31°F (-0.4°C)	99°F (37°C)	113°F (45°C)
pH	4.4	7.0	9.4
a_w	0.92	-	-
Other	Non-sporeformer		
Atmosphere	Facultative - grows with or without oxygen		

Sources: ICMSF 1995 and Bad Bug Book 2nd edition

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Cronobacter Sakazakii

- **Recently reclassified** bacteria (2006-07), formerly known as ***Enterobacter sakazakii***
- The **Genus *Cronobacter*** was derived from the Greek term "Cronos," a Titans of ancient mythology who swallowed each of his infants as soon as they were born (he was afraid to be replaced by his infants).
- The **species name, *sakazakii***, is named in honor of the Japanese microbiologist, Riichi Sakazaki, when the bacterium was first explained in 1980.
- Gram-negative, rod-shaped bacteria.
- Facultative anaerobic
- The growing temperature range is 6°C-45°C
- Primarily associated with **Powered Infant Formula**
- There has been several outbreaks associated with the bacterium and neonatal meningitis and death including two outbreaks in **Tennessee** (**1998 and 2001**).

APHA Compendium of Methods, Salfinger and Lou Tortorello, Fifth Edition

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FDA U.S. FOOD & DRUG ADMINISTRATION

Outbreak Investigations & Safety Advisories
Center for Food Safety and Applied Nutrition

The following is an update from FDA of concern to our subscribers.

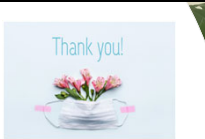
FDA Investigation of Cronobacter Infections: Powdered Infant Formula

March 22, 2022

The FDA, along with CDC and state and local partners are investigating consumer complaints and/or reports of infant illness related to products from Abbott Nutrition's Sturgis, MI facility. All of the ill patients are reported to have consumed powdered infant formula produced from Abbott Nutrition's Sturgis, MI facility.

The FDA is releasing the FDA Form 483s from three inspections conducted at Abbott Nutrition's facility on Sept. 16-24, 2019, Sept. 20-24, 2021, and Jan. 31-March 18, 2022. The inspectional observations

35



Dr. Aliyar Cyrus Fouladkhah,
Faculty Director, Public Health Microbiology Laboratory, Tennessee State University
afouladk@tnstate.edu |
Phone: (970) 690-7392

Photos Courtesy: Adobe Stock, royalty purchased (standard license) by public health microbiology laboratory

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Recommendations and Testing Methods*

Additional Information about the Public Health Microbiology Foundation Could be Accessed at:

https://publichealthmicrobiology.education/

*** Some publicly available references and publications from other institutions were additionally incorporated in this section. Authors are listed on each publications that are not from the foundation.**

Public Health Microbiology Foundation™
Founding Director, Aliyar Cyrus Fouladkhah, PhD, MPH, CFS, CPH
Webpage: https://publichealthmicrobiology.education/food-processing-support
aliyar.fouladkhah@aya.yale.edu (life-time alumni account)| Phone: +1 (970)690-7392

Re: Assistance to Establish Microbiological Testing for Georgian (Zugdidi Region) Stakeholders, Food Processing Support Center of Public Health Microbiology Foundation, Nashville, TN.
CC: Association for Agriculture Development, Zugdidi, Georgia *May 20, 2022*

Ms. Maia Mikava,

I commend you for your endeavors to initiate a microbiological testing program for your region. Based on my recent visit and assessment, establishing this laboratory capability would be of great assistance for stakeholders in the region for ensuring the safety of the products and the public's health and for further business development. This endeavor is particularly important since stakeholders in Zugdidi region are historically underserved due the distance from Tbilisi, the capital of the country. Now that you have acquired some of the needed instrumentations and now that you and your staff have receive some certification and training in this area from the Public Health Microbiology Foudation™, as the first step for this capacity building endeavor, I would recommend you incorporate Aerobic Plate Count as well as Molds and Yeast Enumeration. These will enable your operation to assist stakeholders to determine mesophilic aerobic microorganisms in their product as well as their mold and yeast contents. Establishing and practicing these skills with further assist your operation to assimilate the culture-dependent microbiological analyses that is the foundation for a range of other selective and differential enumeration of microbial pathogens.

Am attaching to this letter two test methods from the U. S. Food and Drug Administration's Bacteriological Analytical Methods and below am summarizing the consumables you would need to have for these procedures as well as suggested item numbers. I would also recommend you further explore regional standards in local language to ensure the compatibility of the proposed method with the regional government. The provided item numbers are not an endorsement of any product by the Public Health Microbiology Foundation™ and they could be replaced by comparable products from other research suppliers. This is not a process authority letter and is not granted as evaluation, recommendation, or approval of the testing procedure for ensuring the safety of the product. I wish you further success in business development and for ensuring the public's health during your future endeavors. Additional information about the Public Health Microbiology Foundation™ programs, services, and activities could be accessed at:

https://publichealthmicrobiology.education/

Best wishes,

Dr. Aliyar Cyrus Fouladkhah, PhD, MS, MPH, MACE, CFS, CPS

Founding Director, Public Health Microbiology Foundation™

Aerobic Plate Count

Reference: Bacteriological Analytical Method of FDA

Consumable Needed*	Fischer Scientific Item # (Catalog #)
Petri dishes (at least 15 × 90 mm)	*09-720-500*
Pipets with pipet tips	*Available in the facility in Zugdidi* *Tips: 02-100-510 (1250 µL)* *Tips: 02-100-508 (200 µL)*
Dilution tubes	*14-933-1B*
Dilution tube racks	*14-810P*
Magnetic stirring bars	*14-512-129*
Magnetic Stirrer Hotplate	*05-405-402*
Hockey stick (glass cell spreader)	*50-121-5126*
Bunsen burner	*04-245-1*
95% ethanol	*AAJ61785AU*
Incubator, refrigerator, and freezer	*Available in the facility in Zugdidi*
Colony counter, dark-field, Quebec, or equivalent	*RE-3325*
Phosphate-buffered dilution	*MP1PBS10X03*
Plate count agar	*OXCM0325B*
Thermometers	*13-201-558*
NIST thermometer as reference	*15-700-327*
Biohazard bags	*11-394-310 and 22-310-406*
Distilled water	*23-749999*

Yeast and Mold Count

Reference: Bacteriological Analytical Method of FDA

Consumable Needed*	Fischer Scientific Item # (Catalog #)
Dichloran rose bengal chloramphenicol (DRBC) agar	CM1149B
Dichloran 18% glycerol (DG18) agar	Dichloran 18% glycerol
Malt agar (MA)	B11401
Malt extract agar	09-100-6817
Potato dextrose agar	R454314
Chloramphenicol (antibiotics stable under autoclave condition)	MT61239RI
NaOCl (commercial bleach) solution, 10%	7495-132

**Alternatively, you can use Petrifilm™ (i.e. 3M™ Petrifilm™ Aerobic Count Plate and 3M™ Petrifilm™ Yeast and Mold Count Plate)*

BAM Chapter 3: Aerobic Plate Count

January 2001

Authors: Larry Maturin (ret.) and James T. Peeler (ret)

For additional information, contact Guodong Zhang (mailto:guodong.zhang@fda.hhs.gov).

The aerobic plate count (APC) is intended to indicate the level of microorganism in a product. Detailed procedures for determining the APC of foods have been developed by the Association of Official Analytical Chemists (AOAC) (3) and the American Public Health Association (APHA) (1). The conventional plate count method for examining frozen, chilled, precooked, or prepared foods, outlined below, conforms to AOAC *Official Methods of Analysis*, sec. 966.23, with one procedural change (966.23C). The suitable colony counting range (10) is 25-250. The automated spiral plate count method for the examination of foods and cosmetics (5), outlined below, conforms to AOAC *Official Methods of Analysis*, sec. 977.27. For procedural details of the standard plate count, see ref. 2.Guidelines for calculating and reporting plate counts have been changed to conform with the anticipated changes in the 16th edition of *Standard Methods for the Examination of Dairy Products* (2) and the *International Dairy Federation* (IDF) procedures (6).

- Conventional Plate Count Method
- Spiral Plate Method
- References

Conventional Plate Count Method

A. **Equipment and materials**

1. Work area, level table with ample surface in room that is clean, well-lighted (100 foot-candles at working surface) and well-ventilated, and reasonably free of dust and drafts. The microbial density of air in working area, measured in fallout pour plates taken during plating, should not exceed 15 colonies/plate during 15 min exposure.
2. Storage space, free of dust and insects and adequate for protection of equipment and supplies
3. Petri dishes, glass or plastic (at least 15 × 90 mm)
4. Pipets with pipet aids (no mouth pipetting) or pipettors, 1, 5, and 10 ml, graduated in 0.1 ml units
5. Dilution bottles, 6 oz (160 ml), borosilicate-resistant glass, with rubber stoppers or plastic screw caps
6. Pipet and petri dish containers, adequate for protection
7. Circulating water bath, for tempering agar, thermostatically controlled to 45 ± 1°C
8. Incubator, 35 ± 1°C; milk, 32 ± 1°C
9. Colony counter, dark-field, Quebec, or equivalent, with suitable light source and grid plate
10. Tally register
11. Dilution blanks, 90 ± 1 ml Butterfield's phosphate-buffered dilution water (R11 (/food/laboratory-methods/bam-r11-butterfields-phosphate-buffered-dilution-water)); milk, 99 ± 2 ml
12. Plate count agar (standard methods) (M124 (/food/laboratory-methods/bam-media-m124-plate-count-agar-standard-methods))
13. Refrigerator, to cool and maintain samples at 0-5°C; milk, 0-4.4°C
14. Freezer, to maintain frozen samples from -15 to -20°C
15. Thermometers (mercury) appropriate range; accuracy checked with a thermometer certified by the National Institute of Standards and Technology (NIST)

B. **Procedure for analysis of frozen, chilled, precooked, or prepared foods**

Using separate sterile pipets, prepare decimal dilutions of 10^{-2}, 10^{-3}, 10^{-4}, and others as appropriate, of food homogenate (**see** Chapter 1 (/food/laboratory-methods/bam-food-samplingpreparation-sample-homogenate) for sample preparation) by transferring 10 ml of previous dilution to 90 ml of diluent. Avoid sampling foam. Shake all dilutions 25 times in 30 cm (1 ft) arc within 7 s. Pipet 1 ml of each dilution into separate, duplicate, appropriately marked petri dishes. Reshake dilution bottle 25 times in 30 cm arc within 7 s if it stands more than 3 min before it is pipetted into petri dish. Add 12-15 ml plate count agar (cooled to 45 ± 1°C) to each plate within 15 min of original dilution. For milk samples, pour an agar control, pour a dilution water control and pipet water for a pipet control. Add agar to the latter two for each series of samples. Add agar immediately to petri dishes when sample diluent contains hygroscopic materials, e.g., flour and starch. Pour agar and dilution water control

plates for each series of samples. Immediately mix sample dilutions and agar medium thoroughly and uniformly by alternate rotation and back-and-forth motion of plates on flat level surface. Let agar solidify. Invert solidified petri dishes, and incubate promptly for 48 ± 2 h at 35°C. Do not stack plates when pouring agar or when agar is solidifying.

C. **Guidelines for calculating and reporting APCs in uncommon cases**

Official Methods of Analysis (3) does not provide guidelines for counting and reporting plate counts, whereas *Standard Methods for the Examination of Dairy Products*, 16th ed. (2) presents detailed guidelines; for uniformity, therefore, use APHA guidelines as modified (6,8). Report all aerobic plate counts (2) computed from duplicate plates. For milk samples, report all aerobic plate (2) counts computed from duplicate plates containing less than 25 colonies as less than 25 estimated count. Report all aerobic plate counts (2) computed from duplicate plates containing more than 250 colonies as estimated counts. Counts outside the normal 25-250 range may give erroneous indications of the actual bacterial composition of the sample. Dilution factors may exaggerate low counts (less than 25), and crowded plates (greater than 250) may be difficult to count or may inhibit the growth of some bacteria, resulting in a low count. Report counts less than 25 or more than 250 colonies as estimated aerobic plate counts (EAPC). Use the following guide:

1. Normal plates (25-250). Select spreader-free plate(s). Count all colony forming units (CFU), including those of pinpoint size, on selected plate(s). Record dilution(s) used and total number of colonies counted.
2. Plates with more than 250 colonies. When number of CFU per plate exceeds 250, for all dilutions, record the counts as too numerous to count (TNTC) for all but the plate closest to 250, and count CFU in those portions of plate that are representative of colony distribution. See ref. 2 for detailed guidelines. Mark calculated APC with EAPC to denote that it was estimated from counts outside 25-250 per plate range (*see* D-3).
3. Spreaders. Spreading colonies are usually of 3 distinct types: 1) a chain of colonies, not too distinctly separated, that appears to be caused by disintegration of a bacterial clump; 2) one that develops in film of water between agar and bottom of dish; and 3) one that forms in film of water at edge or on surface of agar. If plates prepared from sample have excessive spreader growth so that (a) area covered by spreaders, including total area of repressed growth, exceeds 50% of plate area, or (b) area of repressed growth exceeds 25% of plate area, report plates as spreaders. When it is necessary to count plates containing spreaders not eliminated by (a) or (b) above, count each of the 3 distinct spreader types as one source. For the first type, if only one chain exists, count it as a single colony. If one or more chains appear to originate from separate sources, count each source as one colony. Do not count each individual growth in such chains as a separate colony. Types 2 and 3 usually result in distinct colonies and are counted as such. Combine the spreader count and the colony count to compute the APC.
4. Plates with no CFU. When plates from all dilutions have no colonies, report APC as less than 1 times the corresponding lowest dilution used. Mark calculated APC with asterisk to denote that it was estimated from counts outside the 25-250 per plate range. When plate(s) from a sample are known to be contaminated or otherwise unsatisfactory, record the result(s) as laboratory accident (LA).

D. **Computing and recording counts (see refs 6, 8)**

To avoid creating a fictitious impression of precision and accuracy when computing APC, report only the first two significant digits. Round off to two significant figures only at the time of conversion to SPC. For milk samples, when plates for all dilutions have no colonies, report APC as less than 25 colonies estimated count. Round by raising the second digit to the next highest number when the third digit is 6, 7, 8, or 9 and use zeros for each successive digit toward the right from the second digit. Round down when the third digit is 1, 2, 3, or 4. When the third digit is 5, round up when the second digit is odd and round down when the second digit is even.

Examples

Calculated Count	APC
12,700	13,000
12,400	12,000
15,500	16,000
14,500	14,000

1. Plates with 25-250 CFU.

$$N = \frac{\sum C}{[(1\times n_1) + (0.1\times n_2)\times (d)}$$

a. Calculate the APC as follows:

$$\frac{(31+31)\ \text{colonies}}{0.0015\text{ml}} = 4.1\times 10^4$$

where N = Number of colonies per ml or g of product
Σ_c = Sum of all colonies on all plates counted
n_1 = Number of plates in first dilution counted
n_2 = Number of plates in second dilution counted
d = Dilution from which the first counts were obtained

Example

1:100	1:1000
232, 244	33, 28

$$N = \frac{(232 + 244 + 33 + 28)}{[(1\times 2) + (0.1\times 2)]\times 10^{-2}}$$

= 537/0.022
= 24,409
≈ 24,000

b. When counts of duplicate plates fall within and without the 25-250 colony range, use only those counts that fall within this range.

2. All plates with fewer than 25 CFU. When plates from both dilutions yield fewer than 25 CFU each, record actual plate count but record the count as less than 25 × 1/d when d is the dilution factor for the dilution from which the first counts were obtained.

Example

Colonies		
1:100	**1:1000**	**EAPC/ml (g)**
18	2	<2,500
0	0	<2,500

3. **All plates with more than 250 CFU**. When plates from both 2 dilutions yield more than 250 CFU each (but fewer than 100/cm^2), estimate the aerobic counts from the plates (EAPC) nearest 250 and multiply by the dilution.

Example

Colonies		
1:100	**1:1000**	**EAPC/ml (g)**
TNTC	640	640,000

TNTC, too numerous to count.
EAPC, estimated aerobic plate count.

4. All plates with spreaders and/or laboratory accident. Report respectively as Spreader (SPR), or Laboratory Accident (LA).

5. All plates with more than an average of 100 CFU per sq cm. Estimate the APC as greater than 100 times the highest dilution plated, times the area of the plate. The examples below have an average count of 110 per sq cm.

Example

Colonies/Dilution		
1:100	**1:1000**	**EAPC/ml (g)**
TNTC	7,150[a]	>6,500,000 EAPC[b]
TNTC	6,490	>5,900,000 EAPC

[a] Based on plate area of 65 cm^2
[b] EAPC, estimated APC
[c] Based on plate area of 59 cm^2

Spiral Plate Method

The spiral plate count (SPLC) method for microorganisms in milk, foods, and cosmetics is an official method of the APHA (2) and the AOAC (3). In this method, a mechanical plater inoculates a rotating agar plate with liquid sample. The sample volume dispensed decreases as the dispensing stylus moves from the center to the edge of the rotating plate. The microbial concentration is determined by counting the colonies on a part of the petri dish where they are easily countable and dividing this count by the appropriate volume. One inoculation determines microbial densities between 500 and 500,000 microorganisms/ml. Additional dilutions may be made for suspected high microbial concentrations.

A. **Equipment and materials**

1. Spiral plater (Spiral Systems Instruments, Inc., 7830 Old Georgetown Road, Bethesda, MD 20814)

2. Spiral colony counter (Spiral Systems) with special grid for relating deposited sample volumes to specific portions of petri dishes
3. Vacuum trap for disposal of liquids (2-4 liter vacuum bottle to act as vacuum reservoir and vacuum source of 50-60 cm Hg)
4. Disposable micro beakers, 5 ml
5. Petri dishes, plastic or glass, 150 × 15 mm or 100 × 15 mm
6. Plate count agar (standard methods) (M124 (/food/laboratory-methods/bam-media-m124-plate-count-agar-standard-methods))
7. Calculator (optional), inexpensive electronic hand calculator is recommended
8. Polyethylene bags for storing prepared plates
9. Commercial sodium hypochlorite solution, about 5% NaOCl (bleach)
10. Sterile dilution water
11. Syringe, with Luer tip for obstructions in stylus; capacity not critical
12. Work area, storage space, refrigerator, thermometers, tally, incubator, as described for Conventional Plate Count Method, above.
13. Sodium hypochlorite solution (5.25%). Available commercially.

B. **Preparation of agar plates.**

Automatic dispenser with sterile delivery system is recommended to prepare agar plates. Agar volume dispensed into plates is reproducible and contamination rate is low compared to hand-pouring of agar in open laboratory. When possible, use laminar air flow hood along with automated dispenser. Pour same quantity of agar into all plates so that same height of agar will be presented to spiral plater stylus tip to maintain contact angle. Agar plates should be level during cooling.

The following method is suggested for prepouring agar plates: Use automatic dispenser or pour constant amount (about 15 ml/100 mm plate; 50 ml/150 mm plate) of sterile agar at 60-70°C into each petri dish. Let agar solidify on level surface with poured plates stacked no higher than 10 dishes. Place solidified agar plates in polyethylene bags, close with ties or heat-sealer, and store inverted at 0-4.4°C. Bring prepoured plates to room temperature before inoculation.

C. **Preparation of samples.**

As described in Chapter 1, select that part of sample with smallest amount of connective tissues or fat globules.

D. **Description of spiral plater.**

Spiral plater inoculates surface of prepared agar plate to permit enumeration of microorganisms in solutions containing between 500 and 500,000 microorganisms per ml. Operator with minimum training can inoculate 50 plates per h. Within range stated, dilution bottles or pipets and other auxiliary equipment are not required. Required bench space is minimal, and time to check instrument alignment is less than 2 min. Plater deposits decreasing amount of sample in Archimedean spiral on surface of prepoured agar plate. Volume of sample on any portion of plate is known. After incubation, colonies appear along line of spiral. If colonies on a portion of plate are sufficiently spaced from each other, count them on special grid which associates a calibrated volume with each area. Estimate number of microorganisms in sample by dividing number of colonies in a defined area by volume contained in same area. Studies have shown the method to be proficient not only with milk (4) but also with other foods (7,10).

E. **Plating procedure**

Check stylus tip angle daily and adjust if necessary. (Use vacuum to hold microscope cover slip against face of stylus tip; if cover slip plane is parallel at about l mm from surface of platform, tip is properly oriented). Liquids are moved through system by vacuum. Clean stylus tip by rinsing for 1 s with sodium hypochlorite solution followed by sterile dilution water for 1 s before sample introduction. This rinse procedure between processing of each sample minimizes cross-contamination. After rinsing, draw sample into tip of Teflon tubing by vacuum applied to 2-way valve. When tubing and syringe are filled with sample, close valve attached to syringe. Place agar plate on platform, place stylus tip on agar surface, and start motor. During inoculation, label petri plate lid. After agar has been inoculated, stylus lifts from agar surface and spiral plater automatically stops. Remove inoculated plate from platform and cover it. Move stylus back to starting position. Vacuum-rinse system with hypochlorite and water, and then introduce new sample. Invert plates and promptly place them in incubator for 48 ± 3 h at 35 ± 1°C.

F. **Sterility controls**

Check sterility of spiral plater for each series of samples by plating sterile dilution water. CAUTION: Prepoured plates should not be contaminated by a surface colony or be below room temperature (water can well-up from agar). They should not be excessively dry, as indicated by large wrinkles or glazed appearance. They should not have water droplets on surface of agar or differences greater than 2 mm in agar depth, and they should not be stored at 0-4.4°C for longer than l month. Reduced flow rate through tubing indicates obstructions or material in system. To clear obstructions, remove valve from syringe, insert hand-held syringe with Luer fitting containing water, and apply pressure. Use alcohol rinse to remove residual material adhering to walls of system. Dissolve accumulated residue with chromic acid. Rinse well after cleaning.

G. **Counting grid**

1. **Description.** Use same counting grid for both 100 and 150 mm petri dishes. A mask is supplied for use with 100 mm dishes. Counting grid is divided into 8 equal wedges; each wedge is divided by 4 arcs labeled l, 2, 3, and 4 from outside grid edge. Other lines within these arcs are added for ease of counting. A segment is the area between 2 arc lines within a wedge. Number of areas counted (e.g., 3) means number of segments counted within a wedge. Spiral plater deposits sample on agar plate in the same way each time. The grid relates colonies on spiral plate to the volume in which they were contained. When colonies are counted with grid, sample volume becomes greater as counting starts at outside edge of plate and proceeds toward center of plate.

2. **Calibration.** The volume of sample represented by various parts of the counting grid is shown in operator's manual that accompanies spiral plater. Grid area constants have been checked by the manufacturer and are accurate. To verify these values, prepare 11 bacterial concentrations in range of 10^6-10^3 cells/ml by making 1:1 dilutions of bacterial suspension (use a nonspreader). Plate all Incubate both sets of plates for 48 ± 3 h at 35 ± 1°C. Calculate concentrations for each dilution. Count spiral plates over grid surface, using counting rule of 20 (described in H, below), and record number of colonies counted and grid area over which they were counted. Each spiral colony count for a particular grid area, divided by aerobic count/ml for corresponding spirally plated bacterial concentrations, indicates volume deposited on that particular grid area. Use the following formula:

$$\text{Volume (ml) for grid area} = \frac{\text{Spiral Colonies counted in area}}{\text{Baterial count/ml (APC)}}$$

$$\text{Volume (ml)} = \frac{31 + 30 \text{ colonies}}{4.1\times10^4 \text{ bateria/ml}} = 0.0015 \text{ ml}$$

To check total volume dispensed by spiral plater, weigh amount dispensed from stylus tip. Collect in tared 5 ml plastic beaker and weigh on analytical balance (± 0.2 mg).

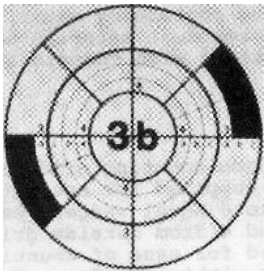

Figure 1. 10 cm plate, area (3b)

$$\frac{(31+31) \text{ colonies}}{0.0015 \text{ ml}} = 4.1\times10^4$$

H. **Examination and reporting of spiral plate counts.**

Counting rule of 20. After incubation, center spiral plate over grid by adjusting holding arms on viewer. Choose any wedge and begin counting colonies from outer edge of first segment toward center until 20 colonies have been counted. Complete by counting remaining colonies in segment where 20th colony occurs. In this counting procedure, numbers such as 3b, 4c (Fig. l) refer to area segments from outer edge of wedge to designated arc line. Any count irregularities in sample composition are controlled by counting the same segments in the opposite wedge and recording results. Example of spirally inoculated plate (Fig. l) demonstrates method for determining microbial count. Two segments of each wedge were counted on opposite sides of plate with 31 and 30 colonies, respectively. The sample volume contained in the darkened segments is 0.0015 ml. To estimate number of microorganisms, divide count by volume contained in all segments counted. See example under Fig. l.

If 20 CFU are not within the 4 segments of the wedge, count CFU on entire plate. If the number of colonies exceeds 75 in second, third, or fourth segment, which also contains the 20th colony, the estimated number of microorganisms will generally be low because of coincidence error associated with crowding of colonies. In this case, count each circumferentially adjacent segment in all 8 wedges, counting at least 50 colonies, e.g., if the first 2 segments of a wedge contain 19 colonies and the third segment contains the 20th and 76th (or more), count colonies in all circumferentially adjacent first and second segments in all 8 wedges. Calculate contained volume in counted segments of wedges and divide into number of colonies.

When fewer than 20 colonies are counted on the total plate, report results as "less than 500 estimated SPLC per ml." If colony count exceeds 75 in first segment of wedge, report results as "greater than 500,000 estimated SPLC per ml." Do not count spiral plates with irregular distribution of colonies caused by dispensing errors. Report results of such plates as laboratory accident (LA). If spreader covers entire plate, discard plate. If spreader covers half of plate area, count only those colonies that are well distributed in spreader-free areas.

Compute SPLC unless restricted by detection of inhibitory substances in sample, excessive spreader growth, or laboratory accidents. Round off counts as described in I-D, above. Report counts as SPLC or estimated SPLC per ml.

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BAM Chapter 18: Yeasts, Molds and Mycotoxins

Bacteriological Analytical Manual (BAM) Main Page (/food/laboratory-methods-food/bacteriological-analytical-manual-bam)

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The large and diverse group of microscopic foodborne yeasts and molds (fungi) includes several hundred species. The ability of these organisms to attack many foods is due in large part to their relatively versatile environmental requirements. Although the majority of yeasts and molds are obligate aerobes (require free oxygen for growth), their acid/alkaline requirement for growth is quite broad, ranging from pH 2 to above pH 9. Their temperature range (10-35°C) is also broad, with a few species capable of growth below or above this range. Moisture requirements of foodborne molds are relatively low; most species can grow at a water activity (a_w) of 0.85 or less, although yeasts generally require a higher water activity.

Both yeasts and molds cause various degrees of deterioration and decomposition of foods. They can invade and grow on virtually any type of food at any time; they invade crops such as grains, nuts, beans, and fruits in fields before harvesting and during storage. They also grow on processed foods and food mixtures. Their detectability in or on foods depends on food type, organisms involved, and degree of invasion; the contaminated food may be slightly blemished, severely blemished, or completely decomposed, with the actual growth manifested by rot spots of various sizes and colors, unsightly scabs, slime, white cottony mycelium, or highly colored sporulating mold. Abnormal flavors and odors may also be produced. Occasionally, a food appears mold-free but is found upon mycological examination to be contaminated. Contamination of foods by yeasts and molds can result in substantial economic losses to producer, processor, and consumer.

Several foodborne molds, and possibly yeasts, may also be hazardous to human or animal health because of their ability to produce toxic metabolites known as mycotoxins. Most mycotoxins are stable compounds that are not destroyed during food processing or home cooking. Even though the generating organisms may not survive food preparation, the preformed toxin may still be present. Certain foodborne molds and yeasts may also elicit allergic reactions or may cause infections. Although most foodborne fungi are not infectious, some species can cause infection, especially in immunocompromised populations, such as the aged and debilitated, HIV-infected individuals, and persons receiving chemotherapy or antibiotic treatment.

The dilution plating and the direct plating methods may be used to detect fungi in foods. The direct plating method is more efficient than the dilution plating method for detecting individual mold species, including most of the toxin producers, but it is less effective in detecting yeasts. It is also used to determine whether the presence of mold is due to external contamination or internal invasion. Methodology for testing the ability of isolates of toxigenic mold species to produce mycotoxins on sterile rice water substrate is included here.

Enumeration of Yeasts and Molds in Food--Dilution Plating Technique

A. **Equipment and materials**

1. Basic equipment (and appropriate techniques) for preparation of sample homogenate, see Chapter 1 (/food/laboratory-methods/bam-food-samplingpreparation-sample-homogenate)
2. Equipment for plating samples, see Chapter 3 (/food/laboratory-methods/bam-aerobic-plate-count)
3. Incubator, 25°C
4. Arnold steam chest
5. pH meter
6. Water bath, 45 ± 1° C

B. **Media** (/food/laboratory-methods/media-index-bam) **and Reagents (/food/laboratory-methods/reagents-index-bam)**

Media

1. Dichloran rose bengal chloramphenicol (DRBC) agar (M183 (/food/laboratory-methods/bam-media-m183-dichloran-rose-bengal-chloramphenicol-drbc-agar))
2. Dichloran 18% glycerol (DG18) agar (M184 (/food/laboratory-methods/bam-media-m184-dichloran-18-glycerol-dg18-agar))
3. Plate count agar (PCA), standard methods (M124 (/food/laboratory-methods/bam-media-m124-plate-count-agar-standard-methods)); add 100 mg chloramphenicol/liter when this medium is used for yeast and mold enumeration. This medium is not efficient when "spreader" molds are present.
4. Malt agar (MA)(M185 (/food/laboratory-methods/bam-media-m185-malt-agar-ma))
5. Malt extract agar (Yeasts and Molds) (MEAYM) (M182 (/food/laboratory-methods/bam-media-m182-malt-extract-agar-yeasts-and-molds-meaym))

6. Potato dextrose agar (PDA), dehydrated; commercially available (M127 (/food/laboratory-methods/bam-media-m127-potato-dextrose-agar))

Antibiotic solutions

Antibiotics are added to mycological media to inhibit bacterial growth. Chloramphenicol is the antibiotic of choice, because it is stable under autoclave conditions. Therefore, media preparation is easier and faster due to the elimination of the filtration step. The recommended concentration of this antibiotic is 100 mg/liter medium. If bacterial overgrowth is apparent, prepare media by adding 50 mg/liter chloramphenicol before autoclaving and 50 mg/liter filter-sterilized chlortetracycline when the media have been tempered, right before pouring plates.

Prepare stock solution by dissolving 0.1 g chloramphenicol in 40 ml distilled water; add this solution to 960 ml medium mixture before autoclaving. When both chloramphenicol and chlortetracycline are used, add 20 ml of the above chloramphenicol stock solution to 970 ml medium before autoclaving. Then, prepare chlortetracycline stock solution by dissolving 0.5 g antibiotic in 100 ml distilled water and filter sterilize. Use 10 ml of this solution for each 990 ml of autoclaved and tempered medium. Refrigerate in the dark and re-use remaining stock solutions for up to a month. Stock solutions should be brought to room temperature before adding to tempered medium.

C. **Procedures:**

Sample preparation

Analyze 25-50 g from each subsample; generally, larger sample sizes increase reproducibility and lower variance compared with small samples. Test individual subsamples or composite according to respective Compliance Program for the food under analysis. Add appropriate amount of 0.1% peptone water to the weighed sample to achieve 10^{-1} dilution, then homogenize in a stomacher for 2 min. Alternatively, blending for 30-60 sec can be used but is less effective. Make appropriate 1:10 (1+9) dilutions in 0.1% peptone water. Dilutions of 10^{-6} should suffice.

Plating and incubation of sample

Spread-plate method. Aseptically pipet 0.1 ml of each dilution on pre- poured, solidified DRBC agar plates and spread inoculum with a sterile, bent glass rod. DG18 is preferred when the water activity of the analyzed sample is less than 0.95. Plate each dilution in triplicate.

Pour-plate method. Use sterile cotton-plugged pipet to place 1.0 ml portions of sample dilution into prelabeled 15 × 100 mm Petri plates (plastic or glass), and immediately add 20-25 ml tempered DG18 agar. Mix contents by gently swirling plates clockwise, then counterclockwise, taking care to avoid spillage on dish lid. After adding sample dilution, add agar within 1-2 min; otherwise, dilution may begin to adhere to dish bottom (especially if sample is high in starch content and dishes are plastic) and may not mix uniformly. Plate each dilution in triplicate.

From preparation of first sample dilution to pouring or surface-plating of final plate, no more than 20 min (preferably 10 min) should elapse. **Note:** Spread plating of diluted sample is considered better than the pour plate method. When the pour plate technique is used, fungal colonies on the surface grow faster and often obscure those underneath the surface, resulting in less accurate enumeration. Surface plating gives a more uniform growth and makes colony isolation easier. DRBC agar should be used for spread plates only.

Incubate plates in the dark at 25°C. Do not stack plates higher than 3 and do not invert. **Note:** Let plates remain undisturbed until counting.

Counting of plates

Count plates after 5 days of incubation. If there is no growth at 5 days, re-incubate for another 48 h. Do not count colonies before the end of the incubation period because handling of plates could result in secondary growth from dislodged spores, making final counts invalid. Count plates containing 10-150 colonies. If mainly yeasts are present, plates with 150 colonies are usually countable. However, if substantial amounts of mold are present, depending on the type of mold, the upper countable limit may have to be lowered at the discretion of the analyst. Report results in colony forming units (CFU)/g or CFU/ml based on average count of triplicate set. Round off counts to two significant figures. If third digit is 6 or above, round off to digit above (e.g., 456 = 460); if 4 or below, round off to digit below (e.g., 454 = 450). If third digit is 5, round off to digit below if first 2 digits are an even number (e.g., 445 = 440); round off to digit above if first 2 digits are an odd number (e.g., 455 = 460). When plates from all dilutions have no colonies, report mold and yeast counts (MYC) as less than 1 times the lowest dilution used.

Isolate individual colonies on PDA or MA, if further analysis and species identification is necessary.

Enumeration of Molds in Foods--Direct Plating Technique for Foods That Can Be Handled with Forceps (Dried Beans, Nuts, Whole Spices, Coffee and Cocoa Beans, etc.)

A. **Equipment and materials**

1. Freezer, -20° C
2. Beakers, sterile, 300 ml
3. Forceps, sterile
4. Arnold steam chest
5. Water bath, 45 ± 1° C
6. Incubator, 25° C

B. **Media and Reagents**

1. Dichloran rose bengal chloramphenicol (DRBC) agar (M183 (/food/laboratory-methods/bam-media-m183-dichloran-rose-bengal-chloramphenicol-drbc-agar))
2. Dichloran 18% glycerol (DG18) agar (M184 (/food/laboratory-methods/bam-media-m184-dichloran-18-glycerol-dg18-agar))

3. Antibiotic solutions (see previous section)
4. NaOCl (commercial bleach) solution, 10%
5. Sterile distilled water

C. **Analysis of non-surface-disinfected (NSD) foods**

Sample and media preparation

Before plating, hold sample at -20° C for 72 h to kill mites and insects that might interfere with analysis.

Prepare DRBC agar as described in the appendix. If DRBC is not available, or the water activity of the analyzed sample is less than 0.95, use DG18 agar. Media should be prepared no more than 24 h prior to use.

Plating and incubation of sample

From each sample, transfer about 50 g into a sterile 300 ml beaker. Using 95% ethanol-flamed forceps place intact food items on surface of solidified agar, 5-10 items per plate (depending on size of food item) 50 items total per sample.

Flame forceps between plating of each item. Use several forceps alternately to avoid overheating. Do not plate visibly moldy or otherwise blemished items.

Align 3-5 plates in stacks and identify with sample number plus date of plating. Incubate stacks, undisturbed in the dark at 25°C for 5 days. If there is no growth at 5 days of incubation, re-incubate for another 48 h to allow heat- or chemically-stressed cells and spores enough time to grow.

Reading of plates

Determine occurrence of mold in percentages. If mold emerged from all 50 food items, moldiness is 100%; if from 32 items, moldiness is 64%. Determine percent occurrence of individual mold genera and species in like manner. Experienced analysts may identify *Aspergillus, Penicillium* and most other foodborne mold genera directly on medium with low power (10-30X) magnification.

D. **Analysis of surface-disinfected (SD) foods**

Perform disinfection in clean laboratory sink, not stainless steel, free from any acid residues, with tap water running (precautions against chlorine gas generation). Wear rubber gloves and transfer about 50 g of sample into a sterile 300 ml beaker. Cover with 10% chlorine (commercial bleach) solution for 2 min, while swirling beaker contents gently but constantly in clockwise-counterclockwise motion. Decant 10% chlorine solution and give beaker contents two 1-min rinses with sterile distilled water. Prepare plates; plate sample, incubate, and read plates as in non-surface disinfected direct plating method, above. Compare NSD and SD results from the same sample to determine if moldiness was due mainly to surface contamination or to internal invasion and growth. Isolate individual colonies on PDA or MA.

Fluorescence Microscopy Procedure for Quantitation of Viable and Nonviable Yeasts in Beverages

Methods for counting viable yeasts by plating are described above. A direct microscopic procedure for counting nonviable and viable yeasts in beverages and other liquid samples is presented here. Quantitating yeast cells by microscopy eliminates the need for extended incubation, thus reducing the analytical time required. All yeasts can be counted, and living and dead yeast cells can be differentiated.

A. Millipore disk filter holders for standard syringes
B. Millipore filters: AABG, 0.8 µm, black, gridded; 25 mm diameter
C. Syringes, disposable
D. Pipets
E. Forceps
F. Bibulous paper
G. Microscope slides and 24 × 24 mm coverslips
H. Fluorescence microscope: blue excitation; l0X eyepieces with Howard mold count or other eyepiece grid; 20× or 40× objective

- **Reagents**
  1. Aniline blue; 1% in M/15 K_2HPO_4 (M/15 is equivalent to 11.6 g/L), adjusted to pH 8.9 with K_3PO_4. A stock solution can be made; age improves fluorescence.
  2. NaOH; 25 g in 100 ml water
- **Sample preparation for filterable liquids (e.g. water and grape juice)**
  Filter aliquot (usually 10 ml) of sample through Millipore filter (AABG, 0.8 µm, black, gridded).(Portion size can be increased or decreased, depending on level of contamination). Use Millipore disk filter holder which attaches to standard syringe. Make sure that syringe is accurate. If not, remove plunger, attach syringe to filter holder, and pipette 10 ml into syringe. Press all of sample through filter. Do this with air cushion of about 3 ml between plunger and sample. Keep filter holder vertical to ensure even distribution of sample over filter. Remove filter from filter holder and place on microscope slide; grid should be parallel to edges of slide to facilitate counting.
- **Sample preparation for non-filterable liquids that clog the filter (e.g. orange juice)**
  To suppress background interference in fluorescence microscope, mix 4 ml sample with 1 ml sodium hydroxide (25 g in 100 ml water). Shake well and wait 10 min. Place Millipore filter (AABG, 0.8 µm, black, gridded) on a piece of bibulous paper and spread 0.1 or 0.01 ml (depending on level

of contamination) of sample over filter. When filter surface is dry, place filter on microscope slide, keeping grid parallel to edges of slide to facilitate counting.

- **Microscopic counting procedure**

  Cover filter with a drop of aniline blue, 1% in M/15 (11.6 g/L) K_2HPO_4, adjusted to pH 8.9 with K_3PO_4. Spread aniline blue stain over whole filter with glass rod or coverslip without touching filter itself. Wait about 5 min; then cover filter with 24 × 24 mm coverslip.

  Count yeasts, using fluorescence microscope with blue excitation. Use 10X eyepiece with Howard mold count or other eyepiece grid, and 20X (or 40X) objective. Count 3 squares of eyepiece grid in each field of filter not covered by gasket. Count budding yeasts as 1 cell if daughter cell is obviously smaller than mother cell. If they are approximately equal in size, count them as 2 cells. Count all yeasts located completely within an eyepiece square and all yeasts touching left and lower border of eyepiece square. Do not count yeasts touching right and upper borders.

  This method also differentiates dead (heat- or formaldehyde-killed) and living yeast cells. Dead cells show fairly uniform fluorescence, and plasma may be granular. In living cells, the cell wall stains brighter and is more defined than the plasma, which is less prominent and uniformly stained.

- **Calculations to determine number of yeasts per ml**

  Determine area of filter covered by 1 square of eyepiece grid, using objective (stage) micrometer. For filtered samples, the working area of the Millipore filter (portion not covered by the gasket) is 380 mm^2. For nonfiltered samples, it is the entire filter, or 491 mm^2, since no gasket is used.

$$\text{No. of yeasts per ml} = \frac{\text{No. of yeasts counted}}{\text{No. of eyepiece squares examined}} \times \frac{\text{working area of filter}}{\text{area of one eyepiece square}} \times \frac{1}{\text{volume of liquid}}$$

- **NOTE:** For non-filterable liquids, volume includes only net amount used and not volume of NaOH added (i.e., 80% of total volume applied to filter).
- For background information on the method, including photographs of dead and living yeast cells, **see** Koch et al., ref. 8, below.

Methods for Determining Toxin Production by Molds

A. **Equipment and materials**

1. Erlenmeyer flasks, 300 ml, wide-mouth
2. Cotton, nonabsorbent
3. Funnels, short-stem glass, 90-100 mm diameter
4. Filter paper, 18 cm diameter, folded (Schleicher & Schuell No. 588)
5. Boiling chips, silicon carbide
6. Fume hood equipped with steam bath; air-flow rate, 100 cubic ft/min
7. Blender, high speed, explosion-proof
8. Thin layer chromatographic apparatus or high-performance liquid chromatograph
9. Incubator, 22-25°C

B. **Media and reagents**

1. Long or short grain polished rice
2. Chloroform for extraction of aflatoxins, ochratoxins, sterigmatocystin, xanthomegnin, luteoskyrin, patulin, penicillic acid, citrinin, T-2 toxin, zearalenone
3. Methanol for extraction of deoxynivalenol
4. Appropriate mycotoxin standards
5. NaOCl solution, 5%

C. **Toxin production**

Into 300 ml wide-mouth Erlenmeyer flask, add 50 g rice and 50 ml distilled water. Plug flasks with cotton and autoclave 20 min at 121°C and 15 psi. Aseptically multispore-inoculate separate cooled flasks with individual mold isolates. Incubate inoculated flasks at 22-25°C until entire surface is covered with growth, and mycelium has penetrated to bottom of flask (15-20 days). To each flask, add 150 ml chloroform (150 ml methanol if toxin in question is deoxynivalenol), using short-stem glass funnel inserted alongside unremoved cotton plug (to minimize mold spore dissemination). Heat flask contents in fume hood on steam bath until solvent begins to boil. (Conduct all subsequent steps in fume hood.) With spatula, break up moldy rice cake and transfer flask contents into explosion-proof blender and blend at high speed for 1 min. Filter blender contents through filter paper inserted into short-stem glass funnel. Collect filtrate in 300 ml Erlenmeyer flask. Return rice cakes to blender, add 100 ml unheated solvent and blend 1 min at high speed. Filter as above and combine filtrates. Add boiling chips to flask containing filtrates and evaporate with steam to 20-25 ml. If analysis is not to follow immediately, evaporate to dryness and store flask in the dark. Rinse all glassware,

etc., used for extraction in 5% NaOCl solution before soap and water cleansing. Submerge rice cake in 5% NaOCl solution for 72 h before autoclaving and disposal.

D. **Toxin analysis**

Appropriate mycotoxin standards are required for both qualitative and quantitative analysis of toxin. Use either thin layer chromatography as described in references 16 or 17 or high performance liquid chromatography,as described in reference 15a, to determine mycotoxins extracted from mold cultures. Naturally occurring mycotoxins in foods or feeds can best be determined by methods described in *Official Methods of Analysis* (16).

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Media

Dichloran 18% glycerol (DG18) agar (M184)

Reagent	Quantity
Glucose	10.0 g
Peptone	5.0 g
KH_2PO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
Dichloran (2,6-dichloro-4-nitroaniline) solution (0.2% (w/v) in ethanol)	1.0 ml
Chloramphenicol	0.1 g
Agar	15.0 g
Distilled water	800 ml

Mix above items and steam to dissolve agar, then bring volume to 1000 ml with distilled water. Add 220 g glycerol (analytical reagent grade), and sterilize by autoclaving at 121°C for 15 min. Temper medium to 45° C and pour plates under aseptic conditions. The final a_w of this medium is 0.955. DG18 agar is used as a general purpose mold enumeration medium and is preferred when the a_w of the analyzed food is less than 0.95. The low water activity of this medium reduces interference by bacteria and fast-growing fungi. When both yeasts and molds must be enumerated, DRBC agar should be used.

Dichloran rose bengal chloramphenicol (DRBC) agar (M183)

Reagent	Quantity
Glucose	10.0 g
Bacteriological peptone	5.0 g
KH_2PO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
Rose bengal (5% aqueous soln., w/v)	0.5 ml
Dichloran (0.2% in ethanol, w/v)	1.0 ml
Chloramphenicol	0.1 g
Agar	15.0 g
Distilled water	1.0 liter

Final pH should be 5.6

Mix ingredients, heat to dissolve agar and sterilize by autoclaving at 121°C for 15 min. Temper to 45 ± 1° C in a water bath and pour plates.

Notes: DRBC agar is especially useful for analyzing samples containing "spreader" molds (e.g. *Mucor, Rhizopus*, etc.), since the added dichloran and rose bengal effectively slow down the growth of fast-growing fungi, thus readily allowing detection of other yeast and mold propagules, which have lower growth rates.

Media containing rose bengal are **light-sensitive**; relatively short exposure to light will result in the formation of inhibitory compounds. Keep these media in a dark, cool place until used. DRBC agar should be used for spread plates only.

Malt Agar (MA) (M185)

Reagent	Quantity
Malt extract, powdered	20.0 g
Agar	20.0 g
Distilled water	1.0 liter

Mix ingredients, steam to dissolve agar and sterilize for 15 min at 121° C. Temper medium to 45° C and pour plates under aseptic conditions. To prepare slants dispense 5-6 ml of steamed medium (before autoclaving) into each of several 16 × 125 mm screw-cap tubes, loosely cap tubes and sterilize as above. After autoclaving lay tubes in a slanting position and let them cool. This medium is recommended as a general maintenance medium.

Malt Extract Agar (Yeasts and Molds)(MEA) (M182)

Reagent	Quantity
Malt extract, powdered	20.0 g
Glucose	20.0 g
Peptone	1.0 g
Agar	20.0 g
Distilled water	1.0 liter

Mix ingredients, heat to dissolve agar and sterilize at 121° C for 15 min. Temper media to 45° C and pour plates under aseptic conditions. Dehydrated MEA is commercially available, but since more than one MEA formula exists, check for the appropriate composition. This medium is recommended for the identification of *Aspergillus* and *Penicillium*.

Hypertext Source: Bacteriological Analytical Manual, 8th Edition, Revision A, 1998. Chapter 18.

Public Health Microbiology Foundation™
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Webpage: https://publichealthmicrobiology.education/food-processing-support
aliyar.fouladkhah@aya.yale.edu (life-time alumni account)| Phone: +1 (970)690-7392

Re: Assistance to Establish Microbiological Testing for Georgian (Zugdidi Region) Stakeholders, Food Processing Support Center of Public Health Microbiology Foundation, Nashville, TN- **Aflatoxin Testing, Rapid Method**
CC: Association for Agriculture Development, Zugdidi, Georgia *May 23, 2022*

Ms. Maia Mikava,

Per our conversation and needs in your region, here is additional information about aflatoxin testing for corn and additional commodities. Although there are many methods for testing for these group of secondary metabolites in various products, this specific method has lower initial capital investment. Additionally, implementation of this rapid testing is manageable based on current competency of the staff in your association as currently no one has chromatography experiences and purchasing gas and/or high-performance liquid chromatography was not financially possible based on information you provided. This testing method has been approved by USDA's Federal Grain Inspection Service for quantitative analyses of aflatoxins in corn, corn products, and additional commodities. The certificate for this approval and similar quotes obtained from the company are attached to this letter. Additionally, list of suggested supplies for conducting the test and a training video from the company is provided below.

Testing for aflatoxins is very important for the public's health and further business development in regional, national, and global commerce, and I commend your associations willingness to learn and implement new and emerging methodologies. For additional information about mycotoxins please refer to training participation manual that I provided during the multi-day workshop completed in the week of May 16, 2022. I wish you further success in business development and for ensuring the public's health during your future endeavors. Additional information about the Public Health Microbiology Foundation™ programs, services, and activities could be accessed at: https://publichealthmicrobiology.education/

Best wishes,

Dr. Aliyar Cyrus Fouladkhah, PhD, MS, MPH, MACE, CFS, CPS

Founding Director, Public Health Microbiology Foundation™

Aflatoxin Testing, Rapid Method*

Please watch this video for details of testing procedure:

https://www.youtube.com/watch?v=_FJYjRbB7wU&t=292s

** Please note that with the same unit, you will be able to conduct additional testing (e.g. Ochratoxin, antibiotic residue in milk, etc.). You could discuss these with the suppliers' technical team to explore additional testing available for the same unit.*

Consumable Needed	**Fischer Scientific Item # (Catalog #)**
Grinder	Available in the facility in Zugdidi
Timer	Available in the facility in Zugdidi
Scale for measuring 10.0 g± 0.1 g	Available in the facility in Zugdidi
Sample rack	03-448-340
Graduated cylinder- 50 ml	08-557C
65% ethanol	NC1853112
100 and 500 µL pipette	Available in the facility in Zugdidi
Tips for 100 and 500 µL pipette	Available in the facility in Zugdidi
Sample collection cup	14-904-53
Sample collection tubes with caps	14-959-11B
Grade 4 Qualitative Filter Papers	09-790-12G
Funnel	03-865
Equipment Needed	**Neogene Company Item #**
Raptor Diagnostic Reader	9680
Cartridge and Kits needed	**Neogene Company Item #**
Raptor Vortex Cartridges	9681
Reveal Q+ MAX for Aflatoxin**	8088

*** Each kit is sufficient for 25 analyses and has expiration date. Since shipping the kits to your facility may take some time, I would propose you purchase sufficient kits for your testing needs with careful consideration of the kit's shelf-life.*

Sample Quote from a Previous Purchase and USDA Certification of the Products

USDA

United States Department of Agriculture

Agricultural Marketing Service

Federal Grain Inspection Service
Technology and Science Division
10383 North Ambassador Drive
Kansas City, MO 64153

July 12, 2019

Mr. Alex Kostin
Neogen Corporation
620 Lesher Place
Lansing, MI 48912

Dear Mr. Kostin:

I am pleased to inform you that the Reveal Q+ MAX for Aflatoxin using the AccuScan Gold Reader has been approved by the Federal Grain Inspection Service for the quantitative determination of aflatoxins in corn and additional commodities. The Certificate of Conformance, FGIS 2019-125, is enclosed, along with the evaluation data contained in Attachment I.

If you have any questions about the performance verification, please contact Dr. Ajit Ghosh at (816) 891-0417 or by e-mail at Ajit.K.Ghosh@usda.gov. For questions regarding FGIS policies on test kit evaluations, please contact me at (816) 891-0449.

Sincerely,

Thomas A. Weber, Chief
Analytical Chemistry Branch
Technology and Science Division

Enclosure: COC FGIS 2019-125
Attachment: Attachment I – Summary of Verification Data for Test Kit (2019159 QN)

United States Department of Agriculture
Agricultural Marketing Service
Federal Grain Inspection Service
Technology and Science Division
10383 North Ambassador Drive
Kansas City, MO 64153

Attachment I - Summary of Verification Data for Test Kit (2019159 QN)
Aflatoxins in Corn

AccuScan Gold Reader, Model #9595

5 ppb Level		20 ppb Level		300 ppb Level	
Analyst	Reading	Analyst	Reading	Analyst	Reading
1	5.3	1	16	1	285
1	4.4	1	14	1	275
1	5.6	1	16	1	291
1	5.0	1	16	1	294
1	5.6	1	16	1	265
1	5.4	1	18	1	274
1	5.5	1	17	1	293
2	4.4	2	14	2	204†
2	5.0	2	17	2	304
2	5.6	2	19	2	214
2	5.2	2	16	2	248
2	5.1	2	16	2	273
2	5.3	2	14	2	268
2	4.7	2	14	2	229
3	4.7	3	19	3	230
3	5.0	3	16	3	228
3	4.1	3	16	3	240
3	4.8	3	16	3	232
3	4.7	3	15	3	238
3	4.8	3	15	3	250
3	4.8	3	15	3	242
Total Out-of-Range	**0**		**0**		**1**
Acceptable Ranges	CRV ± 2*0.25*CRV		CRV ± 2*0.20*CRV		CRV ± 2*0.16*CRV

† Out-of-range
CRV – Certified Reference Value

U.S. DEPARTMENT OF AGRICULTURE
Federal Grain Inspection Service

CERTIFICATE NO.: FGIS 2019-125

CERTIFICATE OF CONFORMANCE

Quantitative test kit for aflatoxin in corn (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings) and additional commodities listed under TEST 3.

For:	Neogen Corporation	Submitted by:	Neogen Corporation. 620 Lesher Place Lansing, MI 48912 Telephone: (800) 234-5333 Contact: Mr. Alex Kostin
Method:	Lateral Flow Strip		

Standard Features and Options

Model:	Reveal Q+ MAX for Aflatoxin, Product #8088
Sample Preparation:	Grind sample so that at least 95% passes through a 20 mesh sieve
Extraction Method:	Blend 50-gram sample with one packet of MAX-1 G50 extraction powder in 250 mL of distilled or deionized water for 30 seconds.
Temperature Range:	18 – 30 °C (64 – 86 °F)
Aflatoxin Level:	5.0 – 300 ppb
Detection Technique:	AccuScan Gold Reader, Model #9595

Test kits must be operated according to the FGIS-issued instructions.

This test kit underwent an initial verification of performance under the authority of Section 7B (c) of the United States Grain Standards Act, as amended, and was found to meet all test performance criteria as defined in "Design Criteria and Test Performance Specifications for Quantitative Aflatoxin Test Kits," June 2018 version. Evaluation tests that passed are summarized in Attachment I.

For further information, contact:

USDA, Federal Grain Inspection Service
Technology and Science Division
Analytical Chemistry Branch
10383 N. Ambassador Drive
Kansas City, Missouri 64153-1394 Telephone: (816) 891-0401

Thomas A. Weber, Chief
Analytical Chemistry Branch
Technology and Science Division

Date: 7-12-2019

Certificate Expires Three Years from the Date Signed

Note: The mention of firm name or trade products does not imply that they are endorsed or recommended by the United States Department of Agriculture over other firms or similar products not mentioned.

Type Evaluation
Certificate No.: FGIS 2019-125

ATTACHMENT I

Manufacturer: Neogen Corporation.
620 Lesher Place
Lansing, MI 48912
Telephone: (800) 234-5333
Contact: Mr. Alex Kostin

TEST 1: TIME REQUIRED FOR COMPLETION OF AN ANALYSIS.

The data submitted by the manufacturer indicated that the analysis time required for one sample was less than the maximum limit of 30 minutes.

TEST 2: COMPARATIVE ACCURACY OF TEST KITS ON CORN SAMPLES NATURALLY CONTAMINATED WITH AFLATOXINS.

The data submitted by the test kit manufacturer for four naturally-contaminated corn samples, containing approximately 5, 20, 100, and 300 ppb total aflatoxins, met the performance criteria.

TEST 3: ADDITIONAL COMMODITIES.

The manufacturer submitted data supporting the performance of this kit for the following additional commodities: pearl or pearled barley (including quick pearl barley), popcorn, brown rice, sorghum, and wheat (including whole grain wheat flour, wheat middlings, wheat red dog, wheat flour 2nd clear, and wheat screenings).

TEST 4: AVOIDANCE OF TOXIC OR HAZARDOUS SUBSTANCES.

The Safety Data Sheets provided by the manufacturer confirmed this test kit meets safety requirements.

TEST 5: SENSITIVITY TO ELECTROMAGNETIC FIELDS (EMF).

A statement of certification has been provided that indicated AccuScan Gold Reader, Model #9595 met the EMF sensitivity requirements.

TEST 6: TEMPERATURE SENSITIVITY.

The data submitted by the test kit manufacturer supported performance of the kit at 18 °C, 24 °C, and 30 °C.

TEST 7: STABILITY.

The data submitted by the test kit manufacturer supported storage and stability claims.

TEST 8: FGIS PERFORMANCE VERIFICATION.

The data generated by FGIS staff showed the test kit is capable of quantifying aflatoxins in corn in the range of 5.0 – 300 ppb total aflatoxins. The evaluation was conducted using AccuScan Gold Reader, Model #9595.

Quote

Quote Valid from: 4/21/2021 to 5/21/2021

Bill To: TEN020-0001
Tennessee State University, Public Health Microbiology Lab
Carp Building Lab 122
3500 John A Merritt Blvd
Nashville, TN 37209-1500 United States
(970) 690-7392

Ship To:
Tennessee State University, Public Health Microbiology Lab
Carp Building Lab 122
3500 John A Merritt Blvd
Nashville, TN 37209-1500 United States
(970) 690-7392

Attn: Dr Aliyar Cyrus Fouladkhah

Quote Date 4/21/2021
Quote# 203576-G5Y5N6
Revision# 0

PO Number	Ship via	Payment Terms	Salesperson	Entered By
	UPS 2 DAY	Net 30 Days	Kirkpatrick, Darcy	Kirkpatrick, Darcy

Product Description	Lot #	Quantity	Price/Unit	Ext Amount
9680 - Raptor Diagnostic Reader		1	$2700.00	$2,700.00
9681 - Raptor Vortex Cartridges		1	$18.00	$18.00
8088 - Reveal Q+ MAX for Aflatoxin		1	$220.50	$220.50
8688 - Reveal Q+ Max for Ochratoxin		1	$299.25	$299.25
			Sub-Total	$3,237.75
Shipping Charges: Charge for Shipping			Freight Amount	$0.00
			TOTAL	**$3,237.75**

If applicable, tax charges will be applied once invoiced

Shipping charges will be determined at later time

Quote

Quote Valid from: 8/3/2021 to 9/2/2021

Bill To:	TEN020-0001	**Ship To:**	**Quote Date** 8/3/2021
Tennessee State University, Public Health Microbiology Lab		Tennessee State University, Public Health Microbiology Lab	**Quote#** 208375-D3D1Q5
Carp Building Lab 122		Carp Building Lab 122	**Revision#** 0
3500 John A Merritt Blvd		3500 John A Merritt Blvd	
Nashville, TN 37209-1500 United States		Nashville, TN 37209-1500 United States	
(615) 963-7605		(970) 690-7392	

Attn: Dr Aliyar Cyrus Fouladkhah

PO Number	Ship via	Payment Terms	Salesperson	Entered By
Quote requested	UPS 2 DAY	Net 30 Days	Kirkpatrick, Darcy	Williams, Mary

Product Description	Lot #	Quantity	Price/Unit	Ext Amount
8088 - Reveal Q+ MAX for Aflatoxin		2	$245.00	$490.00
8688 - Reveal Q+ Max for Ochratoxin		2	$332.50	$665.00
9681 - Raptor Vortex Cartridges		2	$18.00	$36.00
			Sub-Total	$1,191.00
Shipping Charges: Charge for Shipping			Freight Amount	$160.79
			TOTAL	**$1,351.79**

If applicable, tax charges will be applied once invoiced

Shipping charges will be determined at later time

Public Health Microbiology Foundation™
Founding Director, Aliyar Cyrus Fouladkhah, PhD, MPH, CFS, CPH
Webpage: https://publichealthmicrobiology.education/food-processing-support
aliyar.fouladkhah@aya.yale.edu (life-time alumni account)| Phone: +1 (970)690-7392

Re: Assistance to Establish Microbiological Testing for Georgian (Zugdidi Region) Stakeholders, Food Processing Support Center of Public Health Microbiology Foundation, Nashville, TN- **Gram Staining and Antibiotic Stewardship**
CC: Association for Agriculture Development, Zugdidi, Georgia *May 23, 2022*

Ms. Maia Mikava,

As you know, your operation is prescribing various antibiotics for agricultural and domestic/companion animals, and the use of therapeutic and sub-therapeutic antibiotics could lead to challenges associated with public's health. Based on needs and discussions I had with laboratory staff and the information I discussed during the multi-day workshop, would strongly recommend an antibiotic stewardship program in your operation. Since now you have functional microscopic instrumentation and training to utilize them, would recommend a two-step approach for prescription of antibiotics. Upon determination by a clinician that the antibiotic prescription is potentially needed, would first suggest a Gram-staining procedures that could provide information in very short amount of time on where the symptoms are caused by bacterial pathogens and what classes of antibiotics are needed. You could consult this weblink that recommends different classes of antibiotic for treating Gram-positive and Gram-negative microorganisms in various species:

https://cvm.msu.edu/vdl/laboratory-sections/bacteriology-mycology/antimicrobial-susceptibility-testing-ast-resources/use-of-antimicrobial-susceptibility-data-to-guide-therapy

In short:

Small Animals Standard MIC panel for Gram-Positive Bacteria: Amikacin, Amoxicillin/Clav, Ampicillin, Cefazolin, Cefovecin, Cefpodoxime, Cephalothin, Chloramphenicol, Clindamycin, Doxycycline, Enrofloxacin, Erythromycin, Gentamicin, Marbofloxacin, Minocycline, Nitrofurantoin, Oxacillin, Penicillin, Pradofloxacin, Rifampin, Tetracycline, Trimethoprim/SMZ.

Small Animals Standard MIC panel for Gram-Negative Bacteria: Amikacin, Amoxicillin/Clav,, Ampicillin, Cefazolin, Cefovecin, Cefpodoxime, Ceftazidime, Cephalexin, Chloramphenicol, Doxycycline, Enrofloxacin, Gentamicin, Marbofloxacin, Orbifloxacin, Piperacillin/Tazo, Pradofloxacin, Tetracycline, Trimethoprim/SMZ

The second step would be incorporating an antimicrobial susceptibility test for further pinpointing the best and most efficacious antibiotic(s) to treat the infection. This could be achieved in 24 to 72 hours after initiating the test. For this, you could utilize a standard disk-based method or using a 96-well microplate preloaded with antibiotics.

Below am incorporating some testing methods and consumables needed for conduct of these two procedures. Incorporating these will assist your operation to best serve the stakeholder's while protecting the public's health against the threat of antibiotics resistant microorganisms.

I commend your association's willingness to learn and implement new and emerging methodologies. For additional information about therapeutic and sub-therapeutic use of antibiotics in food production, please refer to training participation manual that I provided during the multi-day workshop completed in the week of May 16, 2022.

I wish you further success in business development and for ensuring the public's health during your future endeavors. Additional information about the Public Health Microbiology Foundation™ programs, services, and activities could be accessed at: https://publichealthmicrobiology.education/

Best wishes,

Dr. Aliyar Cyrus Fouladkhah, PhD, MS, MPH, MACE, CFS, CPS

Founding Director, Public Health Microbiology Foundation™

Grain-Staining and Antibiotic Susceptibility Test*

Consumable Needed for Gram-Staining	Fischer Scientific Item # (Catalog #)
PROTOCOL™ Gram Stain Sets	23-005-83
Consumable Needed for Antibiotic Susceptibility	**Fischer Scientific Item # (Catalog #)**
Ceftazidime	11973812
Ceftriaxone	11963812
Azithromycin	10660125
Enrofloxacin	11962992
Colistin	11963972
Cefpodoxime	10547293
Bacitracin	10671845
Novobiocin	10046292
Other relevant antibiotics	As needed by the operation, based on the antibiotics used to treat infections.

** Please note that the susceptibility test could be conducted using standard disks, a serial dilution, or a 96-well microplate. Please review the accompanying article for additional information.*

Procedures for Gram-Staining and Antibiotic Susceptibility Test

Gram Stain Protocols

Created: Friday, 30 September 2005

Author

- Ann C. Smith
- Marise A. Hussey

Information

History

The Gram stain was first used in 1884 by Hans Christian Gram (Gram,1884). Gram was searching for a method that would allow visualization of cocci in tissue sections of lungs of those who had died of pneumonia. Already available was a staining method designed by Robert Koch for visualizing turbercle bacilli. Gram devised his method that used Crystal Violet (Gentian Violet) as the primary stain, an iodine solution as a mordant followed by treatment with ethanol as a decolorizer. This staining procedure left the nuclei of eukaryotic cells in tissue samples unstained while the cocci found in the lungs of those who had succumbed to pneumonia were stained blue/violet. Gram found that his stain worked for visualizing a series of bacteria associated with disease such as the "cocci of suppurative arthritis following scarlet fever". He found however that Typhoid bacilli were easily decolorized after the treatment with crystal violet and iodine, when ethanol was added. We now know that those organisms that stained blue/violet with Gram's stain are **gram-positive** bacteria and include *Streptococcus pneumoniae* (found in the lungs of those with pneumonia) and *Streptococcus pyogenes* (from patients with Scarlet fever) while those that were decolorized are **gram-negative**bacteria such as the *Salmonella* Typhi that is associated with Typhoid fever.

Purpose

The Gram stain is fundamental to the phenotypic characterization of bacteria. The staining procedure differentiates organisms of the domain Bacteria according to cell wall structure. Gram-positive cells have a thick peptidoglycan layer and stain blue to purple. Gram-negative cells have a thin peptidoglycan layer and stain red to pink.

Theory

The Gram stain, the most widely used staining procedure in bacteriology, is a complex and differential staining procedure. Through a series of staining and decolorization steps, organisms in the Domain Bacteria are differentiated according to cell wall composition. Gram-positive bacteria

have cell walls that contain thick layers of peptidoglycan (90% of cell wall). These stain purple. Gram-negative bacteria have walls with thin layers of peptidoglycan (10% of wall), and high lipid content. These stain pink. This staining procedure is not used for Archeae or Eukaryotes as both lack peptidoglycan. The performance of the Gram Stain on any sample requires four basic steps that include applying a primary stain (crystal violet) to a heat-fixed smear, followed by the addition of a mordant (Gram's Iodine), rapid decolorization with alcohol, acetone, or a mixture of alcohol and acetone and lastly, counterstaining with safranin.

Details of the chemical mechanism of the Gram stain were determined in 1983 (Davies et al.,1983 and Beveridge and Davies, 1983). In aqueous solutions crystal violet dissociates into CV^+ and Cl^-ions that penetrate through the wall and membrane of both gram-positive and gram-negative cells. The CV^+ interacts with negatively charged components of bacterial cells, staining the cells purple. When added, iodine (I^- or I_3^-) interacts with CV^+ to form large CVI complexes within the cytoplasm and outer layers of the cell. The decolorizing agent, (ethanol or an ethanol and acetone solution), interacts with the lipids of the membranes of both gram-positive and gram-negative Bacteria. The outer membrane of the gram-negative cell is lost from the cell, leaving the peptidoglycan layer exposed. Gram-negative cells have thin layers of peptidoglycan, one to three layers deep with a slightly different structure than the peptidoglycan of gram-positive cells (Dmitriev, 2004).With ethanol treatment, gram-negative cell walls become leaky and allow the large CV-I complexes to be washed from the cell. The highly cross-linked and multi-layered peptidoglycan of the gram-positive cell is dehydrated by the addition of ethanol. The multi-layered nature of the peptidoglycan along with the dehydration from the ethanol treatment traps the large CV-I complexes within the cell. After decolorization, the gram-positive cell remains purple in color, whereas the gram-negative cell loses the purple color and is only revealed when the counterstain, the positively charged dye safranin, is added. At the completion of the Gram stain the gram-positive cell is purple and the gram-negative cell is pink to red.

Some bacteria, after staining with the Gram Stain yeild a pattern called gram-variable where a mix of pink and purple cells are seen. The genera *Actinomyces, Arthrobacter, Corynebacterium, Mycobacterium,* and *Propionibacterium* have cell walls particularly sensitive to breakage during cell division, resulting in gram-negative staining of these gram-positive cells. In cultures of *Bacillus, Butyrivibrio,* and *Clostridium* a decrease in peptidoglycan thickness during growth coincides with an in increasing number cells that stain gram-negative (Beveridge, 1990). In addition, in all bacteria stained using the Gram stain, the age of the culture may influence the results of the stain.

Some bacteria do not stain as expected with the Gram stain. For example, members of the genus*Acinetobacter* are gram-negative cocci that are resistant to the decolorization step of the Gram stain.*Acinetobacter* spp. often appear gram-positive after a well prepared Gram stain (Visca et al. 2001). For *Mycobacterium* spp., the waxy nature of the coat renders the bacteria not readily stainable with dyes used in

the Gram stain, though the bacteria are considered to be gram positive (Saviola and Bishai, 2000). *Gardnella* has an unusual gram-positive cell wall structure that causes bacteria of this genus to stain gram-negative or gram-variable (Sadhu et al 1989).

Misinterpretation of the Gram stain has led to misdiagnosis or delayed diagnosis of infectious disease (Visca et al., 2001, Noviello et al., 2004)

RECIPE

(Gephardt et al., 1981)This is Hucker's modification of the Gram Stain method. Gram originally used Gentian Violet as the primary stain in the Gram stain. Crystal violet is generally used today. In Hucker's method ammonium oxalate is added to prevent precipitation of the dye (McClelland, 2001) and uses an alcoholic solution of the counterstain. Burke's modification of the Gram Stain adds sodium bicarbonate to the crystal violet solution. Sodium bicarbonate prevents the acidification of the solution as iodine oxidizes (McClelland, 2001) and uses an aqueous solution of Safranin for the counterstain (Gephardt et al., 1981).

The reagents listed below can be made or purchased commercially from biological supply houses

1. Primary Stain: Crystal Violet Staining Reagent.

Solution A for crystal violet staining reagent

Crystal violet (certified 90% dye content), 2g
Ethanol, 95% (vol/vol), 20 ml

Solution B for crystal violet staining reagent

Ammonium oxalate, 0.8 g
Distilled water, 80 ml

Mix A and B to obtain **crystal violet staining reagent.** Store for 24 h and filter through paper prior to use.

2. Mordant: Gram's Iodine

Iodine, 1.0 g
Potassium iodide, 2.0 g
Distilled water, 300 ml

Grind the iodine and potassium iodide in a mortar and add water slowly with continuous grinding until the iodine is dissolved. Store in amber bottles.

3. Decolorizing Agent

Ethanol, 95% (vol/vol)

*Alternate Decolorizing Agent

Some professionals prefer an acetone decolorizer while others use a 1:1 acetone and ethanol mixture. Commercially, a variety of mixtures are available, most using 25 – 50% acetone with the ethanol. A few include a small quantity of isopropyl alcohol and/or methanol in the formulation.

Acetone, 50 ml
Ethanol (95%), 50 ml

4. Counterstain: Safranin

Stock solution:

2.5g Safranin O
100 ml 95% Ethanol

Working Solution:

10 ml Stock Solution
90 ml Distilled water

PROTOCOL (Gephardt et al, 1981, Feedback from ASMCUE participants, ASMCUE , 2005)

1. Flood air-dried, heat-fixed smear of cells for 1 minute with crystal violet staining reagent. **Please note** that the quality of the smear (too heavy or too light cell concentration) will affect the Gram Stain results.
2. Wash slide in a gentle and indirect stream of tap water for 2 seconds.
3. Flood slide with the mordant: Gram's iodine. Wait 1 minute.
4. Wash slide in a gentle and indirect stream of tap water for 2 seconds.
5. Flood slide with decolorizing agent. Wait 15 seconds or add drop by drop to slide until decolorizing agent running from the slide runs clear (see Comments and Tips section).
6. Flood slide with counterstain, safranin. Wait 30 seconds to 1 minute.
7. Wash slide in a gentile and indirect stream of tap water until no color appears in the effluent and then blot dry with absorbent paper.
8. Observe the results of the staining procedure under oil immersion using a Brightfield microscope. At the completion of the Gram Stain, gram-negative bacteria will stain pink/red and gram-positive bacteria will stain blue/purple.

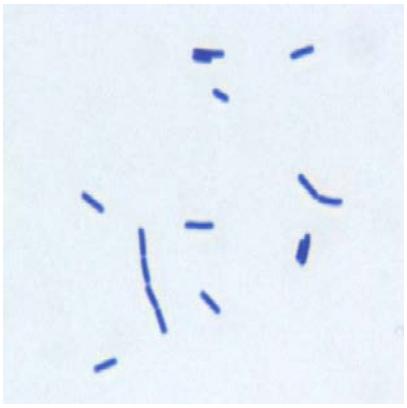

FIG. 1

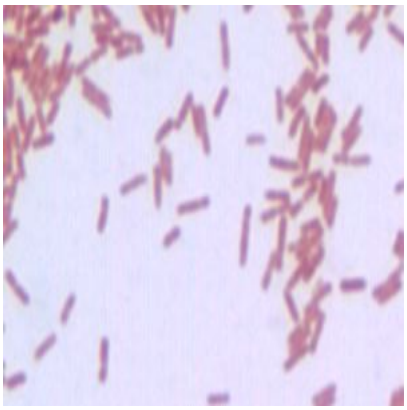

FIG. 2

In a smear that has been stained using the Gram Stain protocol, the shape, arrangement and gram reaction of a bacterial culture will be revealed. FIG. 1. shows gram-positive (blue/purple) rods and FIG. 2. shows gram-negative (pink/red) rods.

SAFETY

The ASM advocates that students must successfully demonstrate the ability to explain and practice safe laboratory techniques. For more information, read the laboratory safety section of the ASM Curriculum Recommendations: Introductory Course in Microbiology and the Guidelines for Biosafety in Teaching Laboratories.

MSDS links:

Acetone information:
http://www.jtbaker.com/msds/englishhtml/A0446.htm
Ammonium oxalate information:
http://www.jtbaker.com/msds/englishhtml/A6072.htm
Crystal violet Information:
http://www.jtbaker.com/msds/englishhtml/C5720.htm
Ethanol information:

http://www.jtbaker.com/msds/englishhtml/D0210.htm
Iodine Information:
http://www.jtbaker.com/msds/englishhtml/I2680.htm
Potassium iodide information:
http://www.jtbaker.com/msds/englishhtml/P5906.htm
Safranin Information:
http://www.jtbaker.com/msds/englishhtml/S0240.htm

COMMENTS AND TIPS

Comments and tips come from discussions at ASM Conference for Undergraduate Educators 2005.

The thickness of the smear used in the Gram stain will affect the result of the stain. **The step that is most crucial in effecting the outcome of the stain is the decolorizing step.** Over-decolorizing will lead to an erroneous result where gram-positive cells may stain pink to red indicating a gram-negative result, and under-decolorizing will lead to an erroneous result where gram-negative cells may appear blue to purple indicating a gram-positive result. The degree of decolorizing required is determined by the thickness of the smear (number of cells on the slide). The Gram stain was discussed in detail at the American Society for Microbiology Conference for Undergraduate Educators in 2005 when this site was reviewed. The group recommends that cells be prepared with a thin smear with no areas of clumping or inconsistency. When staining the thin smear a short decolorizing time should be used. Some individuals will flood the slide for 15 seconds or less with decolorzing agent, while others recommend adding decolorizing agent drop wise for 5- 15 seconds or until the color of the decolorizing agent running from the slide no longer shows any color.

It is recommended that young, actively growing cultures be used for gram staining. An intact cell wall is required for an accurate gram stain. Older cultures may have breaks in the cell wall and often give gram-variable results where a mixture of pink/red cells are seen among blue/purple cells.

Using a gram stain control is recommended. On the same slide as the test culture include a sample of cells with a known gram stain reaction to serve as a control for success in the gram stain technique.

Gram-stained bacteria should be viewed with a brightfield microscope at 1000X magnification with oil immersion. If the smear of cells is crowded it will be difficult to note cell shape and arrangement.

When viewing slides use brightfield microscopy and adjust the brightness sufficiently to reveal the color of the specimen.

Freshly made staining reagents are recommended. With older staining reagents, filter stains before use.

In the Gram Stain technique, two positively charged dyes are used: crystal violet and safranin. The use of the designation “gram-positive”

should not be confused with the concept of staining cells with a simple stain that has a positive charge.

KOH string test may be used as a confirmatory test for the Gram Stain (Powers, 1995, Arthi et al., 2003): The formation of a string (DNA) in 3% KOH indicates that the isolate is a gram-negative organism.
Procedure:
Place a drop of 3% KOH onto a glass slide.
Emulsify in KOH a loopful of the culture from a BA incubated for 18-24 hours.
Continue to mix the suspension for 60 sec and by slowly lifting the loop, observe for the formation of a string.
Interpretation:
Gram-negative cells form a string within 60 seconds.
Gram-positive cells are not affected.

Various formulations of decolorizing agents may be used (acetone, acetone/ethanol, ethanol). Acetone is the most rapid decolorizer followed by acetone/ethanol and then ethanol. Ethanol is recommended for student use to prevent over-decolorization of samples (McClelland, 2001)

When the counterstain is added this positively charged dye will replace the crystal violet dye in the gram-positive bacteria as well as stain the gram-negative bacteria, although the presence of the mordant slows this process considerably it is important not to overexpose cells to counterstain (McClelland, 2001)

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Bacterial Culture and Antibiotic Susceptibility Testing

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Abstract: *Complicated bacterial infections should prompt clinicians to pursue a definitive diagnosis. Two methods of bacterial culture and antibiotic susceptibility testing are commonly used in veterinary medicine: (1) the disk diffusion technique and (2) the broth dilution technique. Both methods identify the infecting pathogen and the antibiotics that are likely to inhibit its growth. The broth dilution test also provides the minimal inhibitory concentration, which can help in making the best antibiotic choice.*

Bacterial infections in companion animals are frequently self-limiting, causing discomfort but not serious illness. The types of bacteria responsible for these infections are frequently predictable. The choice of antibiotics to treat simple infections can usually be made based on historical data and the clinician's experience.

More serious bacterial illness should prompt clinicians to identify the responsible pathogen and determine the medication most likely to be efficacious in inhibiting or killing the bacteria. Bacterial culture and antibiotic susceptibility testing should be considered for life-threatening illnesses; recurrent, nonresponsive, or chronic infections; and illnesses with a history of previous antibiotic therapy.

Reasons for Bacterial Culture and Antibiotic Susceptibility Testing

Bacterial culture and antibiotic susceptibility testing are important for confirming the presence of bacterial infection, identifying the responsible pathogen, and directing antibiotic choices.[1] Additional variables such as route and frequency of administration, cost, and potential adverse effects can then be considered when choosing the most appropriate antibiotic for the patient. Using susceptibility testing to help in choosing the most effective antibiotic can reduce the expense and client frustration that may occur with blind antibiotic trials; lower the risks of complications and of promoting antibiotic resistance; and improve the chance and speed of a patient's recovery.

Specimen Collection

Samples must be collected and handled properly to obtain reliable results. Poor collection techniques may result in lack of bacterial growth or abundant growth of contaminants. To avoid contamination, aseptic technique is necessary when obtaining samples. Submission of fluid, effusion, exudate, and tissue samples is preferred to simply submitting swabs of these samples.[2]

Collection of samples early in the disease process is recommended to reduce the possibility of pathogenic bacteria dying or being overgrown by other bacteria.[2] Samples should be collected before antibiotic therapy to assure the best growth of the pathogen. If antibiotic therapy has already been instituted, samples should be collected just before the next dose is administered.[2]

Identifying Bacteria

When submitting samples to a laboratory for bacterial culture and antibiotic susceptibility testing, the clinician should include information about the site of sample collection and the type of lesion. This information assists the microbiologist in deciding which nutrient media and growth conditions to use. Samples for bacterial culture are applied to plates of various growth media with a sterile loop, effectively spreading bacterial organisms over the surface of each plate in a single layer.[3] Once inoculated, the plates are incubated in an environment with controlled temperature, humidity, and oxygen and carbon dioxide levels that are optimum for replication of the suspected bacteria.

Each bacterial organism grows into a small cluster, called a *colony*, and individual colonies are inoculated onto new, separate media, creating pure samples.[3] Identification of the cultured bacteria is based on the characteristics of colony growth and appearance as well as biochemical testing of the individual colonies.[3]

FIGURE 1

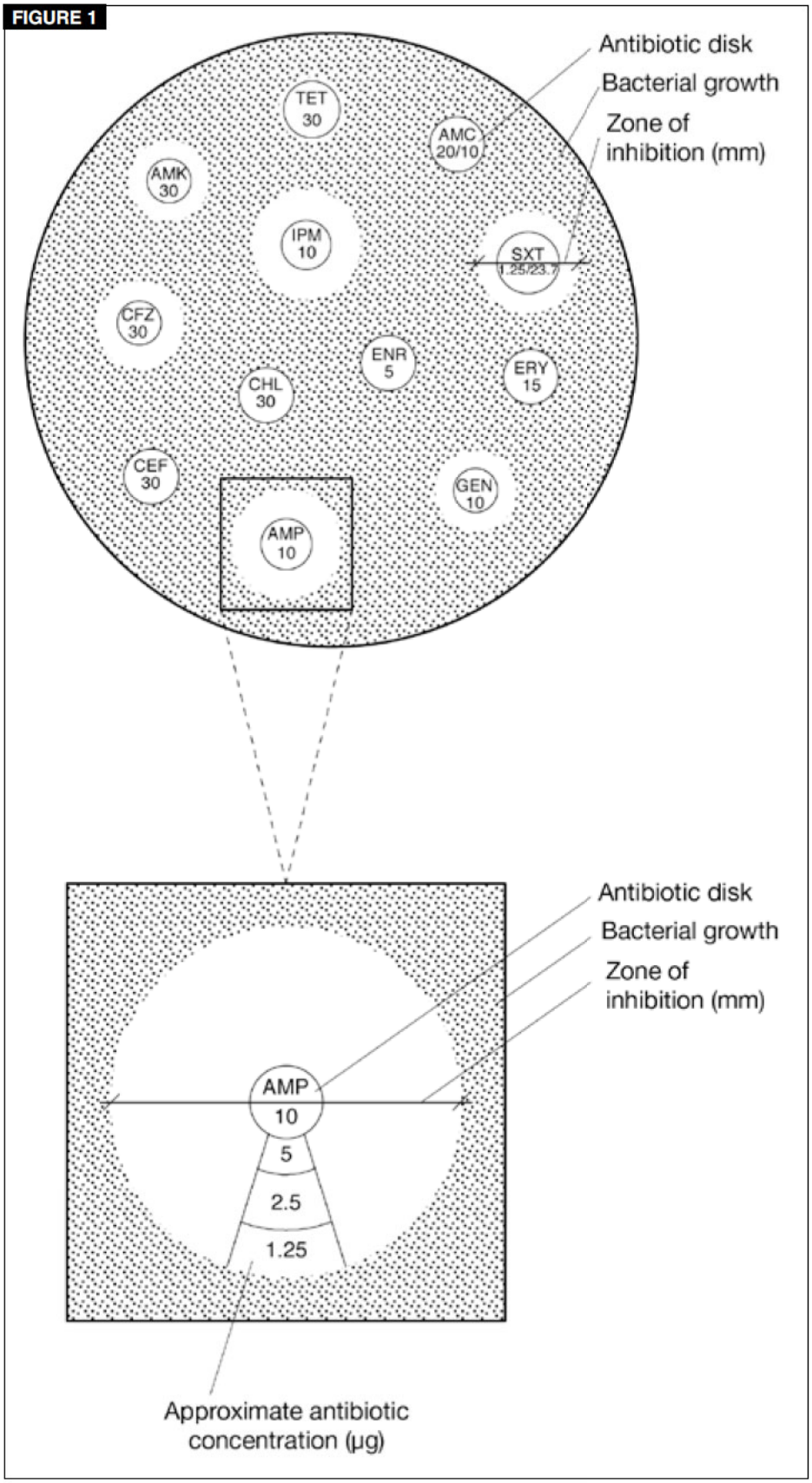


Diagram of the disk diffusion method. The antibiotic concentration in the agar decreases as the drug diffuses away from the disk.

Testing Bacteria for Antibiotic Susceptibility

Once identified, the bacteria undergo testing to identify the antibiotics most likely to inhibit their growth. The most common methods of antibiotic susceptibility testing used in veterinary laboratories are the disk diffusion and broth dilution techniques.[4]

Disk Diffusion Technique

The disk diffusion technique (Kirby-Bauer method) historically has been and continues to be the method most commonly used in the veterinary field for determining antibiotic susceptibility. In this technique, a fixed volume of nutrient broth containing a standard concentration of bacteria is smeared evenly onto the surface of an agar plate.[5] Next, disks of filter paper, each impregnated with a standard concentration of an antibiotic, are applied to the plate surface.[6] The plate is incubated, and as the bacteria grow on the surface of the plate, the antibiotics diffuse from the paper disks out into the agar. Each antibiotic diffuses at a different rate, achieving different concentrations in the surrounding agar based on its molecular size and chemical properties. The concentration of antibiotic in the agar decreases as the antibiotic diffuses further from the disk. Eventually, the antibiotic concentration in the agar drops below that needed to inhibit the growth of the bacteria. The area around the disk in which the antibiotic concentration is high enough to inhibit bacterial growth is called the *zone of inhibition* (**FIGURES 1** and **2**).

The zone of inhibition is measured in millimeters by a laboratory technician. The edge of this zone correlates with the antibiotic concentration that inhibits the growth of the bacteria. The width of the zone is compared to a standard

table of predetermined zone widths representing antibiotic concentrations in the agar that correlate with the concentration of the antibiotic achievable in the plasma of a patient using the manufacturer's recommended dosage. If the zone of inhibition is wider than the predetermined zone, the bacterial species is considered to be susceptible (S) to the antibiotic. If bacteria grow within the predetermined zone width, the species is considered resistant (R). An intermediate (I) designation is used if the zone of inhibition approximates the predetermined zone width.

Laboratories using the disk diffusion technique report the bacteria isolated, a list of the antibiotics tested, and the designation S, I, or R. A disadvantage of the disk diffusion technique is that the exact concentration of the antibiotic that inhibited bacterial growth is not known.

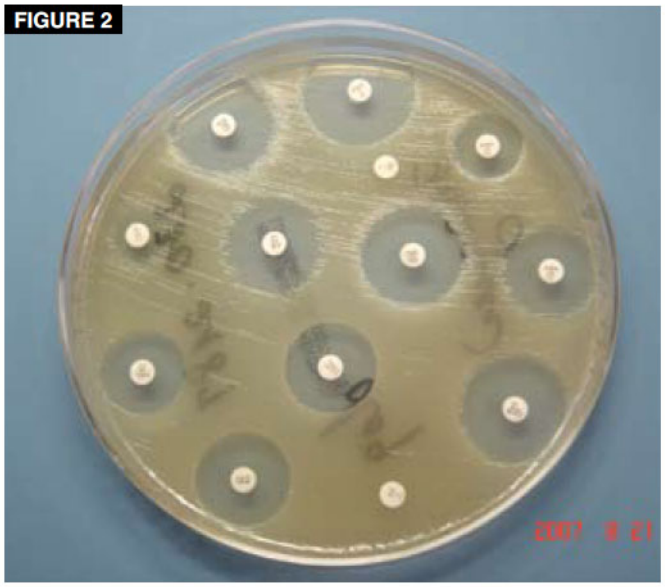
Broth Dilution Technique

The broth dilution technique of antibiotic susceptibility testing is also known as the *minimal inhibitory concentration (MIC)* technique. Test tubes or wells containing increasing concentrations of each antibiotic to be tested, from 0.0312 to 512 µg/mL, are inoculated with a fixed volume of nutrient broth containing a standard concentration of bacteria.[7] The concentration of the antibiotic in each tube is double that in the previous tube. Very few laboratories evaluate bacterial growth at the full range of antibiotic concentrations.[8] More often, a smaller range of dilutions is used to evaluate bacterial growth based on previous experience with antibiotic susceptibility testing for the particular pathogen.

The tubes are incubated and examined for turbidity. A turbid sample is an indication of bacterial growth, whereas a clear sample is an indication of inhibition of bacterial growth (**FIGURE 3**). The MIC is the lowest concentration of the antibiotic being tested that inhibits the growth of the bacteria, resulting in a sample that lacks turbidity (**FIGURE 4**). To determine whether the pathogen is susceptible, intermediate, or resistant, the MIC is compared with the concentration of antibiotic that can be achieved in the plasma of a patient using the manufacturer's recommended dosage. (The concentration of antibiotic achievable in the plasma using the recommended dosage is also sometimes called the *breakpoint* or MIC_{BP}). Ideally, the clinician should have the breakpoint values available when evaluating the test results (**TABLE 1**[9]).

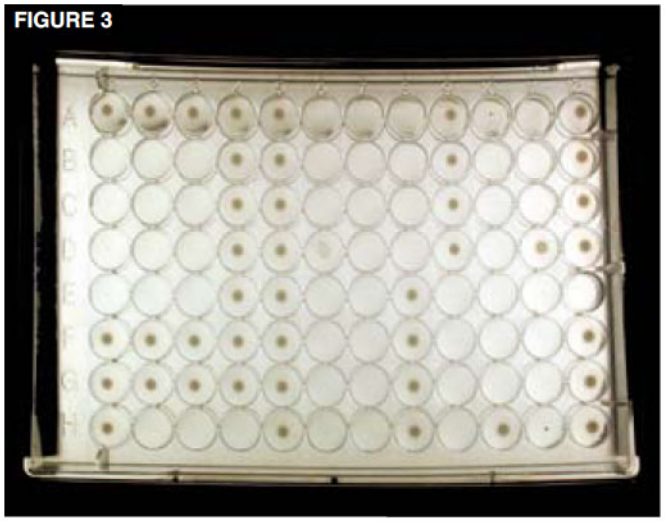
When reporting broth dilution results, laboratories typically note the species of bacteria isolated, the antibiotics tested, the MIC for each antibiotic tested, and a final interpretation of S, I, or R for each antibiotic. A less than (<) notation is used if the MIC was less than the range of antibiotic concentration tested (i.e., there was no bacterial growth even at the lowest antibiotic concentration tested), whereas a greater than (>) notation is used if the MIC was greater than the range of antibiotic concentration tested (i.e., there was bacterial growth in all of the antibiotic concentrations tested). Some laboratories also report the antibiotic dosage used to make the interpretation of susceptibility.

FIGURE 2



Disk diffusion plate. Courtesy of Cynthia Essmyer, MD, DABIM, DABPMM, Saint Luke's Hospital, Kansas City, Missouri

FIGURE 3



Broth microdilution tray. Courtesy of William Fales, PhD, DACVM (Hon), University of Missouri Veterinary Medical Diagnostic Laboratory

The broth dilution method is considered to be superior to the disk diffusion method because it provides the MIC in addition to an interpretation of S, I, or R.[5] Comparing the MIC to the achievable antibiotic plasma concentration allows consideration of the relative susceptibility of the bacteria to each antibiotic. The lower the MIC compared with the achievable antibiotic plasma concentration (MIC_{BP}), the more likely the therapy is to be effective.

Interpretation of Antibiotic Susceptibility Results

The Clinical Laboratory Standards Institute (CLSI) is respon-

FIGURE 4

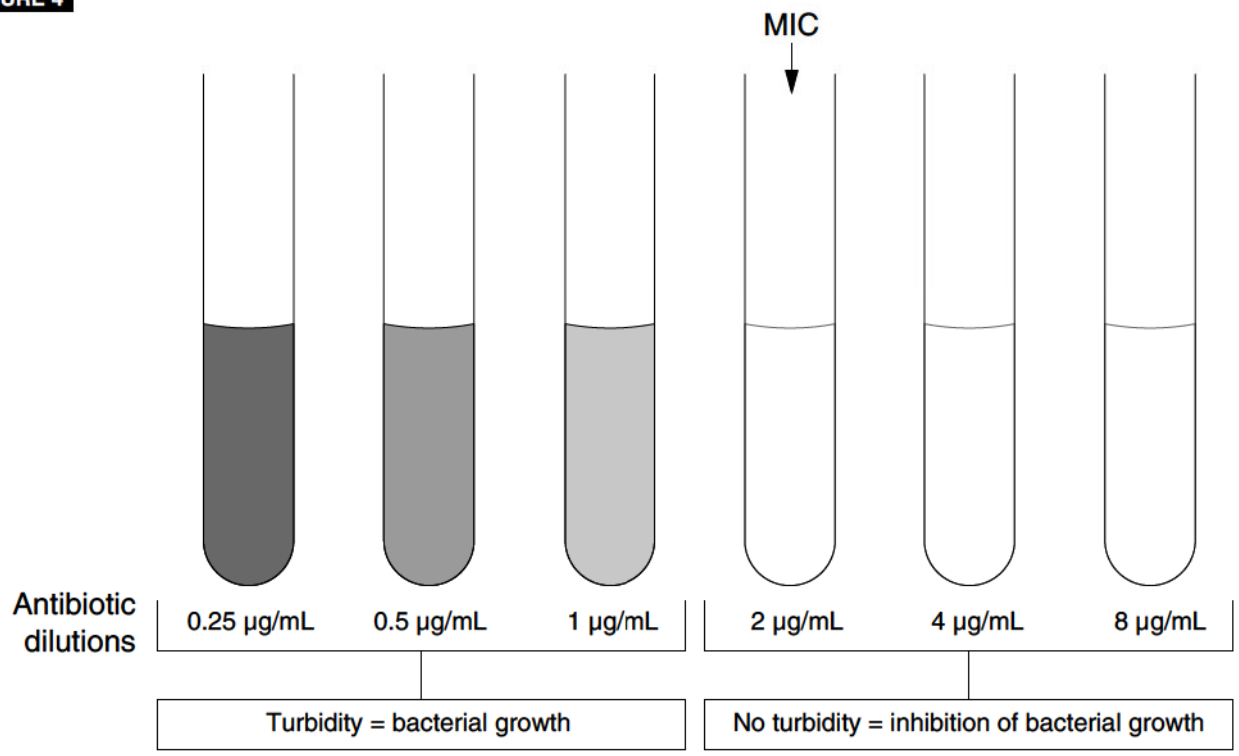


Diagram of the broth dilution method. The MIC is the lowest concentration of antibiotic that lacks turbidity (i.e., has no bacterial growth).

sible for determining the standards used to interpret bacterial culture and antibiotic susceptibility results. Antibiotic pharmacokinetic and pharmacodynamic studies performed by the CLSI, independent researchers, and antibiotic manufacturers provide the information used to formulate these standards. These standards differ between humans and animals; therefore, it is important that the laboratory use the standards created for animals.

The CLSI is responsible for reporting the concentration of each antibiotic to which a bacterial pathogen is to be considered susceptible or resistant. For bacteria to be considered susceptible to an antibiotic, their growth must be inhibited at a concentration lower than the plasma concentration achievable using the manufacturer's labeled dosage. A bacterial pathogen is considered resistant to a particular antibiotic if its growth is inhibited at a concentration that is higher than the concentration that can be reasonably achieved in plasma.[5] An intermediate classification suggests that the concentration of antibiotic needed to inhibit the growth of the organism is very close to the plasma levels achievable at the recommended dosage.

Occasionally, manufacturers provide a dosage range to treat infections. If a dosage range for a drug is given, the highest dose in the range is used to determine whether the pathogen is considered susceptible, intermediate, or resistant to the respective antibiotic.

Guidelines for Evaluating Antibiotic Susceptibility Results

Ideally, clinicians should always choose a drug to which the identified bacteria are considered susceptible and should avoid agents to which they are intermediate or resistant. If MIC information is available, an antibiotic with an MIC that is much lower than the achievable antibiotic plasma concentration should be considered because it is much more likely to be effective in treating the infection.[10]

Antibiotic doses can also be altered based on MIC values. If the MIC is much lower than achievable antibiotic plasma concentrations based on the recommended manufacturer's dosage, a lower dose or dosing interval may still be effective. If the MIC is near the achievable antibiotic plasma concentration, a higher dose or dosing interval should be used.

Limitations of Bacterial Culture and Antibiotic Susceptibility Testing

Selecting antibiotics based on susceptibility data does not guarantee clinical success. Susceptibility tests are conducted in vitro and cannot completely predict the behavior of the pathogen or the antibiotic in vivo. The determination of S, I, or R is based on the expected concentration of the drug in the plasma, not in the tissue at the site of infection.[9] The concentration of bacteria at the site of infection may be higher than that used during the antibiotic susceptibility

testing, resulting in a reduced effectiveness of the drug. False-positive results can occur if normal flora or nosocomial bacteria are isolated. False-negative results are possible if the sample is improperly collected or stored for culture or if the patient has previously received antibiotic therapy. Factors such as the cost of therapy, pharmacodynamics and pharmacokinetics of the drug, and location and character of the infection must also be taken into account when choosing the best antibiotic for each particular patient.[5]

Conclusion

The broth dilution and disk diffusion techniques are the most commonly used methods of bacterial culture and antibiotic susceptibility testing in veterinary medicine. Both methods can be used to identify the likely pathogen involved in a bacterial infection and the antibiotic most likely to inhibit the bacteria.

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TABLE 1 Antibiotic Breakpoints of Common Antibiotics

Antimicrobial	Susceptible (µg/mL)[a]	Resistant (µg/mL)
Amikacin	≤16	≥64
Amoxicillin–clavulanic acid	≤8/4[b]	≥32/16
Ampicillin	≤8[b]	≥32
Cefazolin	≤8	≥32
Cefotaxime	≤8	≥64
Cefoxitin	≤8	≥32
Ceftazidime	≤8	≥32
Cephalothin[c]	≤8	≥32
Chloramphenicol	≤8	≥32
Ciprofloxacin	≤1	≥4
Clindamycin	≤0.5	≥4
Difloxacin	≤0.5	≥4
Enrofloxacin	≤0.5[d]	≥4
Erythromycin	≤0.5[b]	≥8
Gentamicin	≤2	≥8
Imipenem (and meropenem)	≤4	≥16
Marbofloxacin	≤1	≥4
Orbifloxacin	≤1[d]	≥8
Oxacillin[e]	≤2	≥4
Rifampin	≤1	≥4
Tetracycline	≤4	≥16
Ticarcillin	≤64[b]	≥128
Trimethoprim–sulfamethoxazole	≤2/38	≥4/76
Vancomycin	≤4	≥32

[a]Values between the susceptible and resistant ranges are interpreted as intermediate.
[b]There are exceptions for interpreting some pathogens: for ampicillin, susceptibility is ≤0.25 µg/mL for staphylococci and streptococci; for amoxicillin–clavulanate, susceptibility is ≤4/2 µg/mL for staphylococci; for ticarcillin, susceptibility is ≤64 µg/mL for *Pseudomonas* spp and ≤16 µg/mL for enteric gram-negative bacteria; for erythromycin, susceptibility is ≤0.25 µg/mL for streptococci.
[c]Cephalothin is used as a marker to test for susceptibility to cephalexin and cefadroxil.
[d]For enrofloxacin and orbifloxacin, the intermediate category may require higher doses.
[e]If organisms are resistant to oxacillin, they should be considered also resistant to other β-lactam antibiotics.
Adapted with permission from Papich MG, Bidgood T. Antimicrobial drug therapy. In: Ettinger SJ, Feldman ED, eds. *Textbook of Veterinary Internal Medicine.* 6th ed. St. Louis: Elsevier Saunders; 2005:498-503.

3 CE CREDITS

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1. **Bacterial culture and susceptibility testing would be important to perform in a patient with**
   - **a.** a life-threatening illness.
   - **b.** a recurrent infection.
   - **c.** an illness previously treated with antibiotic therapy.
   - **d.** any of the above

2. **Which sample is ideal for submission for bacterial culture and antibiotic susceptibility testing?**
   - **a.** a syringe of whole blood in an EDTA tube obtained during routine venipuncture
   - **b.** a syringe of purulent exudate collected during surgery
   - **c.** a swab of voided urine collected in a bowl
   - **d.** a swab of a superficial skin wound

3. **Samples for bacterial culture and sensitivity testing should ideally be collected**
   - **a.** as early in the disease process as possible.
   - **b.** after the first dose of antibiotic has been given.
   - **c.** after the animal has become febrile.
   - **d.** when the white blood cell count is elevated.

4. **Which characteristic is indicative of bacterial growth using the broth dilution technique?**
   - **a.** visualization of bacteria in the sample using light microscopy
   - **b.** turbidity of the sample
   - **c.** viscosity of the fluid
   - **d.** none of the above

5. **Which laboratory finding can be used to help predict the most effective antibiotic selection?**
   - **a.** diameter of the zone of inhibition (disk diffusion technique)
   - **b.** number of bacteria per high-power field on direct microscopic examination (broth dilution technique)
   - **c.** MIC (broth dilution technique)
   - **d.** characteristics of colony growth on the agar plate (disk diffusion technique)

6. **Which antibiotic would be most likely to result in successful treatment of a bacterial infection at the MIC given?**
   - **a.** enrofloxacin; MIC = 0.25 µg/mL
   - **b.** cefazolin; MIC = 1 µg/mL
   - **c.** ampicillin; MIC = 16 µg/mL
   - **d.** clindamycin; MIC = 1 µg/mL

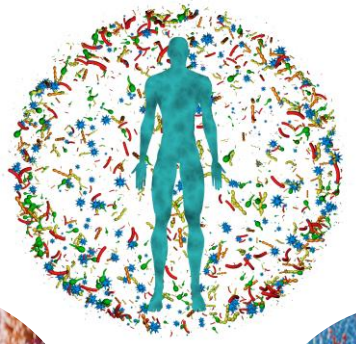
7. **Based on the disk diffusion technique, the most effective antibiotic is likely to be**
   - **a.** the one with the largest zone of inhibition.
   - **b.** the one the smallest zone of inhibition.
   - **c.** designated S.
   - **d.** the one with the lowest MIC.

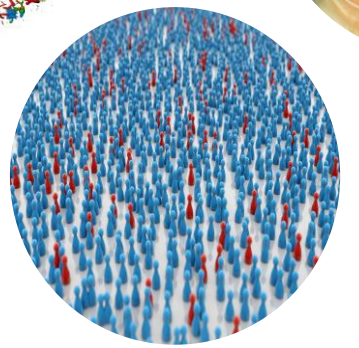
8. **Which of the following is not a limitation of bacterial culture and antibiotic susceptibility testing?**
   - **a.** They are in vitro tests.
   - **b.** They assume equal plasma and tissue concentrations of antibiotic.
   - **c.** False-positive and false-negative results are possible.
   - **d.** They can limit the chance of antimicrobial resistance.

9. **When a manufacturer supplies a dosage range for an antibiotic, susceptibility testing is based on**
   - **a.** the low end of the dosage range.
   - **b.** the middle of the dosage range.
   - **c.** the high end of the dosage range.
   - **d.** a dose 10% above the high end of the dosage range.

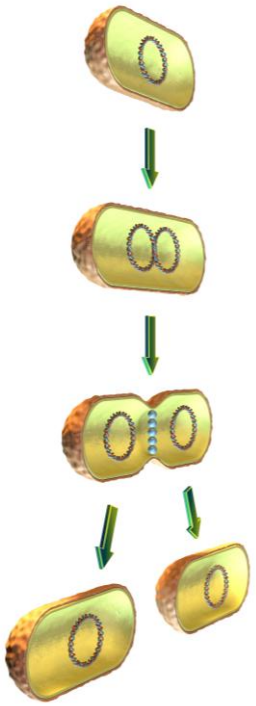
10. **The susceptibility or resistance of a pathogen is determined by comparing the MIC with the**
    - **a.** achievable plasma drug concentration.
    - **b.** diameter of the zone of inhibition.
    - **c.** minimum bactericidal concentration.
    - **d.** characteristics of colony growth.

Bacterial Multiplication

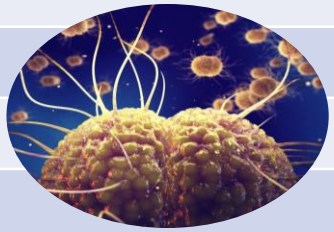




Binary Fission: 20 minutes or less when intrinsic and extrinsic factors are optimal.



Time	# of Bacteria
0 minutes	1
20 minutes	2
40 minutes	4
1 hour	8
2 hours	64
4 hours	4,096
6 hours	262,144
8 hours	16,777,216
12 hours	68,719,476,736



Bacteria	Estimated Infective Dose*
Salmonella serovars	<10 cells
Shiga toxin-producing *E. coli*	10 to 100 cells
Cronobacter sakazakii	10 to 100 cells
Listeria monocytogenes	<1000 cells
Campylobacter spp.	5000 to 10,000 cells
Staphylococcus aureus	>100,000 cells
Vibrio cholerae	1,000,000 cells

Information and photos are modified and adopted from BBB of Food and Drug Administration, BAM Resources of Centers for Disease Control and Prevention. Photo Courtesy: Adobe Stock (standard license of photos purchased by the Public Health Microbiology laboratory).

** Calculated for oral ingestion based on epidemiological data from outbreaks and human feeding trials of volunteers. Data obtained from BBB of Food and Drug Administration (2nd edition).*

Public Health Microbiology Laboratory: Education, Research, Outreach, and Technical Assistance: https://publichealthmicrobiology.education/

FARMER TO FARMER
The USAID John Ogonowski and Doug Bereuter Farmer-to-Farmer Program

Volunteer Certification Form (VCF)

- Submit one VCF per assignment
- This VCF is subject to examination by USAID and ACDI/VOCA's external auditors. Please be as specific as possible and provide back-up documentation (training attendance sheets, proof of third-party contributions, etc.) to the Farmer-to-Farmer (F2F) Field Staff
- Volunteer submits the Final Report to the Country Director and F2FVolunteer@acdivoca.org within two weeks after the assignment, if not submitted in-country (see template in the Welcome Packet)
- Volunteer submits the Volunteer Expense Report (VER to F2FVolunteer@acdivoca.org with scanned receipts and boarding passes within 30 days of assignment (see template in the Welcome Packet)
- Information related to outreach activities in the U.S. (media appearances, presentations, etc.) will be collected through a bi-annual survey distributed separately via Survey Monkey.

Assignment Data – To Be Completed by the Volunteer

Assignment Number (refer to the Scope of Work):	GEO-2014-061
Volunteer Name:	Dr. Aliyar Cyrus Fouladkhah, PhD, MPH
Country:	Georgia
Assignment Title:	Training in Agricultural Microbiology
Host Organization(s):	Agro House Ltd
Assignment Dates:	5/16-5/3/2022
PERSUAP Number, if applicable (refer to the Scope of Work):	N/A

Number of Persons Trained (include training attendance sheets):

Male	**2**
Female	**7**
Non-Binary	**0**
Out of these: - Youth (Ages 15-29)	**0**

Volunteer Certification Timesheet

Type	Dates (Range)	# of Days
Pre-Assignment Days (1 Day = 8 Hours) *If more than 3 pre-assignment days, please provide a description of activities	5/13-5/15/2022	**3**
International Travel to Assignment		
Assignment Days	5/16-5/3/2022	**18**
International Travel Home	**5/3/2022-5/3/2022**	
Personal Days	**0**	
	Total # of Days	**21**

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Number of Recommendations, per Host (no more than 6 per host)

1. Implementation of Aflatoxins testing based on provided information in training sessions and activities.
2. Implementation of Antibiotic stewardship program based on information provided and the testing.
3. Implementation of aerobic plate counts, yeast and molds counts and culture-dependent microbiological analyses based on information discussed in the laboratory and training sessions.

Did you conduct any radio or TV broadcasts or publications while in the host country?

If yes, please explain: N/A

Additional Contributions – To Be Completed with Field Staff

I. Host Contributions

If the host contributed any resources to assist with the volunteer assignment, please list them here:

Description	Unit Value USD	# of Units	Total
Meals – Breakfasts	$		
Meals – Lunches	$13	14	$195
Meals – Dinners	$	0	$
Lodging – Nights	$	0	$
Interpreters – Days	$60	16	$960
Transportation – Days	$20	16	$320
		Total of Contributions, Host 1	$ 1,475
		Total of Contributions, Host 2	$
		Total of Contributions, Host 3	$

II. Volunteer Contributions – *Please include receipts in the Volunteer Expense Report*

Contributions from the Volunteer (Founder/Director of the Public Health Microbiology Foundation™)

Description of Item	Unit Value USD	# of Units	Total
Certification	**$850**	**9**	**7,650**
Books	**$40**	**9**	**450**
		Subtotal:	
		(Minus the amount to be reimbursed to the Volunteer)	
		Total Contributions:	**$8,100**

III. Partner or Third-Party Contributions in the U.S.

If a partner or a third party in the United States (your hometown community, business group, church, etc.) provided a contribution that you brought to the host(s), please list it here.

Contributor	Description of Item	Unit Value in USD	# of Units	Total
			Total of Contributions in USD	

FARMER TO FARMER
The USAID John Ogonowski and Doug Bereuter Farmer-to-Farmer Program

Assignment Summary – To Be Completed by Field Staff

It was a great pleasure and lifetime honor for me to come to the culturally-rich and beautiful country of Georgia for a USAID assignment. First and foremost, I would like to thank great colleagues in the Washington and Tbilisi USAID programs for all they have done for harmonizing the events of the assignment. In week of May 16, 2022 after arrival to the facility in Zugdidi, a very intensive and interactive workshop was conducted discussing important food safety information, impact of climate change on food security, and transboundary infectious diseases. In this week, a form was also developed based on the need of the association and the region for needed testing methodologies in the lab. In week of May 23, 2022, detailed testing procedures were developed for the laboratory and discussed with the laboratory staff based on hands-on activities. Week of May 30, 2022, antibiotic stewardship program was developed and discussed with the laboratory staff and assistance was provided with ongoing instrumentation in the microbiology laboratory to ensure their proper operation and maintenance. Details of these procedures are incorporated into the final report of the project.

PERSUAP Table (if applicable) – To Be Completed by Field Staff

If you interacted with or witnessed pesticide/fungicide use during the assignment, please complete the following USAID table in the greatest detail possible.

Agricultural Pesticide Application Details					**Contact Details**		
Date of Application	Pesticide/Fungicide Name	Application Rate	Crop/Pest	Location	Volunteer	Farmer	Applicator

PERSUAP Summary (if applicable) – To Be Completed by Field Staff

For Type 1 and Type 2 assignments, please write 2-3 sentences about how the Volunteer interacted with or witnessed pesticide use.

None of the pesticides were used during my observation.

Certification Declaration

I certify that the information contained in this document is true and accurate.

Dr. Aliyar Cyrus Fouladkhah 6-1-2022

Signature of Volunteer, Date

_____

Verified and Approved by F2F Field Staff, Date

_____

Received and Reviewed by ACDI/VOCA Headquarters, Date

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