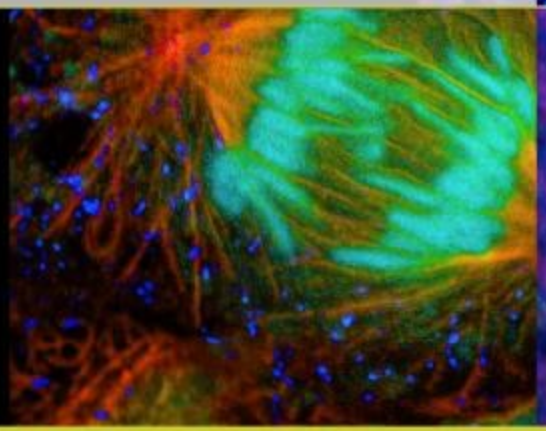
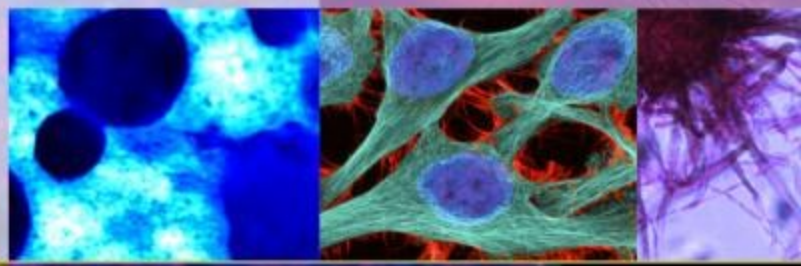
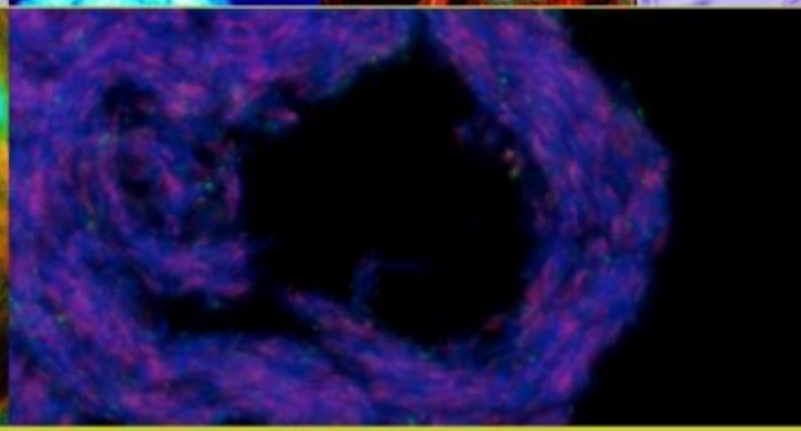
ENHANCING VECTOR-BORNE RESEARCH WITH BIOLOGICAL AND MOLECULAR STANDARDS

David Clawson, M.S.
Lead Biologist, ATCC
March 5, 2015

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About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- World's premiere biological materials resource and standards development organization
- ATCC collaborates with and supports the scientific community with industry-standard products and innovative solutions
- Broad range of biomaterials
  - Continuous cell lines, iPSCs, primary cells, and hTERT immortalized cells
  - Bacteria, fungi, yeasts, protists, and viruses
  - Microbial and tumor cell panels
  - Genomic and synthetic nucleic acids
  - Media, sera, and reagents

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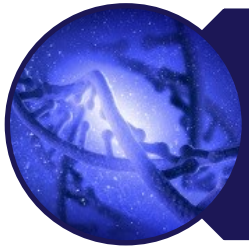
Outline

Introduction and clinical significance

Prevention and treatment



Solutions for vector-borne research

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Photo credit: James Gathany

Vector-borne diseases

- Diseases that result from an infection transmitted to humans and animals by blood-sucking arthropods and some aquatic snails
- Many of these diseases are found in tropical and sub-tropical regions, as well as locations where there is minimal access to potable water and proper sanitation systems
- Various types of vectors:
  - Mosquitoes
  - Ticks
  - Fleas
  - Reduviids
  - Black flies
  - Tsetse flies
  - Sandflies
  - Aquatic snails

The World Health Organization estimates that vector-borne diseases account for 17% of the estimated global burden of all infectious diseases, resulting in more than 1 million deaths annually.

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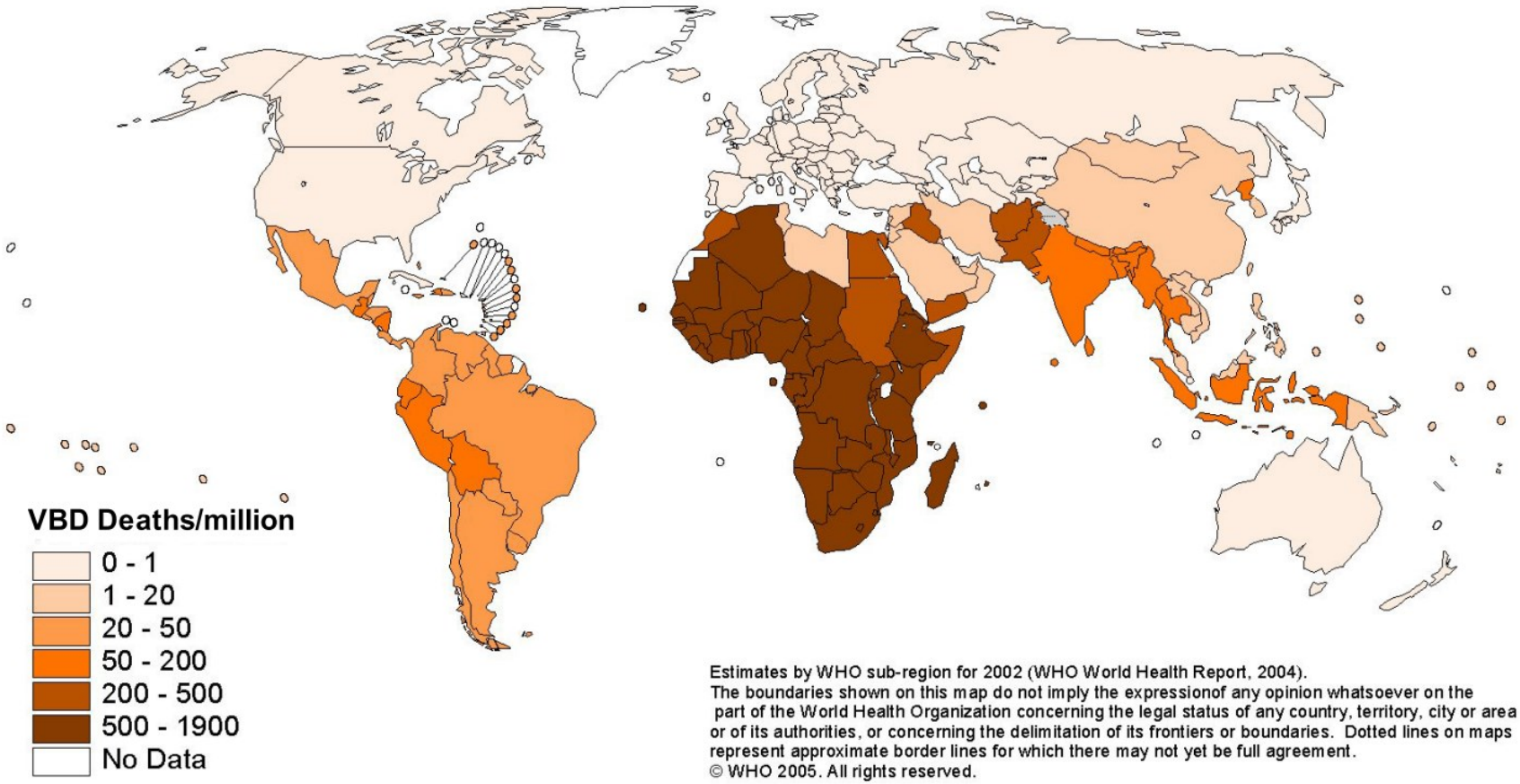
Photo credit: James Gathany and NIAID

Vector-borne diseases



Estimates by WHO sub-region for 2002 (WHO World Health Report, 2004).
The boundaries shown on this map do not imply the expressionof any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

Deaths from vector-borne disease for 2002
WHO World Health Report, 2004

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Vector-borne diseases

Vaccine development

Disability costs

Healthcare costs

Loss of development opportunities

Economic impact

Loss of livestock production

Vector control

Trade embargo

Livestock vaccination

ATCC® Photo credit: James Gathany

Vector-borne diseases

Mosquitoes

Chikungunya, West Nile, Eastern equine encephalitis, St. Louis encephalitis, Dengue, Malaria

Ticks

Lyme disease, Ehrlichiosis, Rocky Mountain spotted fever, Babesiosis, Bartonellosis, Anaplasmosis

Fleas

Plague, Bartonellosis

Reduviids

Chagas disease

Black flies

Filariasis, Onchocerciasis

Tsetse flies

Sleeping sickness

Sand flies

Leishmaniasis

Aquatic snails

Schistosomiasis

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Photo credit: James Gathany, Janice haney Carr, Tam Nguyen, Fred A. Lewis, Yung-San Liang, Nithya Raghavan, Matty Knight

Vector-borne diseases

Mosquitoes

Chikungunya, West Nile, Eastern equine encephalitis, St. Louis encephalitis, Dengue, Malaria

Ticks

Lyme disease, Ehrlichiosis, Rocky Mountain spotted fever, Babesiosis, Bartonellosis, Anaplasmosis

Fleas

Plague, Bartonellosis

Reduviids

Chagas disease

Malaria

- Transmitted by *Anopheles* mosquitoes
- Caused by *Plasmodium* spp.
- Considered to be the most deadly vector-borne disease
- Results in approximately 600,000 deaths annually
- Resistance to antimalarial drugs is a recurring problem

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Photo credit: James Gathany, Janice haney Carr, Mae Melvin

Vector-borne diseases

Mosquitoes

Chikungunya, West Nile, Eastern equine encephalitis, St. Louis encephalitis, Dengue, Malaria

Ticks

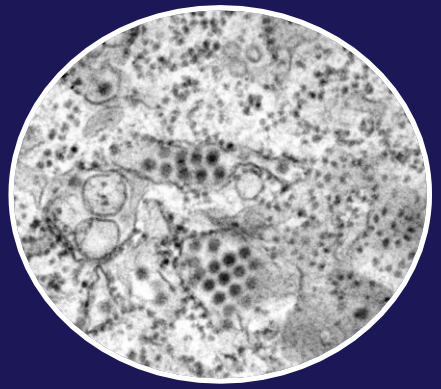
Lyme disease, Ehrlichiosis, Rocky Mountain spotted fever, Babesiosis, Bartonellosis, Anaplasmosis

Fleas

Plague, Bartonellosis

Reduviids

Chagas disease



Dengue

- Transmitted by *Aedes aegypti*
- Caused by Dengue virus serotypes 1, 2, 3, and 4
- Fastest growing vector-borne disease
- Over 40% of the world's population is at risk
- Estimated 50-100 million infections annually

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Photo credit: James Gathany, Janice haney Carr, Frederick Murphey

Vector-borne diseases

Mosquitoes

Chikungunya, West Nile, Eastern equine encephalitis, St. Louis encephalitis, Dengue, Malaria

Ticks

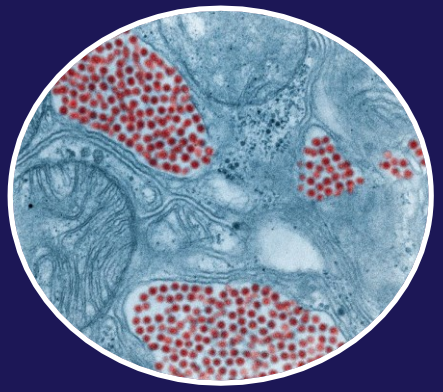
Lyme disease, Ehrlichiosis, Rocky Mountain spotted fever, Babesiosis, Bartonellosis, Anaplasmosis

Fleas

Plague, Bartonellosis

Reduviids

Chagas disease



Eastern equine encephalitis

- Transmitted by *Culiseta melanura,* and some *Aedes* and *Culex* spp.
- Caused by Eastern equine encephalitis virus (EEEV)
- Estimated 30-70% mortality rate in the United States
- Most survivors exhibit significant brain damage

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Photo credit: James Gathany, Janice haney Carr, Fred Murphey, Sylvia Whitfield

Vector-borne diseases

Mosquitoes

Chikungunya, West Nile, Eastern equine encephalitis, St. Louis encephalitis, Dengue, Malaria

Ticks

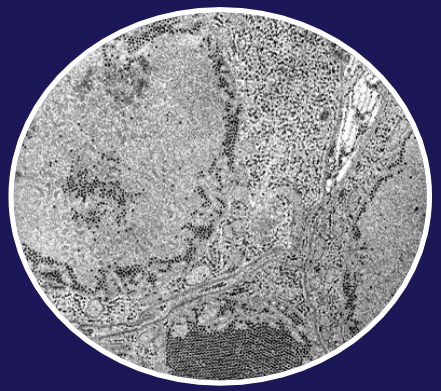
Lyme disease, Ehrlichiosis, Rocky Mountain spotted fever, Babesiosis, Bartonellosis, Anaplasmosis

Fleas

Plague, Bartonellosis

Reduviids

Chagas disease



St. Louis encephalitis

- Transmitted by *Culex* spp.
- Caused by the Saint Louis encephalitis virus (SLEV)
- The principal reservoirs of SLEV include wild birds and domestic fowl
- The mortality rate ranges from 5-30%, with higher rates among the elderly

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Photo credit: James Gathany, Janice haney Carr

Vector-borne diseases

Mosquitoes

Chikungunya, West Nile, Eastern equine encephalitis, St. Louis encephalitis, Dengue, Malaria

Ticks

Lyme disease, Ehrlichiosis, Rocky Mountain spotted fever, Babesiosis, Bartonellosis, Anaplasmosis

Fleas

Plague, Bartonellosis

Reduviids

Chagas disease

Lyme disease

- Transmitted by *Ixodes scapularis* and *Ixodes ricinus*
- Caused by *Borrelia burgdorferi, Borrelia afzelii*, and *Borrelia garinii*
- Reported in 80+ countries worldwide
- Estimated 300,000 infections annually in the United States alone

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Photo credit: James Gathany, Janice haney Carr, Claudia Molins

Vector-borne diseases

Mosquitoes

Chikungunya, West Nile, Eastern equine encephalitis, St. Louis encephalitis, Dengue, Malaria

Ticks

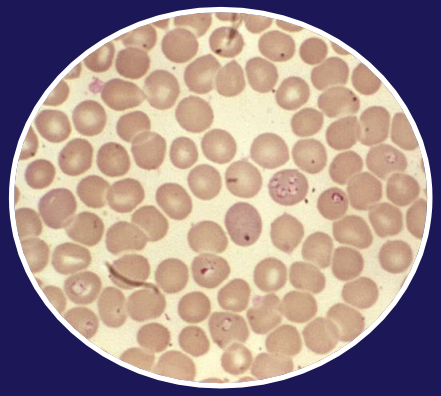
Lyme disease, Ehrlichiosis, Rocky Mountain spotted fever, Babesiosis, Bartonellosis, Anaplasmosis

Fleas

Plague, Bartonellosis

Reduviids

Chagas disease



Babesiosis

- Transmitted by *Ixodes scapularis*
- Caused by *Babesia microti*
- Parasites infect red blood cells
- May be asymptomatic, or result in fatigue, fever, and hemolytic anemia
- Can be transmitted via blood transfusion and from mother to fetus

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Photo credit: James Gathany, Janice haney Carr

Factors contributing to the spread of disease

- Environmental changes
- Global transportation and trade
- Demographic and societal changes
- Poor waste disposal and water storage
- Changes in agricultural practices
- Pathogen evolution

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Prevention

Personal protection/prevention strategies

- Keep skin covered
- Mosquito nets
- Insecticides
- Avoid insect vector habitats
- Preventing access to egg-laying habitats

Environmental management strategies

- Active monitoring and surveillance of vectors and vector environments
- Improved design or operation of water resources development projects
- Biological controls that target vector larvae

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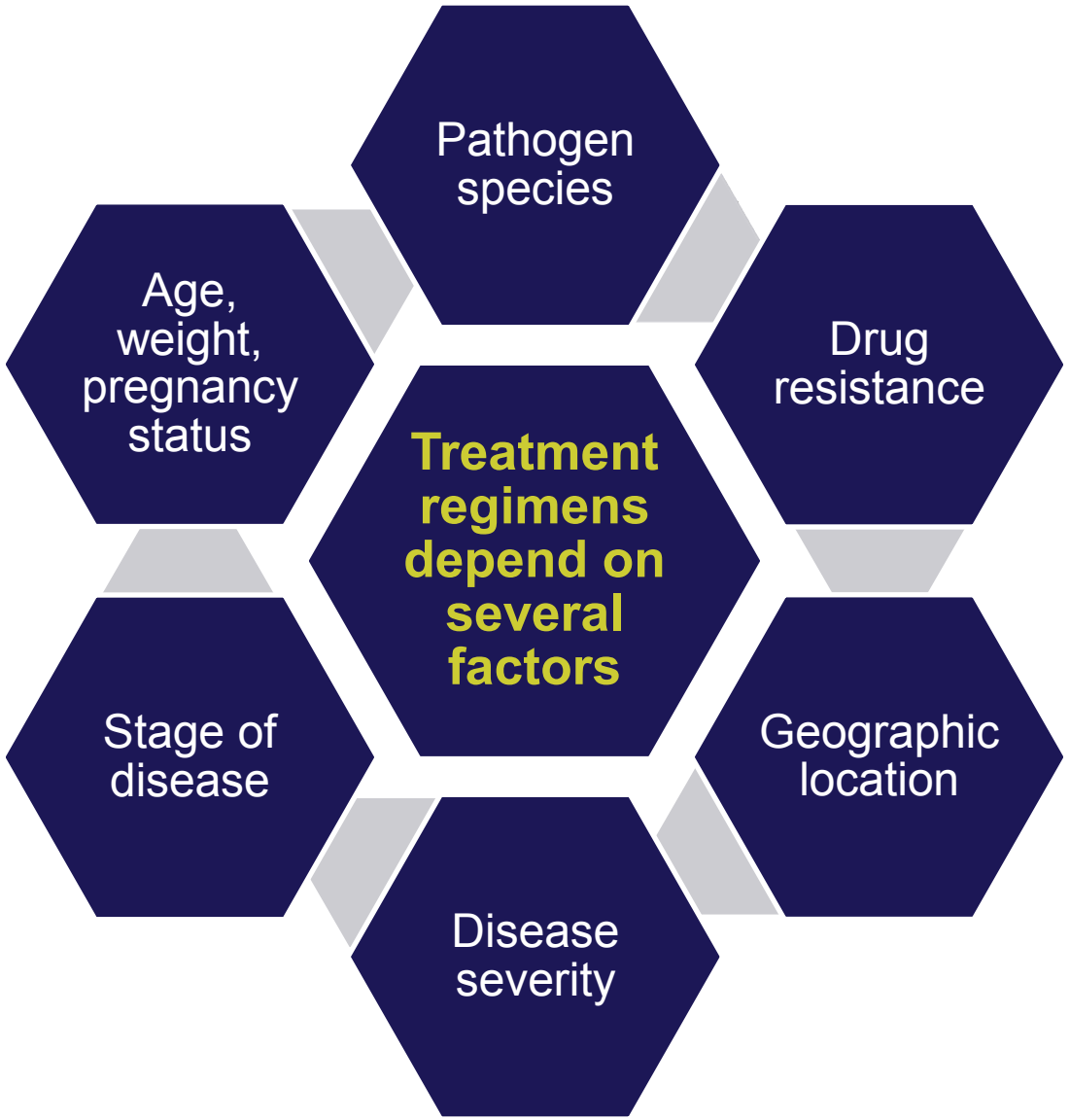
Photo credit: James Gathany

Treatment



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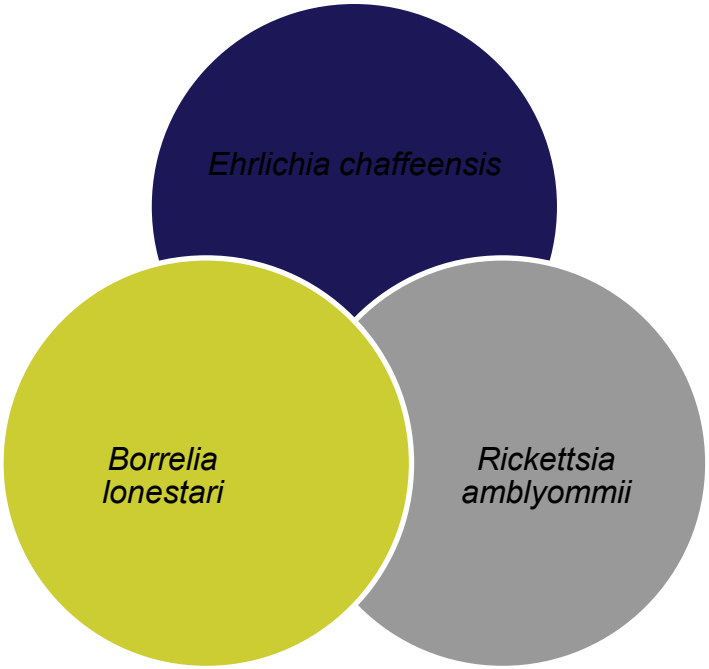
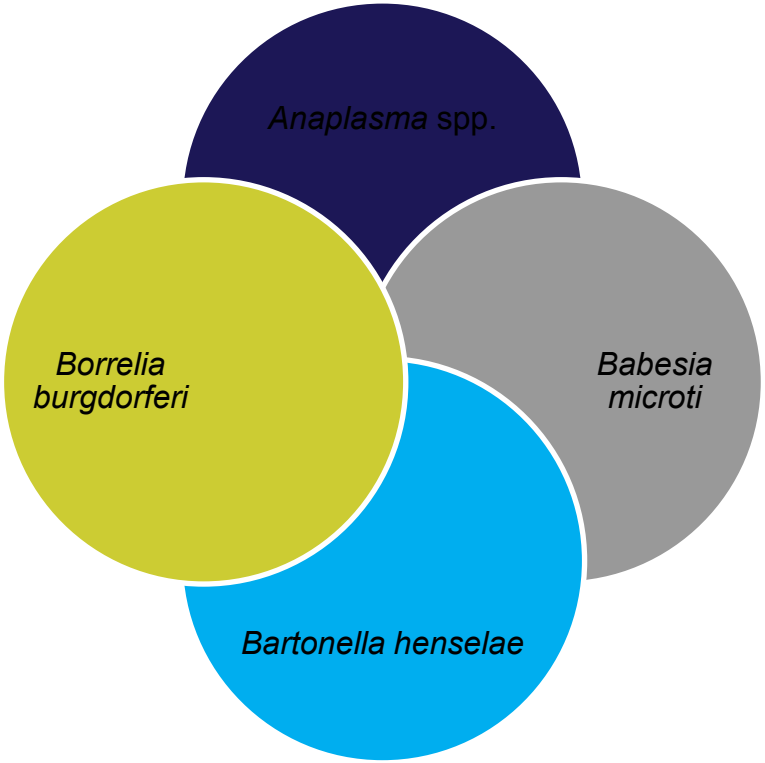
Vector-borne pathogen co-infection



Amblyomma americanum tick vector



Ixodes scapularis tick vector

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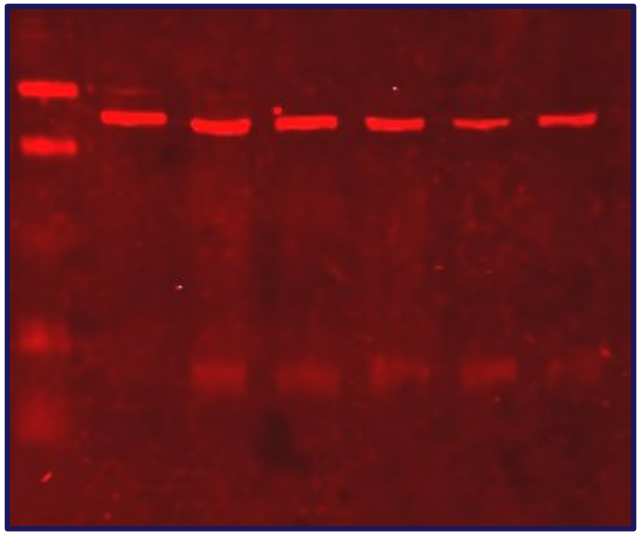
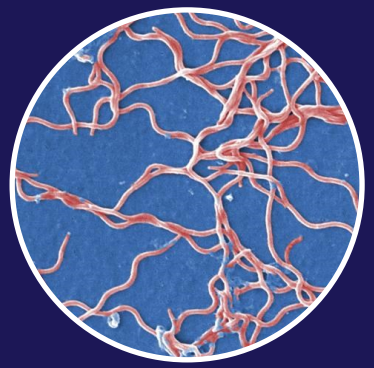
Detection of vector-borne diseases

- Detection Methods:
  - PCR
  - RT-PCR
  - ELISA
  - Western blot
  - Histopathology
  - Blood smear
- What is needed:
  - Field survey kits
  - Fast, accurate, inexpensive laboratory-based surveillance



ATCC® Photo credit: Tim Vickers

ATCC Genuine Cultures®



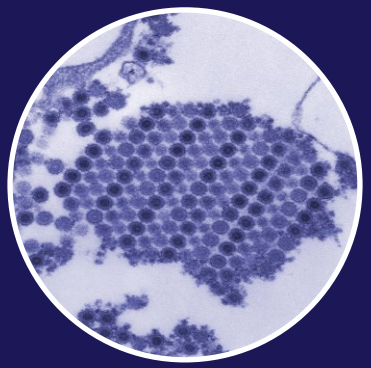
Bacteria

Anaplasma

Borrelia

Ehrlichia

Rickettsia



Viruses

Chikungunya virus

Dengue virus

Western equine encephalitis virus

West Nile virus

Protozoa

Babesia

Leishmania

Plasmodium

Trypanosoma

Visit us online at www.atcc.org/vectorborne

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Photo credit: Janice Haney Carr

ATCC® Genuine Nucleics

Genomic Nucleic Acids

Babesia

Borrelia

Leishmania

Plasmodium

Trypanosoma

Yersina pestis

Synthetic Nucleic Acids

Dengue virus I-IV

Eastern equine encephalitis virus

St. Louis encephalitis virus

West Nile virus

Visit us online at www.atcc.org/vectorborne

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ATCC® Synthetic Molecular Standards

Can be used as a positive control for:

- Difficult to culture or unculturable strains
- Strains requiring BSL-3 containment
- Strains on the commerce control list

Advantages of synthetic nucleic acids:

- Eliminate the need to culture microorganisms
- Can be used in a BSL-1 facility
- No shipping restrictions
- Manufactured under ISO 13485:2003
- Quantified using Droplet Digital™ PCR
- Useful for monitoring assay-to-assay or lot-to-lot variation

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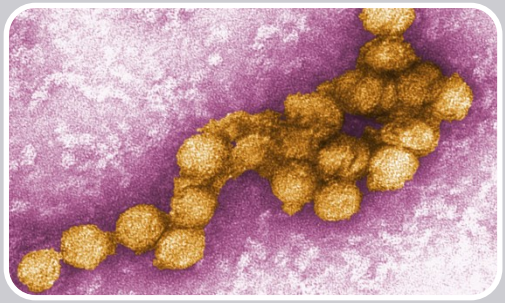
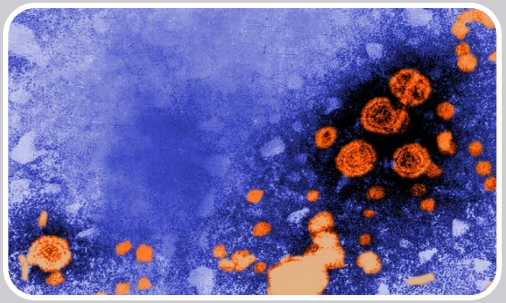
ATCC® Synthetic Molecular Standards

The current collection





Vector-borne disease research

- West Nile virus
- Dengue virus serotypes 1-4
- Eastern equine encephalitis virus
- St. Louis encephalitis virus

Enteric research

- Norovirus GI & GII
- Sapovirus

STI & bloodborne pathogen research

- *Mycoplasma genitalium*
- *Treponema pallidum*
- Hepatitis B
- Hepatitis C
- HPV 16 & 18

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ATCC® Synthetic Molecular Standards

ATCC® No.	Synthetic Genome	Genetic Target
VR-3228SD	Dengue virus type 1	Fragments from the capsid, membrane, and envelope regions
VR-3229SD	Dengue virus type 2	Fragments from the capsid, membrane, and envelope regions
VR-3230SD	Dengue virus type 3	Fragments from the capsid, membrane, and envelope regions
VR-3231SD	Dengue virus type 4	Fragments from the capsid, membrane, and envelope regions
VR-3239SD	Eastern equine encephalitis virus	Fragments from the capsid, NSP1, NSP3, 3' UTR, and the E1 and E2 envelope glycoproteins
VR-3236SD	St. Louis encephalitis virus	Fragments from the NS1 gene, pre-membrane, envelope, NS5 gene, and the 3' UTR regions
VR-3198SD	West Nile virus	Fragments from the 5' UTR, capsid protein C, membrane glycoprotein precursor prM, envelope protein E, nonstructural proteins NS1, NS2A, NS3, NS5, and the 3' UTR regions

Dengue virus

Problem

- Dengue virus is the most common and clinically important arbovirus disease, with an estimated 50-100 million cases annually.
- Surveillance and rapid detection of Dengue infections is important as there are no vaccines or antivirals available.

Concerns

- qRT-PCR is the preferred method for the detection and quantification of Dengue virus in clinical diagnostics and epidemiological surveillance.
- However, full-length RNA on the Commerce Control List, and cannot be shipped internationally without a permit.

Solution

- ATCC Synthetic Dengue virus RNA is not on the Commerce Control List, and can be used in a BSL-1 facility.
- The standards were developed to contain short fragments from the capsid, membrane, and envelope genes, as well as the target regions encompassing the primer sequences from numerous published RT-PCR assays.

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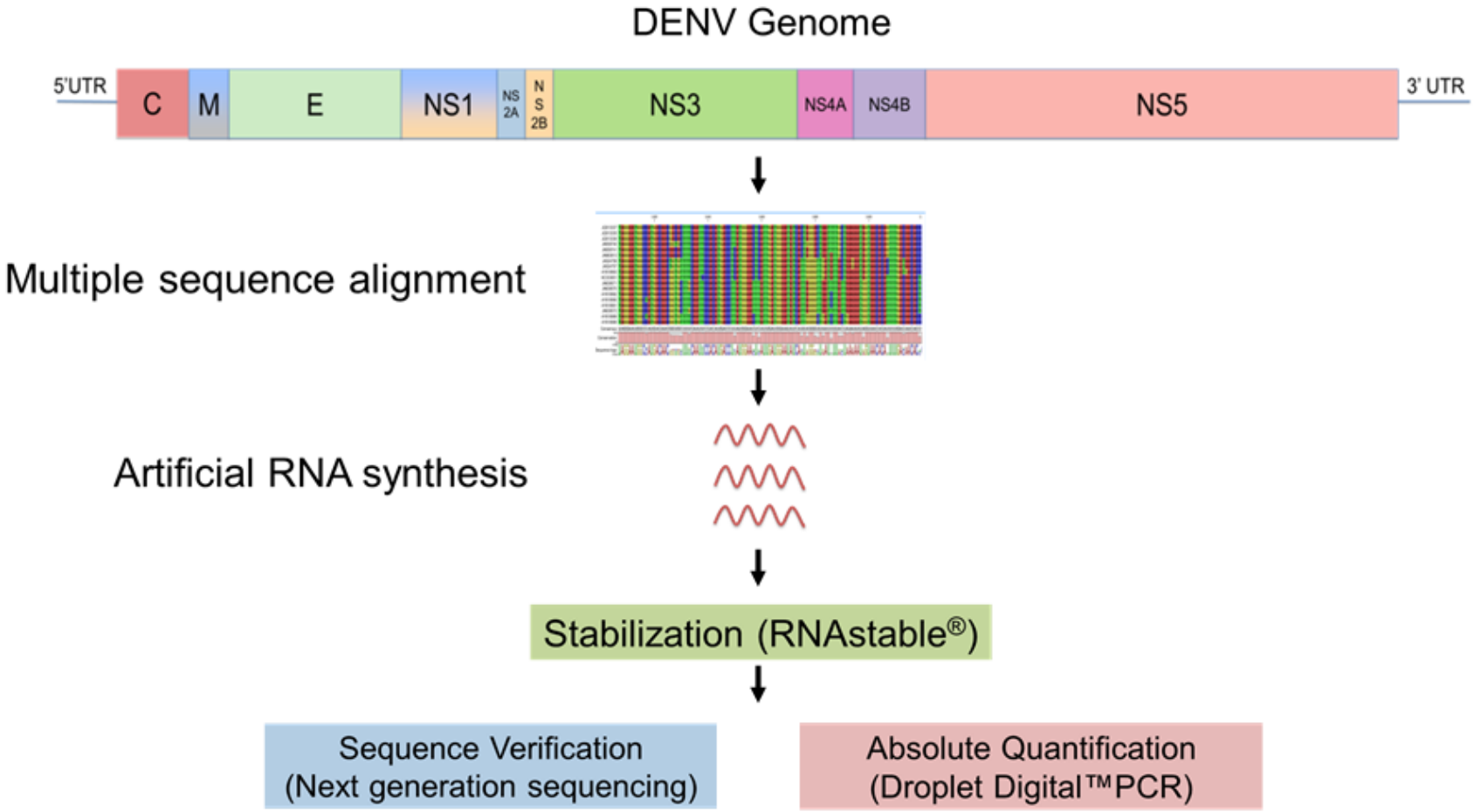
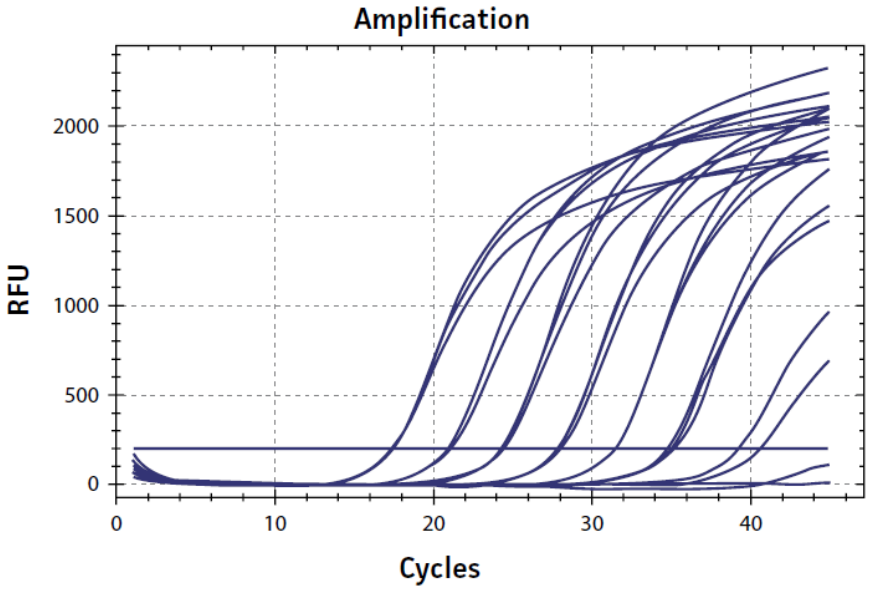
Synthetic Dengue virus RNA

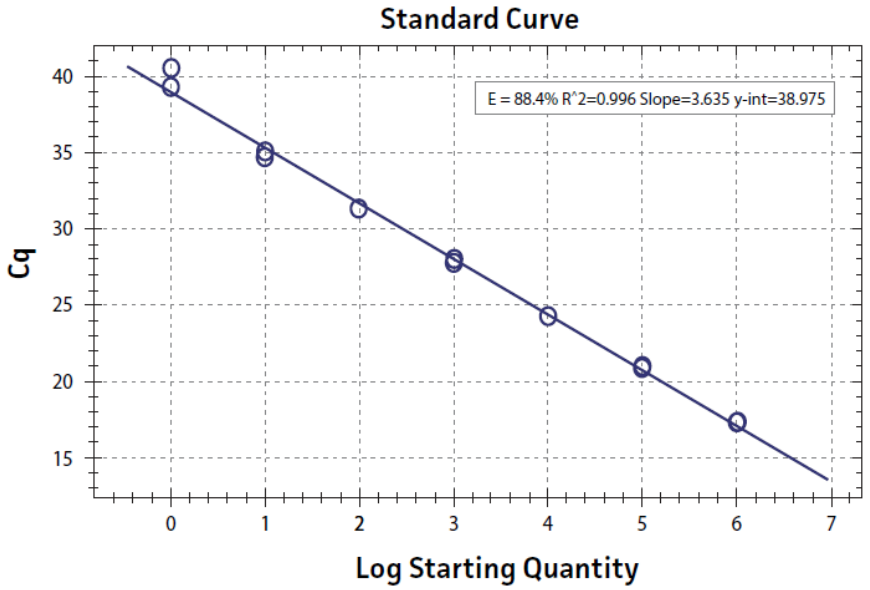


Ashraf S, et al. Development and use of synthetic molecular standards for Dengue virus serotypes 1-4 [Application note], 2014.

Synthetic Dengue virus RNA

Generation standard curves using the DENV-4 Molecular Standard





Primer and Probe		DENV-1	DENV-2	DENV-3	DENV-4
CDC Assay	Slope	-3.244	-3.277	-3.315	-3.642
	R^2	0.990	0.996	0.987	0.996
Waggoner Assay	Slope	-3.536	-3.535	-3.705	-3.775
	R^2	0.991	0.997	0.989	0.996

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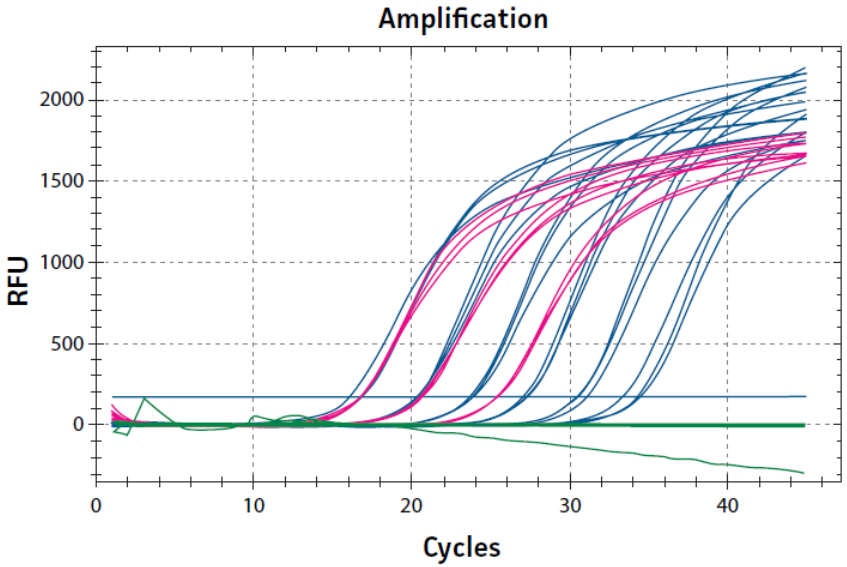
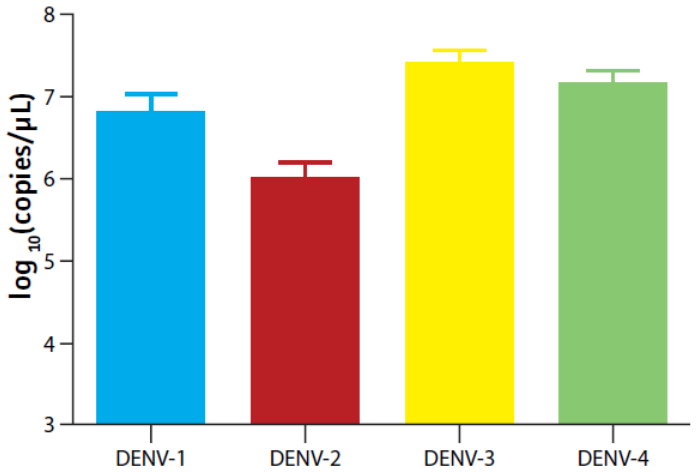
Ashraf S, et al. Development and use of synthetic molecular standards for Dengue virus serotypes 1-4 [Application note], 2014.

Synthetic Dengue virus RNA

Quantification of native DENV RNA





Titers of DENV-1, -2, -3, and -4 samples were determined using the qRT-PCR standard curves generated by the DENV 1-4 molecular standards

DENV 1 = TH-S-man (ATCC® VR-1586™)
DENV 2 = New Guinea C (NR-84, BEI Resources)
DENV 3 = Philippines/H87/1956 (NR-80, BEI Resources)
DENV 4 = H241 (ATCC® VR-1257™)

ATCC® Ashraf S, et al. Development and use of synthetic molecular standards for Dengue virus serotypes 1-4 [Application note], 2014.

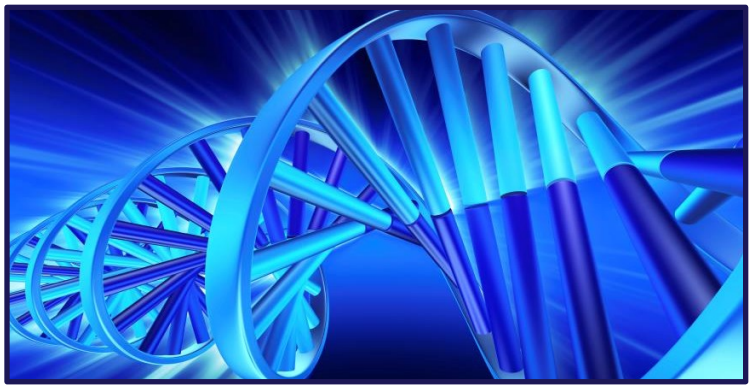
Synthetic Dengue virus RNA

Characteristics

- Fully authenticated
- Quantitated and stable
- Can be handled in BSL-1
- No special permits required
- Exhibit minimal variability
- Compatible with published real-time PCR assays

Applications

- Positive controls in qRT-PCR assays
- Pathogen detection
- Quantify viral load
- Technology can be expanded to other strains



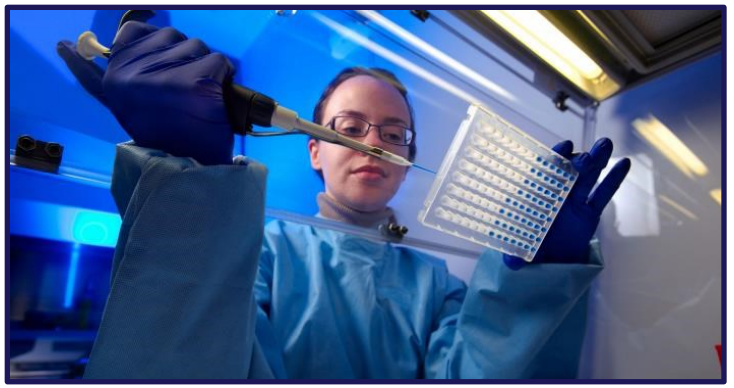


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Photo credit: James Gathany

Conclusion

- Vector-borne diseases are a major cause of morbidity and mortality
- Due to limited preventative and therapeutic treatment options, accurate detection methods are critical
- ATCC offers authenticated strains and nucleic acids that support the development, validation, and employment of novel detection methods
- ATCC Synthetic Molecular Standards
  - Represent key target regions for difficult-to-culture, unculturable, and high containment microorganisms
  - Authenticated, stable, and quantitative
  - Safe to use in BSL-1 facilities
  - Used to detect and quantify bacterial and viral load
  - Generate standard curves
  - Function as positive controls in molecular-based applications

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Thank you!

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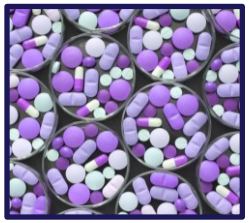
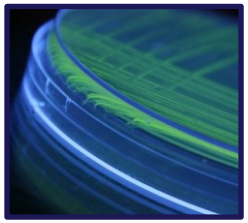


March 19, 2015
10:00 AM, 3:00 PM EST
Scott Sutton, Ph.D., *Principal*, Microbiology Network and
Liz Kerrigan, *Direct, New Product Development, Sales & Marketing*, ATCC
Microbiological quality control of pharmaceutical products



May 21, 2015
10:00 AM, 3:00 PM EST
Jodie Lee, M.S.
Seeing is believing – Reporter-labeled microbial control strains

Thank you for joining today!
Please send additional questions to tech@atcc.org

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