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
Outline

- Introduction and clinical significance
- Prevention and treatment
- Solutions for vector-borne research
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.
Vector-borne diseases

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

- Vaccine development
- Loss of development opportunities
- Vector control

Economic impact

- Disability costs
- Healthcare costs
- Livestock vaccination
- Trade embargo
- Loss of livestock production

Photo credit: James Gathany
### Vector-borne diseases

<table>
<thead>
<tr>
<th>Mosquitoes</th>
<th>Black flies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chikungunya, West Nile, Eastern equine encephalitis, St. Louis encephalitis, Dengue, Malaria</td>
<td>Filariosis, Onchocerciasis</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Ticks</th>
<th>Tsetse flies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyme disease, Ehrlichiosis, Rocky Mountain spotted fever, Babesiosis, Bartonellosis, Anaplasmosis</td>
<td>Sleeping sickness</td>
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</table>

<table>
<thead>
<tr>
<th>Fleas</th>
<th>Sand flies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plague, Bartonellosis</td>
<td>Leishmaniasis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reduviids</th>
<th>Aquatic snails</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chagas disease</td>
<td>Schistosomiasis</td>
</tr>
</tbody>
</table>

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
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

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
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
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
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
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
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
Treatment

Regimens depend on several factors:

- Pathogen species
- Drug resistance
- Geographic location
- Disease severity
- Stage of disease
- Age, weight, pregnancy status
- Disease severity

Disease severity

Pathogen species

Drug resistance

Geographic location

Stage of disease

Age, weight, pregnancy status

Treatment regimens depend on several factors.
Vector-borne pathogen co-infection

- Anaplasma spp.
- Babesia microti
- Bartonella henselae
- Borrelia burgdorferi
- Ehrlichia chaffeensis
- Borrelia lonestari
- Ixodes scapularis tick vector
- Amblyomma americanum tick vector

Amblyomma americanum tick vector

Ixodes scapularis tick vector
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

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

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
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
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
### ATCC® Synthetic Molecular Standards

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Synthetic Genome</th>
<th>Genetic Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>VR-3228SD</td>
<td>Dengue virus type 1</td>
<td>Fragments from the capsid, membrane, and envelope regions</td>
</tr>
<tr>
<td>VR-3229SD</td>
<td>Dengue virus type 2</td>
<td>Fragments from the capsid, membrane, and envelope regions</td>
</tr>
<tr>
<td>VR-3230SD</td>
<td>Dengue virus type 3</td>
<td>Fragments from the capsid, membrane, and envelope regions</td>
</tr>
<tr>
<td>VR-3231SD</td>
<td>Dengue virus type 4</td>
<td>Fragments from the capsid, membrane, and envelope regions</td>
</tr>
<tr>
<td>VR-3239SD</td>
<td>Eastern equine encephalitis virus</td>
<td>Fragments from the capsid, NSP1, NSP3, 3’ UTR, and the E1 and E2 envelope glycoproteins</td>
</tr>
<tr>
<td>VR-3236SD</td>
<td>St. Louis encephalitis virus</td>
<td>Fragments from the NS1 gene, pre-membrane, envelope, NS5 gene, and the 3’ UTR regions</td>
</tr>
<tr>
<td>VR-3198SD</td>
<td>West Nile virus</td>
<td>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</td>
</tr>
</tbody>
</table>
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.
Synthetic Dengue virus RNA

DENV Genome

Multiple sequence alignment

Artificial RNA synthesis

Stabilization (RNAstable®)

Sequence Verification (Next generation sequencing)

Absolute Quantification (Droplet Digital™ PCR)

Synthetic Dengue virus RNA

Generation standard curves using the DENV-4 Molecular Standard

<table>
<thead>
<tr>
<th>Primer and Probe</th>
<th>DENV-1</th>
<th>DENV-2</th>
<th>DENV-3</th>
<th>DENV-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDC Assay</td>
<td>Slope</td>
<td>-3.244</td>
<td>-3.277</td>
<td>-3.315</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.990</td>
<td>0.996</td>
<td>0.987</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.991</td>
<td>0.997</td>
<td>0.989</td>
</tr>
</tbody>
</table>

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™)

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

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
Thank you!

Register for more webinars in the ATCC “Excellence in Research” webinar series at www.atcc.org/webinars.

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