Simplifying assay development with molecular standards: Remove culturing from the equation

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Product Line Business Specialist, ATCC

Credible Leads to Incredible™
Agenda

✓ What ATCC molecular standards provide

✓ Development of ATCC’s synthetic molecular standards

✓ The ATCC molecular standards portfolio
  ✓ Including upcoming new products

✓ Validation data
About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World’s premier biological materials resource and standards development organization
  - 5,000 cell lines
  - 80,000 microorganisms
  - Genomic & synthetic nucleic acids
  - Media/reagents

- ATCC collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Growing portfolio of products and services
- Sales and distribution in 150 countries, 15 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees
Molecular Standards
ATCC molecular standards

- Standards in Science
  - Assay accuracy
  - In-house reproducibility
  - Lab-to-lab reproducibility
ATCC molecular standards

Standards in Science

Assay accuracy

In-house reproducibility

Lab-to-lab reproducibility
ATCC molecular standards

- Standards in Science
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ATCC molecular standards

- Standards in Science
- Assay accuracy
- In-house reproducibility
- Lab-to-lab reproducibility
A culture collection provides good value for research.
A standards organization with a culture collection provides even more!

In 2012, members of the ATCC R&D team realized there was more we could do for the scientific community, and began work on the first four molecular standards: three synthetic viruses and a quantitated bacterial DNA.

Those standards launched in May of 2013:
VR-3198SD (West Nile virus RNA)
VR-3199SD (Norovirus G1 RNA)
VR-3299SD (Norovirus G2 RNA)
29212Q-FZ (Enterococcus faecalis)
ATCC molecular standards

ATCC Molecular Standards

- Quantitative
- BSL 1
- ATCC Quality

Synthetic

- ISO Guide 13485
- Difficult to grow or unculturable

Genomic

- ATCC cultures
- Complete genome
ATCC molecular standards

ATCC Molecular Standards

Quantitative  BSL 1  ATCC Quality

Synthetic  Genomic

ISO Guide 13485  ATCC cultures

Difficult to grow or unculturable  Complete genome

genomes.atcc.org
## Specifications

### Synthetic Standards

<table>
<thead>
<tr>
<th>Authentication</th>
<th>NGS to verify synthetic sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functionality &amp; Identity</td>
<td>qPCR amplification, 3.32 cycles between Cq threshold</td>
</tr>
<tr>
<td>Genome copy number by ddPCR™</td>
<td>$1 \times 10^5$ to $1 \times 10^6$ genome copies/µL</td>
</tr>
<tr>
<td>Fill Volume</td>
<td>100 µL per vial</td>
</tr>
<tr>
<td>Format</td>
<td>Frozen</td>
</tr>
<tr>
<td>Certifications</td>
<td>ISO 13485, ISO 9001, ISO 17025</td>
</tr>
</tbody>
</table>

### Genomic Standards

<table>
<thead>
<tr>
<th>Authentication</th>
<th>Amplicon sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Integrity</td>
<td>High molecular weight DNA, by gel electrophoresis</td>
</tr>
<tr>
<td>Genome copy number by ddPCR™</td>
<td>$1 \times 10^5$ to $1 \times 10^6$ genome copies/µL</td>
</tr>
<tr>
<td>Fill Volume</td>
<td>100 µL per vial</td>
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<td>Frozen</td>
</tr>
<tr>
<td>Certifications</td>
<td>ISO 9001, ISO 17025</td>
</tr>
</tbody>
</table>
Synthetic Molecular Standard Design
Community response to the Norovirus standards

Interest in the standards was high, but feedback showed the synthetic constructs had room for improvement. ATCC modified the design and production processes, presenting the following changes at CVS in 2015.

- **Stability**: Changed from dried to frozen
- **Stability**: Added RNA stabilizer
- **Quantification**: Added ddPCR™ to specifications
- **Certifications**: Added ISO 13485 compliance
- **Versatility**: Added RdRp fragments to construct

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- **Versatility**: Added RdRp fragments to construct
Validating the next generation of standards

Pictured: Standard curves generated with CaliciNet primer and probe set.

Also tested: ECS working group primer and probe set.
- VR-3234SD
  - $R^2 = 0.987$
  - $m = -3.692$
- VR-3235SD
  - $R^2 = 0.998$
  - $m = -3.625$
Old vs. new standards, part 1

CaliciNet primers & probe

ECS working group primers & probe
Old vs. new standards, part 2

CaliciNet primers & probe

ECS primers & probe
A test drive with NIBSC working reagents

NoV-GI

Amplification

RFU

Cycles

R² = 0.996
Slope = -3.513

NoV-GII

Amplification

RFU

Cycles

R² = 1.00
Slope = -3.577

NoV-GI

NIBSC working reagent for NoV-GI

Genome copies/μL

CalicNet Assay
CENT/TC/WG6/TAG4 Assay

NoV-GII

NIBSC working reagent for NoV-GII

Genome copies/μL

CalicNet Assay
CENT/TC/WG6/TAG4 Assay
Design approach – synthetic standards

Data collection
- Literature review
- Bioinformatics sequence data mining
- Technical Review

Construct design
- Sequence analysis
- Choose optimal assay targets
- Condense, trim, and pad construct
- Technical Review

Validation
- Verify construct specificity
- Technical review
- Quality control testing
Design approach – synthetic standards

Construct
- Typically less than 5,000 nucleotides
- Fragments of genes
- Nucleic acid matches organism

Assays
- qPCR reported on ATCC website
- Each gene fragment reported on website
- References indicate other compatible assays

Data
- Sequence is proprietary
- For design support, ATCC will give a yes/no answer to ‘will my primers bind?’
ATCC’s Molecular Standards
Pathogen standards

• BK virus
• Hepatitis B virus
• Hepatitis C virus
• Epstein-Barr virus
• Human immunodeficiency virus 1
• Human T-cell leukemia virus
• Human cytomegalovirus
• Varicella-zoster virus
• Neisseria meningitides
• Plasmodium malariae
• Human parechovirus 3

Blood-borne disease

• Astrovirus
• Cyclospora cayetanensis
• Hepatitis A virus
• Hepatitis E virus
• Norovirus GI
• Norovirus GII
• Sapovirus
• Mycobacterium avium subsp. paratuberculosis
• Clostridiodes difficile
• Salmonella enterica subsp. enterica serovar Typhimurium
• Cryptosporidium parvum
• Human Enterovirus 71
• Rotavirus A
• Dientamoeba fragilis
• Babesia canis
• Giardia lamblia
• Murine norovirus
• Legionella pneumophila subsp. Pneumophila
• Human enterovirus 71 strain H
• Entamoeba histolytica
• E. coli

Gastro-Intestinal disease

• Human bocavirus
• Human coronavirus HKU1
• Human coronavirus NL63
• Human coronavirus 229E
• Middle East respiratory syndrome coronavirus
• Human metapneumovirus
• Bordetella pertussis
• Mycobacterium africanum
• Mycobacterium bovis
• Mycobacterium tuberculosis
• Mycobacterium pinnipedii
• Mycobacterium tuberculosis
• Treponema pallidum
• Chlamydia trachomatis serovar D
• Chlamydia trachomatis serovar E
• Chlamydia trachomatis serovar F
• Chlamydia trachomatis serovar L2
• Neisseria gonorrhoeae
• Human Herpesvirus 1
• Human Herpesvirus 2
• Neisseria meningitides
• Human Herpesvirus 7
• Human Herpesvirus 6
• Mycoplasma genitalium

Respiratory disease

• Hepatitis B virus
• Human immunodeficiency virus 1
• Human papillomavirus 16
• Human papillomavirus 18
• Human papillomavirus 31
• Human T-cell leukemia virus 2
• Treponema pallidum
• Neisseria gonorrhoeae
• Human Herpesvirus 8
• Human Herpesvirus 7
• Human Herpesvirus 6
• Mycoplasma genitalium

Sexually transmitted infections

• Chikungunya virus
• Dengue virus types 1-4
• Eastern equine encephalitis virus
• Plasmodium falciparum
• St. Louis encephalitis virus
• West Nile virus
• Yellow fever virus
• Zika virus
• Borrelia burgdorferi
• Plasmodium falciparum
• Yellow fever virus
• Rift Valley Fever virus

Epidermal & Nosocomial disease

• Staphylococcus aureus subsp. aureus
• Staphylococcus epidermidis
• Streptococcus pyogenes
• Candida albicans
• Pseudomonas aeruginosa

Vector-borne disease

• Chikungunya virus
• Dengue virus types 1-4
• Eastern equine encephalitis virus
• Plasmodium falciparum
• St. Louis encephalitis virus
• West Nile virus
• Yellow fever virus
• Zika virus
• Borrelia burgdorferi
• Plasmodium falciparum
• Yellow fever virus
• Rift Valley Fever virus

www.atcc.org/MolecularStandards
Standards from the microbiome

Skin
- *Staphylococcus aureus*
- *Candida parapsilosis*
- *Staphylococcus epidermidis*
- *Candida tropicalis*

Gut
- *Streptococcus agalactiae*
- *Faecalibacterium prausnitzii*
- *Klebsiella aerogenes*
- *Bacteroides fragilis*
- *Enterococcus faecium*
- *Citrobacter freundii*
- *Escherichia coli*
- *Klebsiella oxytoca*
- *Klebsiella pneumoniae*
- *Enterococcus faecalis*
- *Candida albicans*
- *Helicobacter pylori*

Vaginal
- *Lactobacillus iners*
- *Lactobacillus jensenii*
- *Lactobacillus gasseri*
- *Prevotella bivia*
- *Mobiluncus mulieris*
## Water safety standards

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>29212Q-FZ</td>
<td>Quantitative DNA from <em>Enterococcus faecalis</em></td>
</tr>
<tr>
<td>8739DQ</td>
<td>Quantitative DNA from <em>Escherichia coli</em></td>
</tr>
<tr>
<td>25923DQ</td>
<td>Quantitative DNA from <em>Staphylococcus aureus subsp. aureus</em></td>
</tr>
<tr>
<td>6538DQ</td>
<td>Quantitative DNA from <em>Staphylococcus aureus subsp. aureus</em></td>
</tr>
<tr>
<td>9027DQ</td>
<td>Quantitative DNA from <em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td>13048DQ</td>
<td>Quantitative DNA from <em>Klebsiella aerogenes</em></td>
</tr>
<tr>
<td>10231DQ</td>
<td>Quantitative DNA from <em>Candida albicans</em></td>
</tr>
<tr>
<td>4617DQ</td>
<td>Quantitative DNA from <em>Bordetella bronchiseptica</em></td>
</tr>
<tr>
<td>25285DQ</td>
<td>Quantitative DNA from <em>Bacteroides fragilis</em></td>
</tr>
</tbody>
</table>
Food safety standards

- Big 6 *E. Coli*
- *Campylobacter jejuni*
- *Salmonella enterica*
- *Norovirus*
- *Sapovirus*

<table>
<thead>
<tr>
<th>Designation</th>
<th>ATCC Item Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norovirus GI</td>
<td>VR-3234SD</td>
</tr>
<tr>
<td>Norovirus GII</td>
<td>VR-3235SD</td>
</tr>
<tr>
<td>Murine norovirus</td>
<td>VR-2355SD</td>
</tr>
<tr>
<td><em>E. coli</em> O145</td>
<td>BAA-2192DQ</td>
</tr>
<tr>
<td><em>E. coli</em> O45:H2</td>
<td>BAA-2193DQ</td>
</tr>
<tr>
<td><em>E. coli</em> O26:H11</td>
<td>BAA-2196DQ</td>
</tr>
<tr>
<td><em>E. coli</em> O103:H11</td>
<td>BAA-2215DQ</td>
</tr>
<tr>
<td><em>E. coli</em> O121:H19</td>
<td>BAA-2219DQ</td>
</tr>
<tr>
<td><em>E. coli</em> O111</td>
<td>BAA-2440DQ</td>
</tr>
<tr>
<td><em>C. jejuni</em></td>
<td>700819DQ</td>
</tr>
<tr>
<td><em>S. enterica</em></td>
<td>700720DQ</td>
</tr>
<tr>
<td>Sapovirus</td>
<td>VR-3237SD</td>
</tr>
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### Recently available

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Description</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>VR-3265SD</td>
<td>Quantitative Synthetic Human herpesvirus 7 DNA</td>
<td>Implicated in numerous health complications, and a transplant concern.</td>
</tr>
<tr>
<td>30001DQ</td>
<td>Quantitative Genomic DNA from <em>Trichomonas vaginalis</em></td>
<td>Causative agent of trichomoniasis.</td>
</tr>
<tr>
<td>VR-1360DQ</td>
<td>Quantitative Genomic DNA from <em>Chlamydia pneumoniae</em></td>
<td>A causative agent of pneumonia.</td>
</tr>
<tr>
<td>27853DQ</td>
<td>Quantitative Genomic DNA from <em>Pseudomonas aeruginosa</em></td>
<td>Quality control strain for numerous applications. Opportunistic pathogen.</td>
</tr>
<tr>
<td>15311DQ</td>
<td>Quantitative Genomic DNA from <em>Bordetella parapertussis</em></td>
<td>Causative agent of whooping cough.</td>
</tr>
</tbody>
</table>
Soon available

Coming soon, in 2019

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>VR-1493DQ</td>
<td>Quantitative Genomic DNA from Human herpesvirus 1</td>
</tr>
<tr>
<td>VR-94DQ</td>
<td>Quantitative Genomic RNA from Human respiratory syncytial virus</td>
</tr>
<tr>
<td>22905DQ</td>
<td>Quantitative Genomic DNA from <em>Proteus vulgaris</em></td>
</tr>
<tr>
<td>VR-1826DQ</td>
<td>Quantitative Genomic RNA from Enterovirus D68</td>
</tr>
<tr>
<td>MYA-2876DQ</td>
<td>Quantitative Genomic DNA from <em>Candida albicans</em></td>
</tr>
<tr>
<td>VR-3268SD</td>
<td>Quantitative Synthetic Lassa virus RNA</td>
</tr>
<tr>
<td>VR-3269SD</td>
<td>Quantitative Synthetic Nipah virus RNA</td>
</tr>
<tr>
<td>VR-3266SD</td>
<td>Quantitative Synthetic Human immunodeficiency virus 2 RNA</td>
</tr>
<tr>
<td>VR-3274SD</td>
<td>Quantitative Synthetic West Nile virus</td>
</tr>
<tr>
<td>MYA-4941DQ</td>
<td>Quantitative Genomic DNA from <em>Saccharomyces cerevisiae</em></td>
</tr>
</tbody>
</table>
Validation of Molecular Standards
Validation of synthetic standards for hepatitis viruses

Hepatitis B virus

- VR-3232SD™
- Hepadnaviridae, Orthohepadnavirus
- DNA construct [includes DNastable® (Biomatrica)]
- Portions of precure, core, P, S, and X regions

Hepatitis C virus

- VR-3233SD™
- Flaviviridae, Hepacivirus
- RNA construct [includes RNastable® (Biomatrica)]
- Portions of 5’ UTR, and X-tail region (3’ UTR)

ATCC has also designed synthetic constructs for Hepatitis A virus (VR-3257SD™) and Hepatitis E virus (VR-3258SD™), and ATCC maintains a number of Hepatitis A viral stocks in its collection.
**Hepatitis B virus**

Blue = VR-3232SD™
Red = NIBSC code 10/264 (3rd WHO international working reagent for HBV)

Hepatitis C virus


Blue = VR-3233SD™
Red = NIBSC code 06/102 (4th WHO international standard for HCV)
Hepatitis viruses – ddPCR™

VR-3232SD™ (HBV synthetic standard)

VR-3233SD™ (HCV synthetic standard)
Quantitation of NIBSC Hepatitis standards

- As determined by the WHO:
  - HBV standard = $8.5 \times 10^5$ IU/mL
  - HCV standard = $2.6 \times 10^5$ IU/mL

- qRT-PCR and qPCR quantitation at ATCC:
  - HBV: $9.7 \times 10^6$ genome copies/mL
  - HCV: $1.6 \times 10^7$ genome copies/mL

- Conversion ratio as quantified at ATCC:
  - HBV: 1 IU/mL = 11.4 genome copies
  - HCV: 1 IU/mL = 61.5 genome copies
Human herpes viruses

Human herpes viruses

<table>
<thead>
<tr>
<th></th>
<th>Average Ct</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1 (ATCC® VR-539D™)</td>
<td>29.46</td>
<td>1.14</td>
<td>3.9%</td>
</tr>
<tr>
<td>HSV-2 (ATCC® VR-540D™)</td>
<td>27.27</td>
<td>0.54</td>
<td>2.0%</td>
</tr>
</tbody>
</table>
Other application data and posters

- Association of Molecular Pathology, 7-9 November, Baltimore
- American Society of Tropical Medicine and Hygiene, 20-24 November, National Harbor

https://www.atcc.org/Documents/Learning_Center/Research/Posters.aspx
Summary

- ATCC Molecular Standards are a consistent and reliable control for assay development and validation.

- Genomic standards eliminate the costs of growth and extraction.

- Synthetic standards provide controls for organisms that are difficult to culture.

- 159 standards currently in the portfolio.
  - 115 genomic standards
  - 44 synthetic standards
  - Standards for pathogens, microbiome, & food safety
  - Another 20 to 25 more to launch in 2019!
Thank you to the project team!

ATCC R&D, Technical Transfer, and Marketing Teams

Cincinnati Children’s Hospital, Department of Pathology, Donna Diorio

National Institute for Biological Standards and Control (NIBSC)

Stanford University Medical Center – Benjamin Pinsky
Questions?

Credible Leads to Incredible™
Cultivating collaboration to support global health

Go to www.atcc.org/molecularstandards for more information

Upcoming webinars:

- **EMT/MET reporter cell lines: Elevating biological models of metastasis** October 24, 12:00 ET

- **On the edge of the bubble: Use of exosomes as reference materials in biomedical research** October 31, 12:00 ET

www.atcc.org/webinars