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

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Product Specialist, ATCC

Credible Leads to Incredible™
Agenda

✓ How ATCC molecular standards accelerate assay development
✓ Development process for ATCC’s synthetic molecular standards
✓ Validation data = materials you can trust
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, 19 international distributors
- Talented team of 500+ employees, over one-third with advanced degrees
Molecular Standards
ATCC molecular standards

ATCC Molecular Standards
- Quantitative
- BSL 1
- ATCC Quality

ATCC Molecular Standards Categories:
- Synthetic
- Genomic

ISO 13485 guidance

Difficult to grow or unculturable

ATCC cultures

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><strong>Genome copy number by ddPCR</strong></td>
<td>1 x 10^5 to 1 x 10^6 construct copies/µL</td>
</tr>
<tr>
<td>Fill Volume</td>
<td>100 µL per vial</td>
</tr>
<tr>
<td>Format</td>
<td>Frozen</td>
</tr>
</tbody>
</table>

#### Genomic Standards

<table>
<thead>
<tr>
<th>Authentication</th>
<th>Amplicon sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Integrity</strong></td>
<td>High molecular weight DNA by gel electrophoresis</td>
</tr>
<tr>
<td><strong>Genome copy number by ddPCR</strong></td>
<td>1 x 10^5 to 1 x 10^6 genome copies/µL</td>
</tr>
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<tr>
<td>Format</td>
<td>Frozen</td>
</tr>
</tbody>
</table>
Molecular Diagnostics

Assay development

- Assay Sensitivity
- Limit of Detection
- Inclusivity

- Assay Specificity
- Exclusivity
Assay Sensitivity - Inclusivity testing
A menu for assay design

Blood-borne disease
- BK virus
- Hepatitis B virus
- Hepatitis C virus
- Epstein-Barr virus
- Human immunodeficiency virus 1
- Human T-cell leukemia virus
- Human cytomegalovirus
- Neisseria meningitides
- Plasmodium malariae
- Human parechovirus 3

Gastro-Intestinal disease
- Astrovirus
- Cyclospora cayetanensis
- Hepatitis A 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

Respiratory disease
- SARS-CoV-2
- SARS-CoV
- MERS-CoV
- Human coronavirus OC43
- Human coronavirus HKU1
- Human coronavirus NL63
- Human coronavirus 229E
- Human metapneumovirus
- Bordetella pertussis
- Mycobacterium bovis
- Mycobacterium falomiae
- Mycobacterium microti
- Mycobacterium pinnipedii
- Mycobacterium tuberculosis
- Streptococcus pneumoniae
- Human respiratory syncytial virus
- Influenza B virus (Victoria)
- Influenza B virus (Yamagata)
- Influenza A virus (H3N2)
- Influenza A virus (H1N1)
- Human bocavirus
- Bordetella pertussis
- Haemophilus influenzae
- Adenovirus
- Parainfluenza viruses
- Rhinoviruses
- Chlamydia pneumoniae
- Legionella pneumophila
- Mycoplasma pneumoniae

Sexually transmitted infections
- Neisseria gonorrhoeae
- Human immunodeficiency virus 1
- Human papillomavirus 16
- Human papillomavirus 18
- Human papillomavirus 31
- Human T-cell leukemia virus 2
- Treponema pallidum
- Chlamydia trachomatis LGV I
- Chlamydia trachomatis LGV II
- Chlamydia trachomatis LGV III
- Human herpesvirus 1
- Human herpesvirus 2
- Hepatitis B virus
- Human herpesvirus 8
- Human herpesvirus 7
- Human herpesvirus 6
- Mycoplasma genitalium
- Staphylococcus saprophyticus
- Hamophilus ducreyi

Epidermal & Nosocomial disease
- Powassan virus
- Chikungunya virus
- Dengue virus types 1-4
- Eastern equine encephalitis virus
- Plasmodium malariae
- St. Louis encephalitis virus
- West Nile virus
- Yellow fever virus
- Zika virus
- Borrelia burgdorferi
- Plasmodium falciparum
- Yellow fever virus
- Rift Valley Fever virus

Vector-borne disease
- Staphylococcus aureus subsp. aureus
- Staphylococcus epidermidis
- Streptococcus pyogenes
- Candida albicans
- Pseudomonas aeruginosa
- Candida krusei
Assay scope – strain selection for safety testing

**Water safety**
- Enterococcus faecalis
- Vibrio cholerae
- Cryptosporidium parvum
- Rotavirus
- Pseudomonas aeruginosa
- Escherichia coli serotype O157:H7

**Food safety**
- Norovirus
- Big Six Escherichia coli
- Campylobacter jejuni
- Salmonella enterica
- Listeria monocytogenes
- Sapovirus

Photo credit: CDC, Dr. Charles D. Humphrey
# SARS-CoV-2 molecular standards

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Description</th>
<th>Compatible assays</th>
</tr>
</thead>
</table>
| VR-3276SD™      | Quantitative Synthetic SARS-CoV-2 RNA containing portions of ORF1ab, N, E, nsp12 (RdRp), and ORF1b-nsp14 genes | • China CDC Primers and probes for detection 2019-nCoV (24 January 2020)  
• Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR – Charité, Berlin Germany (17 January 2020)  
• Detection of 2019 novel coronavirus (2019-nCoV) in suspected human cases by RT-PCR – Hong Kong University (23 January 2020)  
• PCR and sequencing protocol for 2019-nCoV - Department of Medical Sciences, Ministry of Public Health, Thailand (Updated 28 January 2020)  
• US CDC panel primer and probes– U.S. CDC, USA (28 January 2020)  |
| VR-3277SD™      | Quantitative Synthetic SARS-CoV-2 RNA containing a portion of Spike 5’ end gene. | • Detection of WN-Human1 sequence from clinical specimen – National Institute of Infectious Diseases Japan (17 January 2020)                                                                                           |
| VR-3278SD™      | Quantitative Synthetic SARS-CoV-2 RNA containing a portion of Spike 3’ end gene. | • PCR and sequencing protocols for 2019-nCoV- National Institute of Infectious Diseases Japan (24 January 2020)                                                                                                     |
| VR-3279SD™      | Quantitative Synthetic SARS-CoV-2 RNA containing portions of the nsp9 and nsp12 (RdRp) genes | • RT-PCR assays for the detection of SARS-CoV-2 with RdRp – Institut Pasteur, Paris (2 March 2020)  
• Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR – Charité, Berlin Germany (17 January 2020)                                                                                     |

## BSL 2

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Genomic RNA from isolate</th>
</tr>
</thead>
</table>
| VR-1986D™       | USA-WA1/2020  
lineage A                                                                                     |
| VR-1991D™       | Hong Kong/VM20001061/2020  
lineage A                                                                                     |
| VR-1992D™       | Italy/INMI1  
lineage B                                                                                     |
| VR-1994D™       | Germany/BavPat1/2020  
lineage B (D614G mutation)                                                                 |
| VR-3326D™       | USA/CA_CDC_5574/2020  
lineage B.1.1.7                                                                               |
| VR-3327D™ *     | USA/MD-HP01542/2021  
lineage B.1.351                                                                                   |
| VR-3338D™ *     | Japan/TY7-503/2021  
lineage P.1                                                                                       |

* In development
A menu for assay design

**Blood-borne disease**
- BK virus
- Hepatitis B virus
- Hepatitis C virus
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- Human immunodeficiency virus 1
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- Human cytomegalovirus
- Varicella-zoster virus
- Neisseria meningitides
- Plasmodium malariae
- Human parechovirus 3

**Gastro-Intestinal disease**
- Astrovirus
- Cyclospora cayetanensis
- Hepatitis A virus
- Hepatitis E virus
- Norovirus GI
- Norovirus GII
- Sapovirus
- Mycobacterium avium subsp. paratuberculosis
- Clostridiodes difficile
- Salmonella enteritisa 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

**Respiratory disease**
- SARS-CoV-2
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- Mycobacterium microti
- Mycobacterium pinnipedii
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- Chlamydia trachomatis LGV III
- Human herpesvirus 1
- Human herpesvirus 2
- Hepatitis B virus
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- Hamophilus ducreyi

**Sexually transmitted infections**
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- Influenza B virus (Yamagata)
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- Bordetella pertussis
- Haemophilus influenza
- Adenovirus
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- Rhinoviruses
- Chlamydia pneumoniae
- Legionella pneumophila
- Mycoplasma pneumoniae

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

**Vector-borne disease**
- Staphylococcus aureus subsp. aureus
- Staphylococcus epidermidis
- Streptococcus pyogenes
- Candida albicans
- Pseudomonas aeruginosa
- Candida krusei

**Inclusivity candidate**
Assay sensitivity - limit of detection

Molecular Diagnostics

Assay Sensitivity

Inclusivity

Limit of Detection

Assay Specificity

Exclusivity
Assay specificity - exclusivity testing
## Resources for SARS-CoV-2 inclusivity/exclusivity

List of recommended pathogens for assay design in the FDA’s emergency-use-authorization letter.

### Viral pathogen formats

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Inactivated / Live</th>
<th>Quantitative Genomic</th>
<th>Synthetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>SARS-CoV-2</td>
<td>2 (heat-killed)</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Human coronavirus 229E</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Human coronavirus OC43</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Human coronavirus HKU1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Human coronavirus NL63</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SARS-CoV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MERS-CoV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenovirus</td>
<td>68</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Human metapneumovirus</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Parainfluenza virus 1-4</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Influenza A &amp; B</td>
<td>82</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Enterovirus</td>
<td>118</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Respiratory syncytial virus</td>
<td>9</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>132</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

### Non-viral pathogen formats

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Inactivated / Live</th>
<th>Quantitative Genomic</th>
<th>Synthetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlamydia pneumoniae</td>
<td>7</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>73</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Legionella pneumophila</td>
<td>35</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>30</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Streptococcus pneumonia</td>
<td>170</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>155</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Bordetella pertussis</td>
<td>30</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Mycoplasma pneumoniae</td>
<td>16</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>225</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>328</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Pneumocystis jirovecii</td>
<td></td>
<td></td>
<td>1*</td>
</tr>
<tr>
<td>Staphylococcus epidermis</td>
<td>33</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Streptococcus salivarius</td>
<td>11</td>
<td>1*</td>
<td></td>
</tr>
</tbody>
</table>

*In development and planning stages.
A menu for assay design

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- E. coli

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- Haemophilus influenza
- Adenovirus
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- Plasmodium malariae
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- West Nile virus
- Yellow fever virus
- Zika virus
- Borrelia burgdorferi
- Plasmodium falciparum
- Yellow fever virus
- Rift Valley Fever virus

Inclusivity candidate
- Neisseria gonorrhoeae
- Human immunodeficiency virus 1
- Human papillomavirus 16

Exclusivity candidate
- Staphylococcus aureus subsp. aureus
- Staphylococcus epidermidis
- Streptococcus pyogenes
- Candida albicans
- Pseudomonas aeruginosa
- Candida krusei
Get the materials

How to find the strains you need

Nucleic Acids

97 Products
Results 1-12 of 97

Search

Refine by

Product category

- Viruses 97

Product type

- Nucleic acid 156
- Molecular standard 97

Product application

- Assay development 88
- Infectious disease res... 81
- Next-generation seq... 76
- Respiratory disease ... 38

Organism

- Influenza A virus (H1... 5
- Human respiratory sy... 4
- Influenza B virus 3
- Human herpesvirus 1 2
- Human enterovirus 71 2

Type strain

- No 7

Nucleic acid type

- Genomic 50
- Synthetic 46

DNA or RNA

- RNA 67
- DNA 30
Ongoing and future efforts

- Quality control strains – pharmacopeia, CLSI, or other citations

- Panels for pathogens
  - Respiratory
  - STI
  - Enteric
  - Oncoviruses
  - Opportunistic
  - Anti-microbial resistant

- What do you need?
  - ATCC exists to be a resource for scientists.
Assay validation - Human herpes viruses

### Assay control - Human herpes viruses

<table>
<thead>
<tr>
<th>Assay Control</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>
Synthetic Molecular Standard Design
Interest in the first 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

**ISO 13485**
- Guidance for manufacturing

**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™
  - $R^2 = 0.998$
  - $m = -3.625$

www.atcc.org/2015posters
Old vs. new standards, Genogroup 1

CaliciNet primers & probe

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

CaliciNet primers & probe

ECS primers & probe
A test drive with NIBSC working reagents

NoV-GI

NoV-GII
Design approach following design control

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

Construct design
- Construct design
- Assay selection and optimization
- Technical review

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

**Construct**
- Genome fragments targeted for assays design
- 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 confirm whether primers bind

For design support, ATCC will confirm whether primers bind.
Validation of Molecular Standards
Validation of synthetic standards for hepatitis viruses

Hepatitis B virus
- ATCC catalog VR-3232SD™
- Hepadnaviridae, Orthohepadnavirus
- DNA construct
- Portions of precore, core, P, S, and X regions

Hepatitis C virus
- ATCC catalog VR-3233SD™
- Flaviviridae, Hepacivirus, Hepacivirus C
- RNA construct
- 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

VR-3232SD™ (HBV synthetic standard)

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

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

qRT-PCR and qPCR quantitation at ATCC:
- HBV: 9.7 x 10^6 genome copies/mL
- HCV: 1.6 x 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
Other application data and posters

www.atcc.org/resources
Summary

- ATCC Molecular Standards are a fast, easy, reliable control for assay development and validation & control.

- Genomic standards eliminate the costs of growth, extraction, and quantitation.

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

- Over 230 standards currently in the portfolio.
  - Over 170 genomic standards
  - Over 60 synthetic standards
  - Standards for pathogens, microbiome, & food safety
  - And more to come!
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, Ph.D.
Questions?

Credible Leads to Incredible™