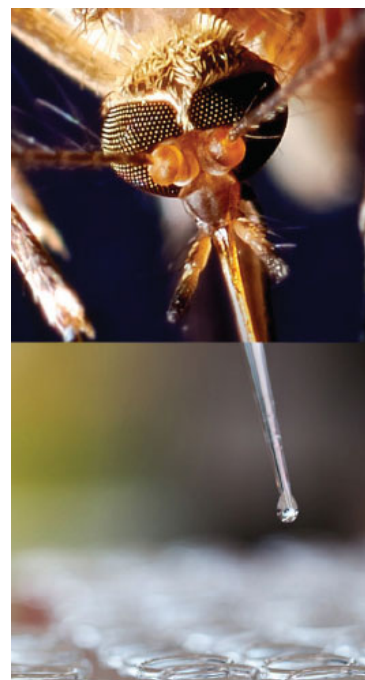
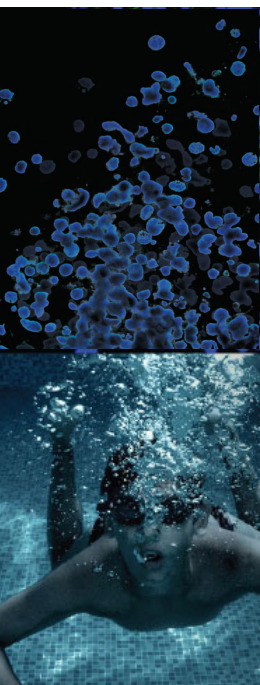




Simplifying assay development with molecular standards: Remove culturing from the equation

Kyle Young, B.S., M.B.A.
Product Line Business Specialist, ATCC

Credible Leads to Incredible™



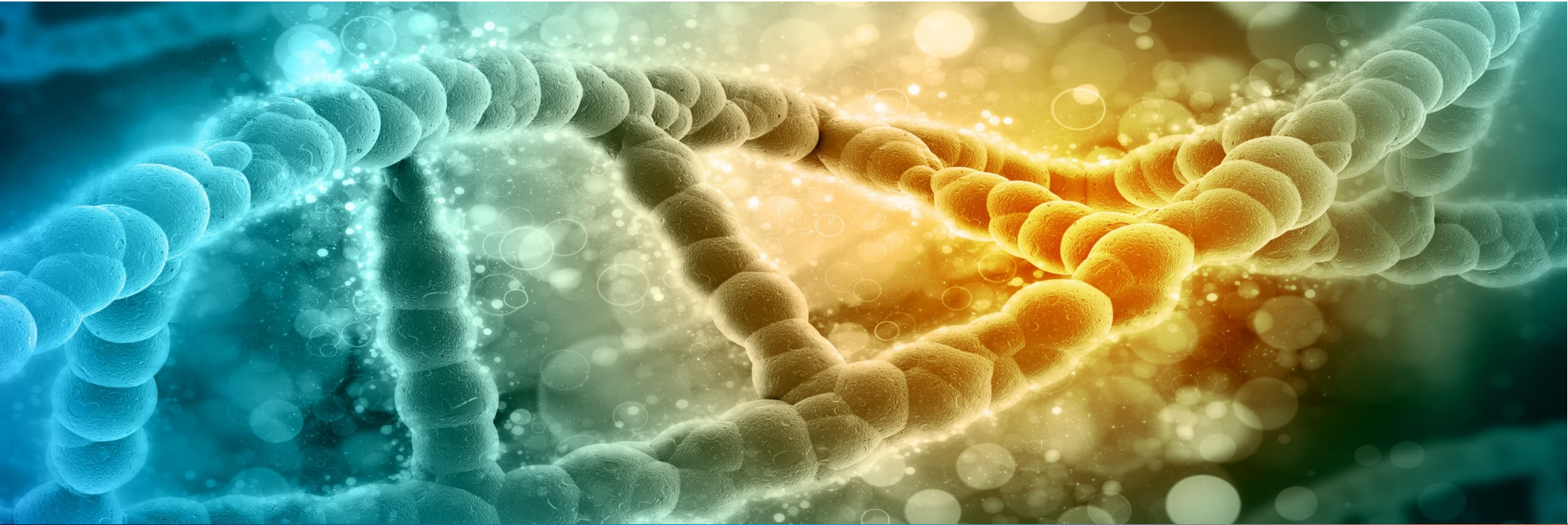
Agenda

- ✓ What ATCC molecular standards provide
- ✓ Development of ATCC's synthetic molecular standards
- ✓ The ATCC molecular standards portfolio
 - ✓ Including upcoming new products
- ✓ Validation data



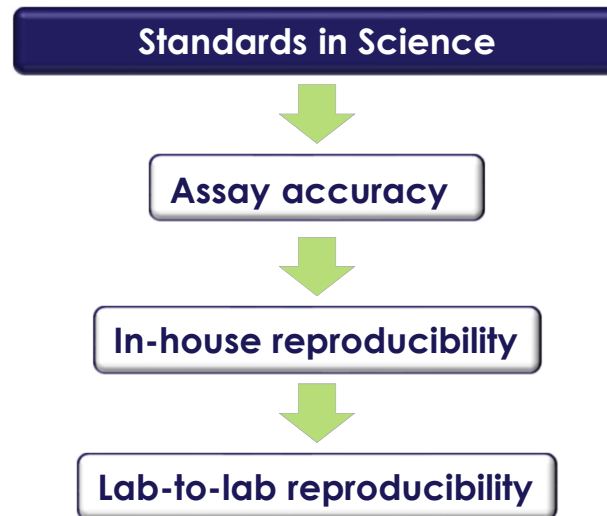
About ATCC

- 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

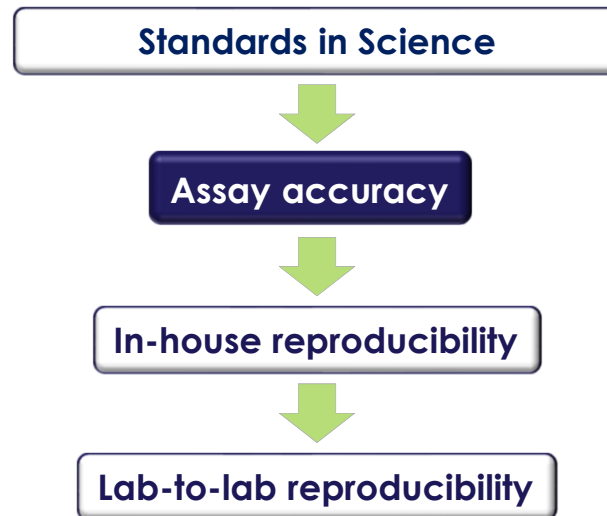


Molecular Standards

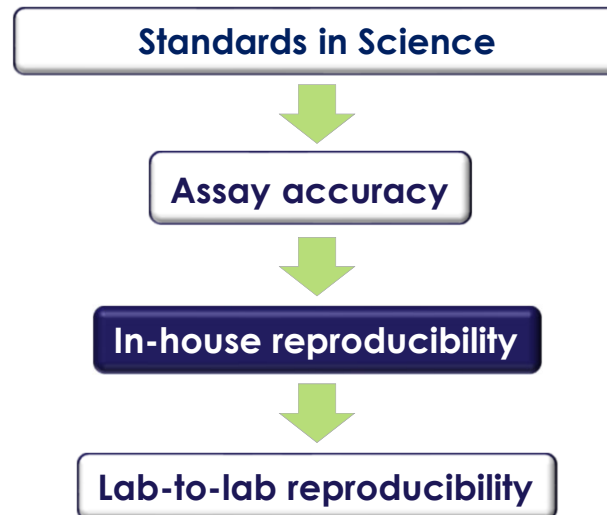
ATCC molecular standards



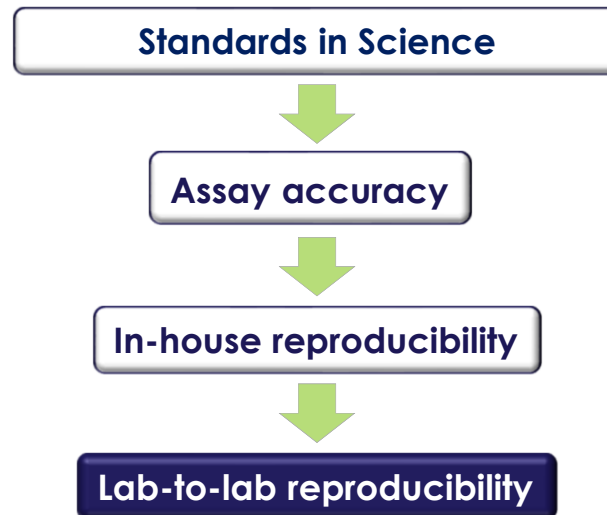
ATCC molecular standards



ATCC molecular standards



ATCC molecular standards



Project history

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)

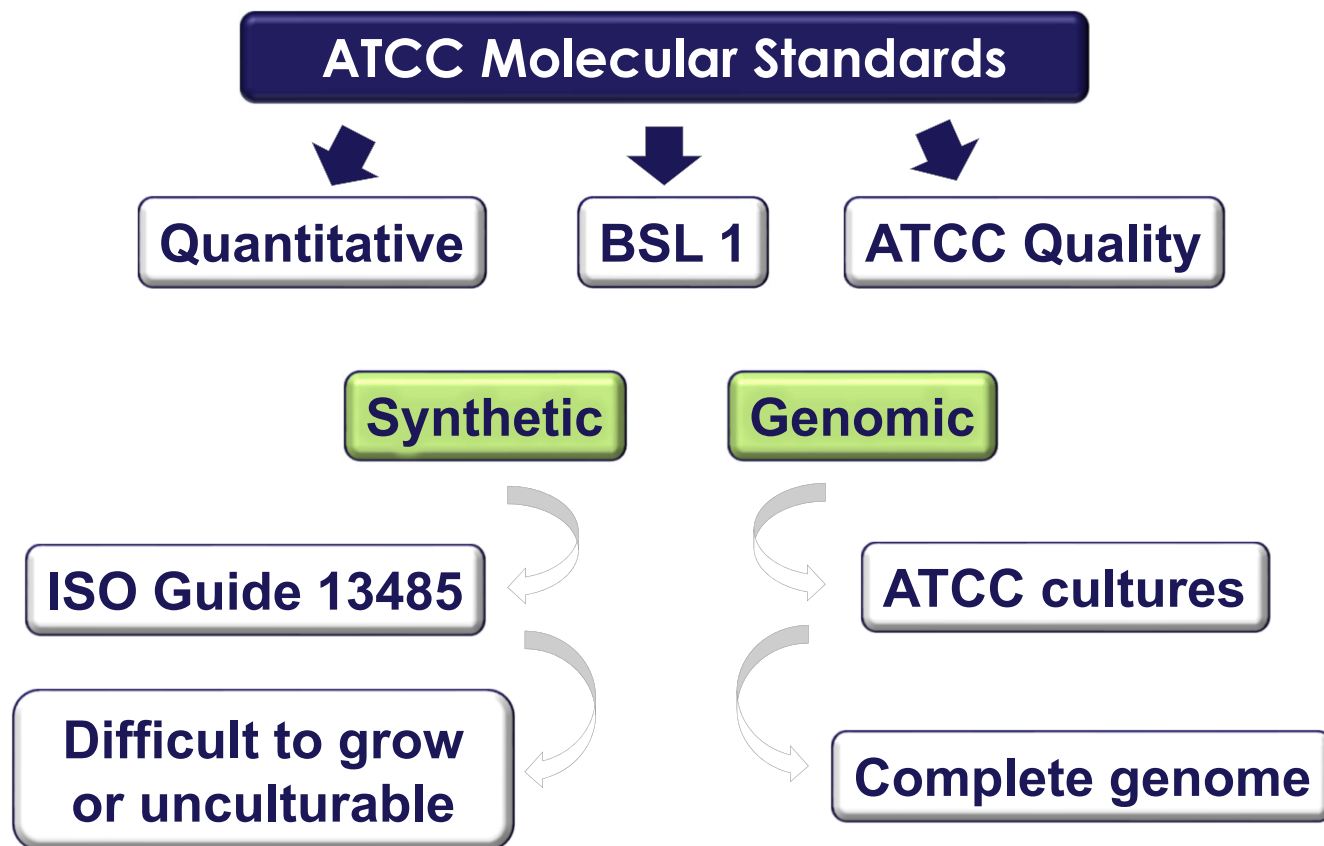
VR-3199SD (Norovirus G1 RNA)

VR-3299SD (Norovirus G2 RNA)

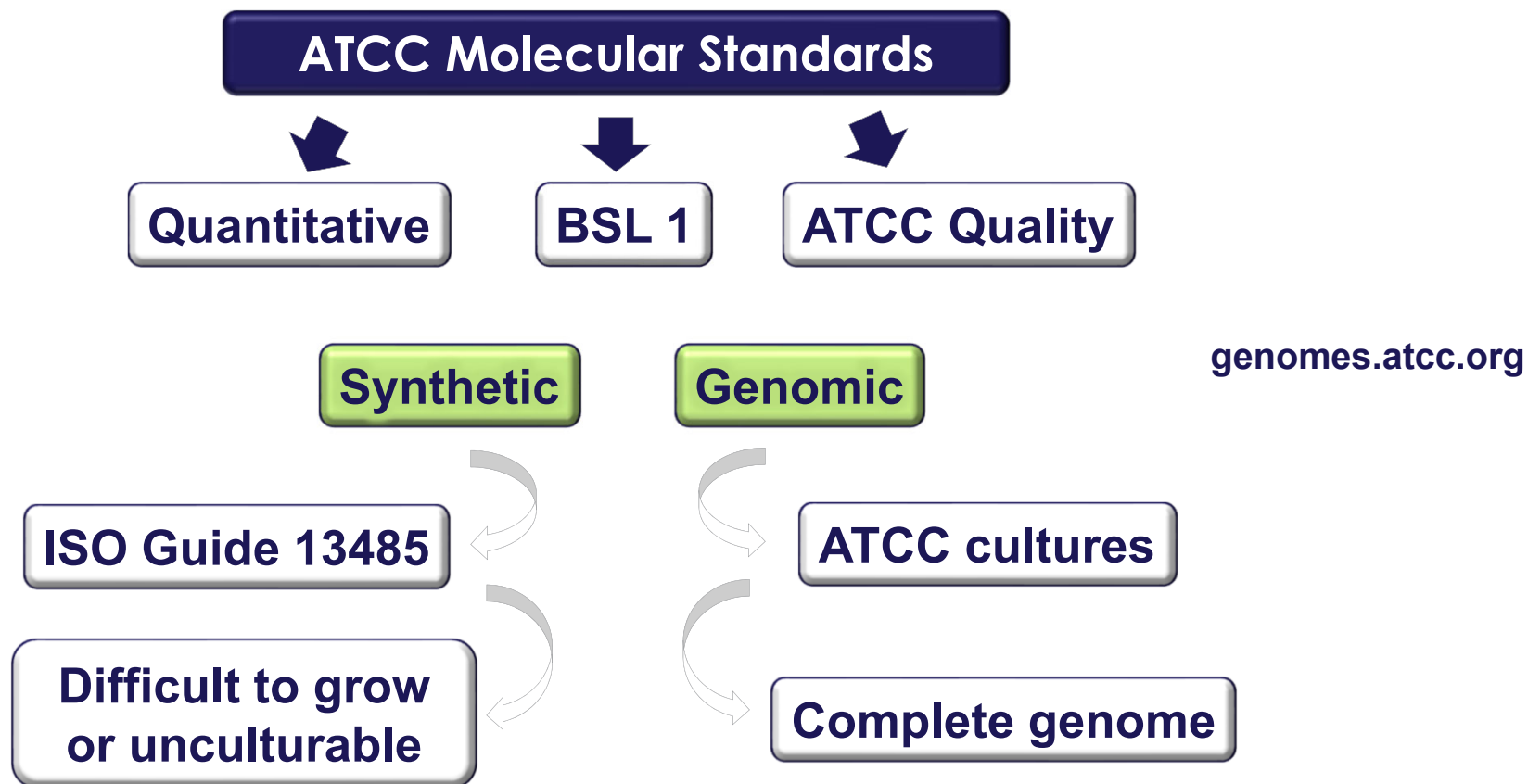
29212Q-FZ (Enterococcus faecalis)



ATCC molecular standards



ATCC molecular standards



Specifications

Synthetic Standards

Genomic Standards

Authentication	NGS to verify synthetic sequence	Authentication	Amplicon sequencing
Functionality & Identity	qPCR amplification, 3.32 cycles between Cq threshold	Integrity	High molecular weight DNA, by gel electrophoresis
Genome copy number by ddPCR™	1 x 10 ⁵ to 1 x 10 ⁶ genome copies/ µL	Genome copy number by ddPCR™	1 x 10 ⁵ to 1 x 10 ⁶ genome copies/ µL
Fill Volume	100 µL per vial	Fill Volume	100 µL per vial
Format	Frozen	Format	Frozen
Certifications	ISO 13485, ISO 9001, ISO 17025	Certifications	ISO 9001, ISO 17025



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

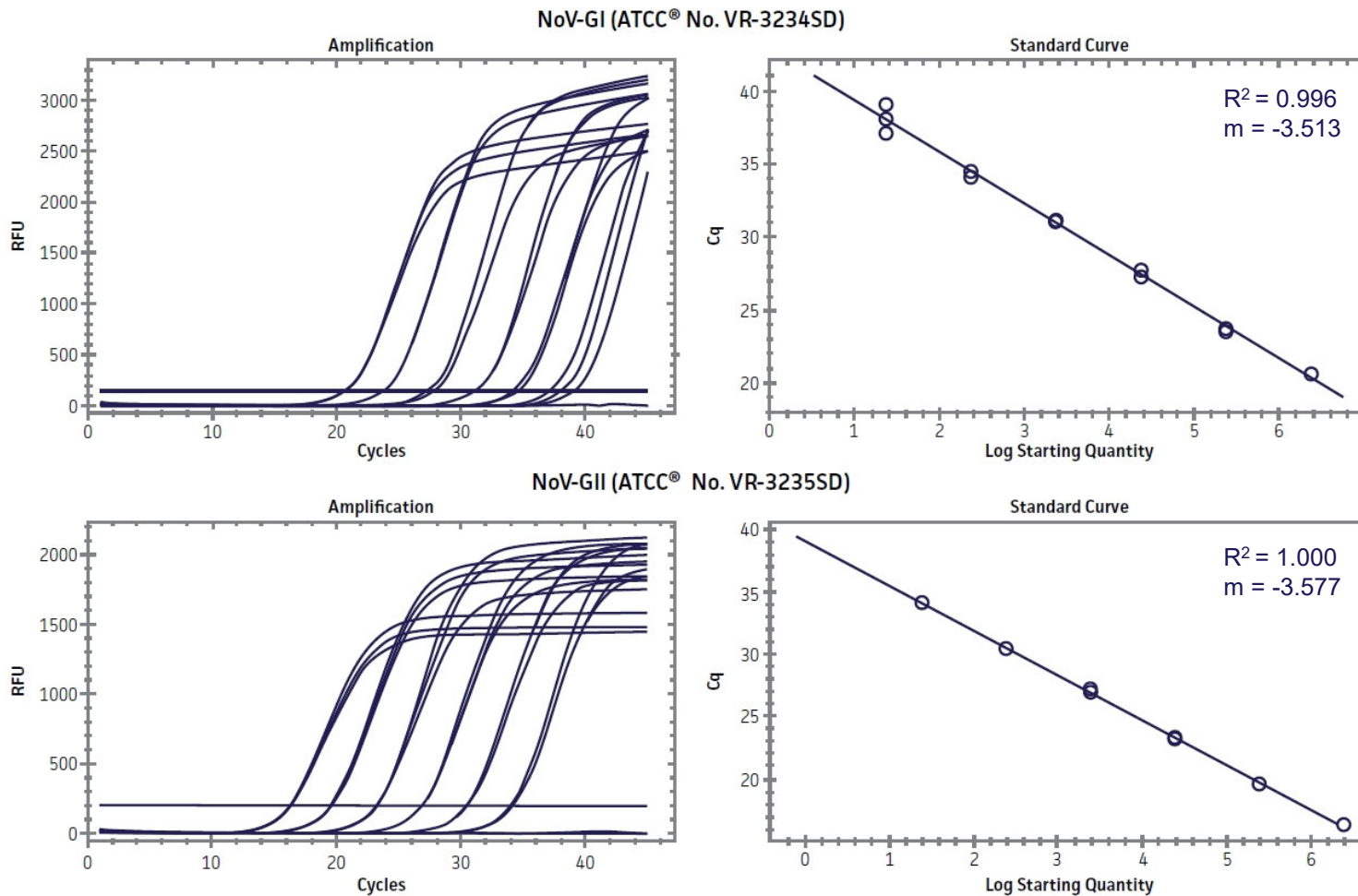
Certifications

Added ISO 13485 compliance

Versatility

Added RdRp fragments to construct

Validating the next generation of standards



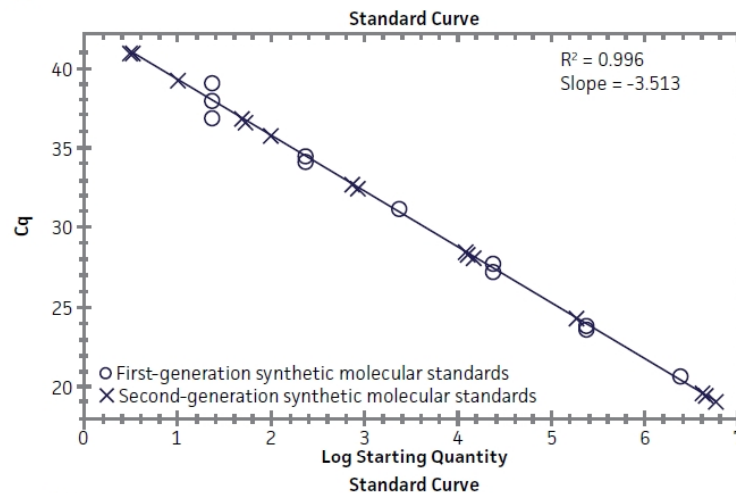
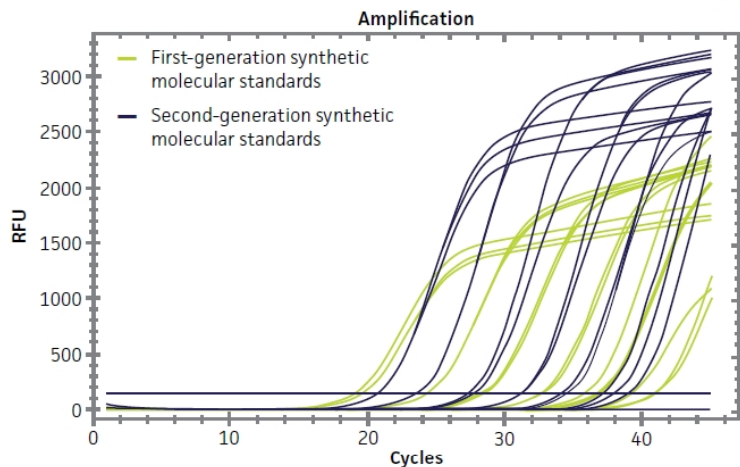
Pictured: Standard curves generated with CaliciNet primer and probe set.

Also tested: ECS working group primer and probe set.

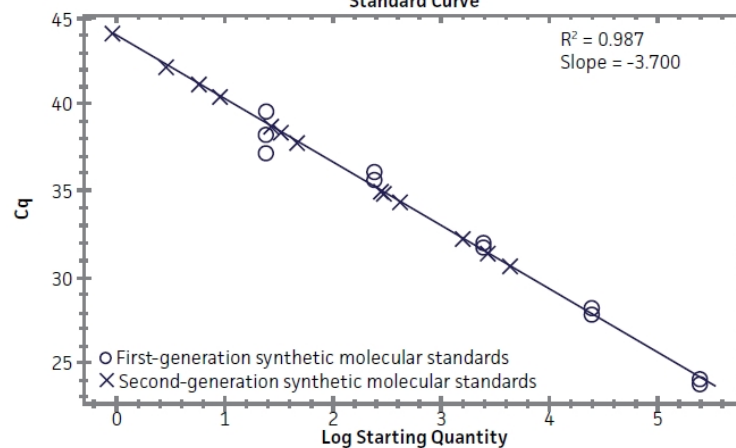
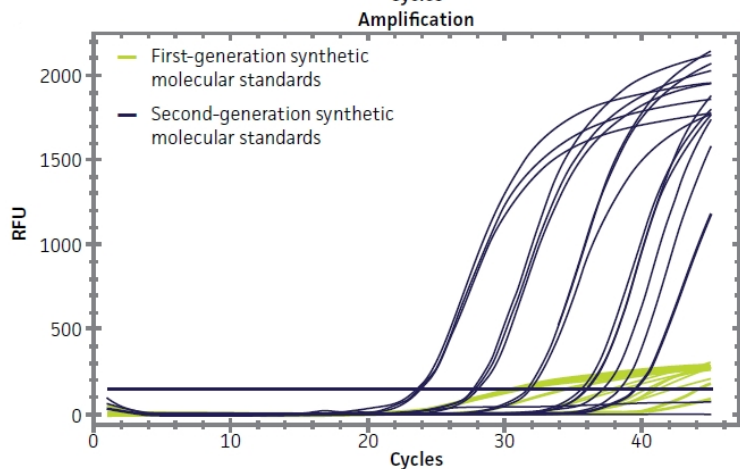
- VR-3234SD
 - $R^2 = 0.987$
 - $m = -3.692$
- VR-3235SD
 - $R^2 = 0.998$
 - $m = -3.625$

Old vs. new standards, part 1

NoV-GI



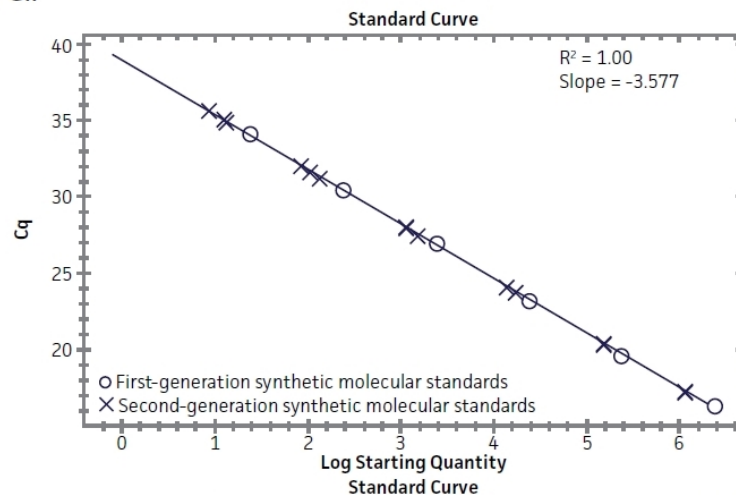
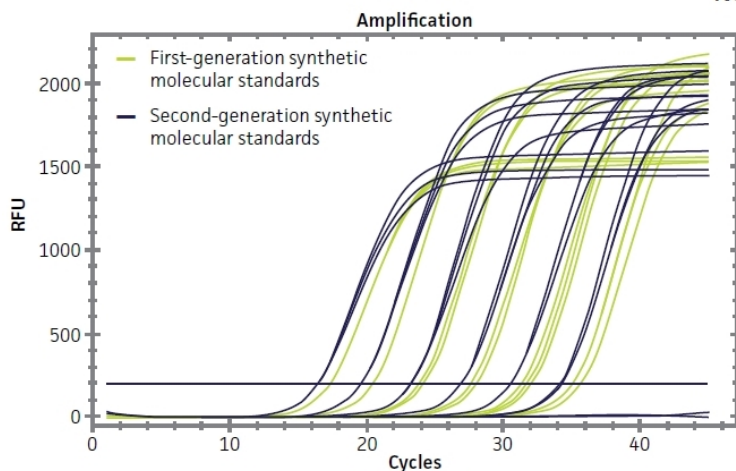
CaliciNet
primers & probe



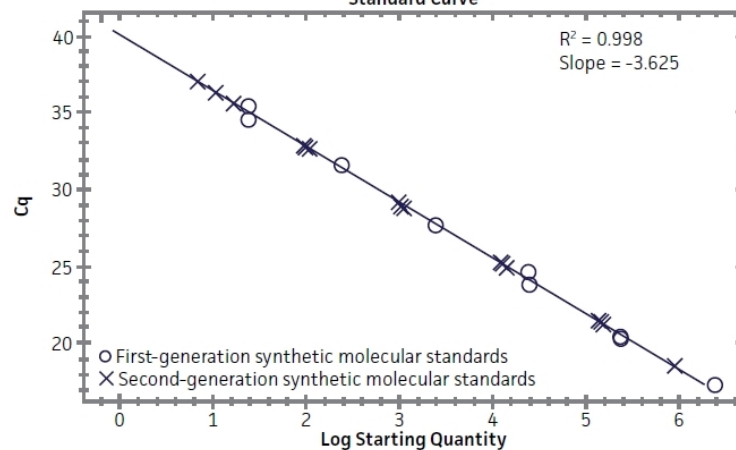
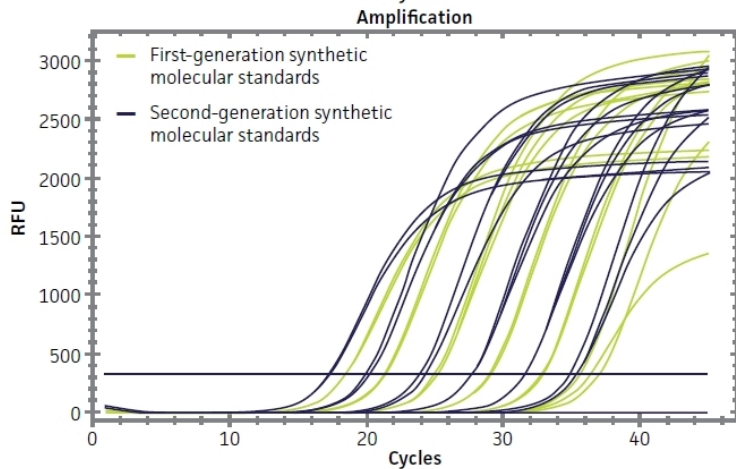
ECS working group
primers & probe

Old vs. new standards, part 2

NoV-GII

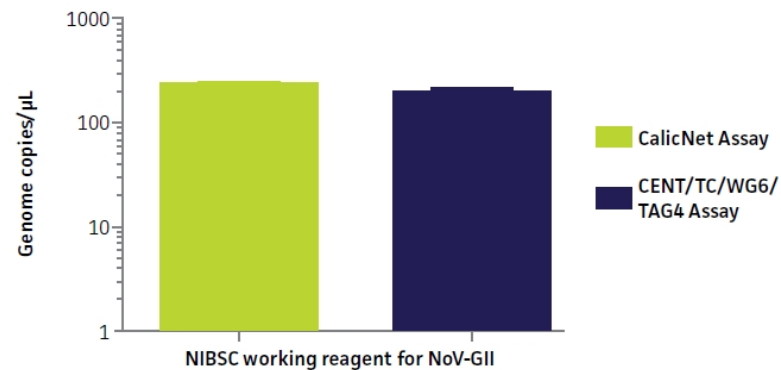
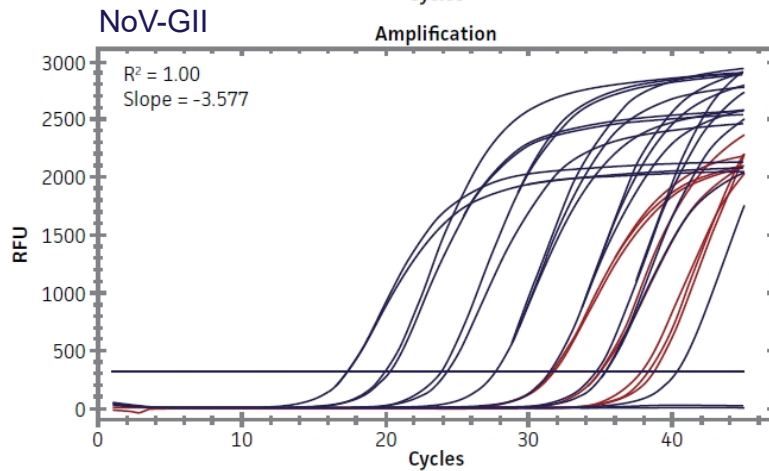
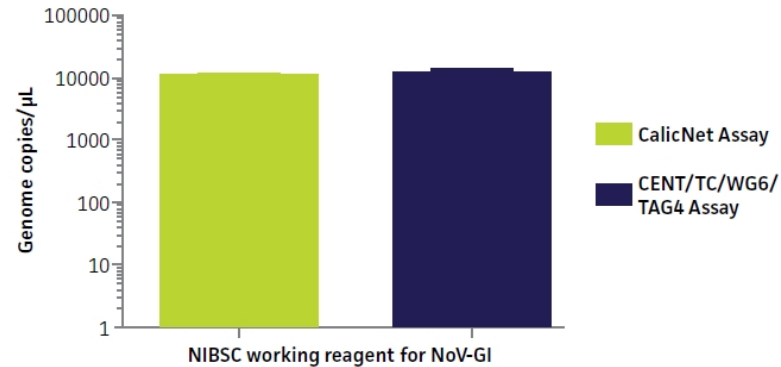
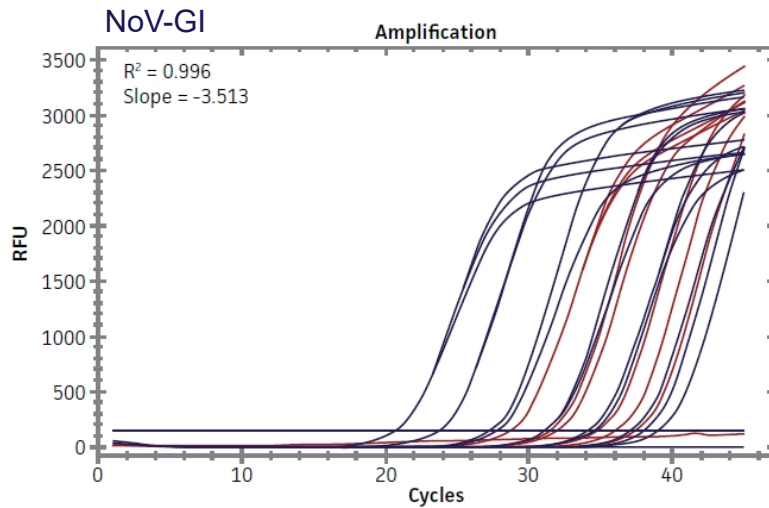


CaliciNet
primers & probe

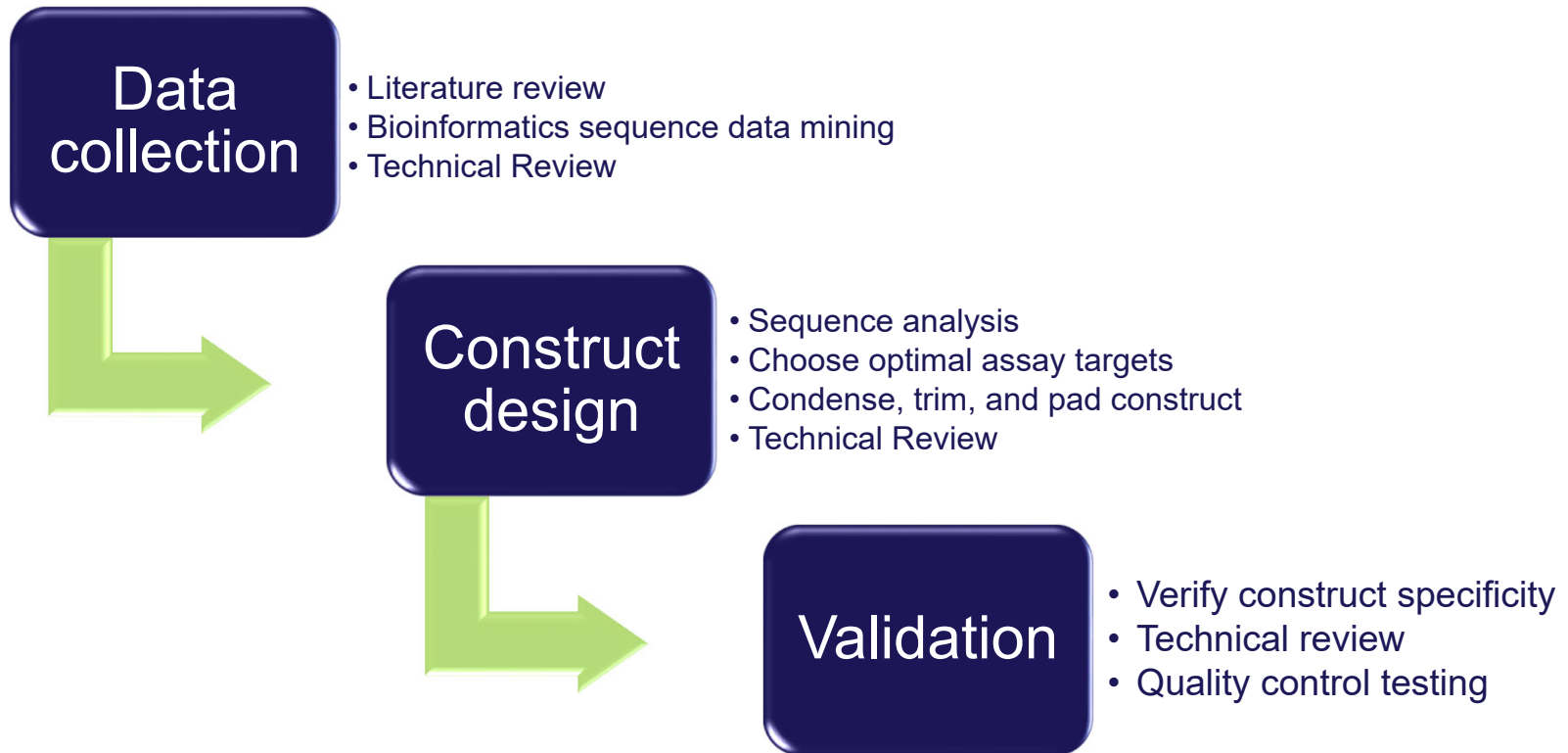


ECS
primers & probe

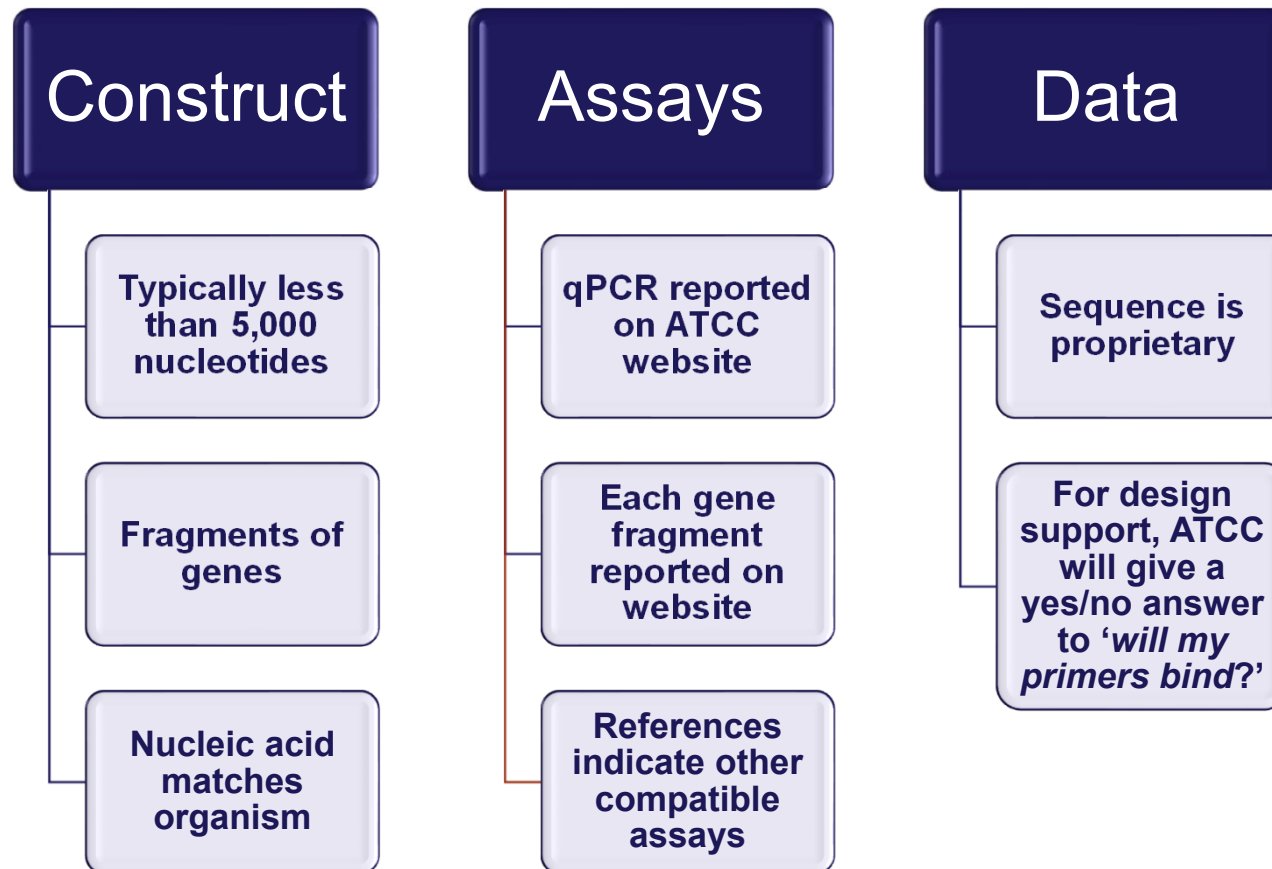
A test drive with NIBSC working reagents



Design approach – synthetic standards



Design approach – synthetic standards





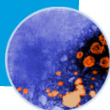
ATCC's Molecular Standards

Pathogen standards

www.atcc.org/MolecularStandards

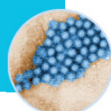
- BK virus
- Hepatitis B virus
- Hepatitis C virus
- Epstein-Barr virus
- Human immunodeficiency virus 1
- Human T-cell leukemia virus
- Human cytomegalovirus
- Varicella-zoster virus
- *Neisseria meningitidis*
- *Plasmodium malariae*
- *Human parechovirus 3*

Blood-borne disease



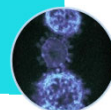
- Astrovirus
- *Cyclospora cayetanensis*
- Hepatitis A virus
- Hepatitis E virus
- Norovirus GI
- Norovirus GII
- Sapovirus
- *Mycobacterium avium* subsp. *paratuberculosis*
- *Clostridioides difficile*
- *Salmonella enterica* subsp. *enterica* serovar Typhimurium
- *Cryptosporidium parvum*
- Human Enterovirus 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 talmoniae*
- *Mycobacterium microti*
- *Mycobacterium pinnipedii*
- *Mycobacterium tuberculosis*
- *Streptococcus pneumoniae*
- Human respiratory syncytial virus strain A2
- Influenza B virus (BY) strain B/Wisconsin/1/2010BX-41A
- Influenza A virus (H3N2) strain A/Wisconsin/15/2009
- Influenza A virus (H1N1) strain A/PR/8/34
- Influenza B virus strain B/Florida/4/2006
- Betacoronavirus 1 strain OC43
- Human rhinovirus 77
- *Bordetella pertussis*
- *Haemophilus influenzae*
- *Streptococcus pneumoniae*

Respiratory disease



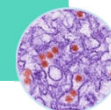
- Hepatitis B virus
- Human immunodeficiency virus 1
- Human papillomavirus 16
- Human papillomavirus 18
- Human papillomavirus 31
- Human T-cell leukemia virus 2
- *Treponema pallidum*
- *Chlamydia trachomatis* serovar I
- *Chlamydia trachomatis* serovar II
- *Chlamydia trachomatis* serovar III
- Human herpesvirus 1
- Human herpesvirus 2
- *Neisseria gonorrhoeae*
- Human Herpesvirus 8
- Human herpesvirus 7
- Human herpesvirus 6
- *Mycoplasma genitalium*

Sexually transmitted infections



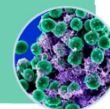
- Chikungunya virus
- Dengue virus types 1-4
- Eastern equine encephalitis virus
- *Plasmodium malariae*
- St. Louis encephalitis virus
- West Nile virus
- Yellow fever virus
- Zika virus
- *Borrelia burgdorferi*
- *Plasmodium falciparum*
- Yellow fever virus
- Rift Valley Fever virus

Vector-borne disease



- *Staphylococcus aureus* subsp. *aureus*
- *Staphylococcus epidermidis*
- *Streptococcus pyogenes*
- *Candida albicans*
- *Pseudomonas aeruginosa*

Epidermal & Nosocomial disease



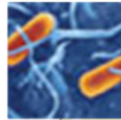
Standards from the microbiome

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

Item Number	Description
29212Q-FZ	Quantitative DNA from <i>Enterococcus faecalis</i>
8739DQ	Quantitative DNA from <i>Escherichia coli</i>
25923DQ	Quantitative DNA from <i>Staphylococcus aureus</i> subsp. <i>aureus</i>
6538DQ	Quantitative DNA from <i>Staphylococcus aureus</i> subsp. <i>aureus</i>
9027DQ	Quantitative DNA from <i>Pseudomonas aeruginosa</i>
13048DQ	Quantitative DNA from <i>Klebsiella aerogenes</i>
10231DQ	Quantitative DNA from <i>Candida albicans</i>
4617DQ	Quantitative DNA from <i>Bordetella bronchiseptica</i>
25285DQ	Quantitative DNA from <i>Bacteroides fragilis</i>

Food safety standards

Big 6 *E. Coli*

Campylobacter jejuni

Salmonella enterica

Norovirus

Sapovirus

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

Recently available

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

Soon available

Coming soon, in 2019

Item Number	Description
VR-1493DQ	Quantitative Genomic DNA from Human herpesvirus 1
VR-94DQ	Quantitative Genomic RNA from Human respiratory syncytial virus
22905DQ	Quantitative Genomic DNA from <i>Proteus vulgaris</i>
VR-1826DQ	Quantitative Genomic RNA from Enterovirus D68
MYA-2876DQ	Quantitative Genomic DNA from <i>Candida albicans</i>
VR-3268SD	Quantitative Synthetic Lassa virus RNA
VR-3269SD	Quantitative Synthetic Nipah virus RNA
VR-3266SD	Quantitative Synthetic Human immunodeficiency virus 2 RNA
VR-3274SD	Quantitative Synthetic West Nile virus
MYA-4941DQ	Quantitative Genomic DNA from <i>Saccharomyces cerevisiae</i>



Validation of Molecular Standards

Validation of synthetic standards for hepatitis viruses

Hepatitis B virus

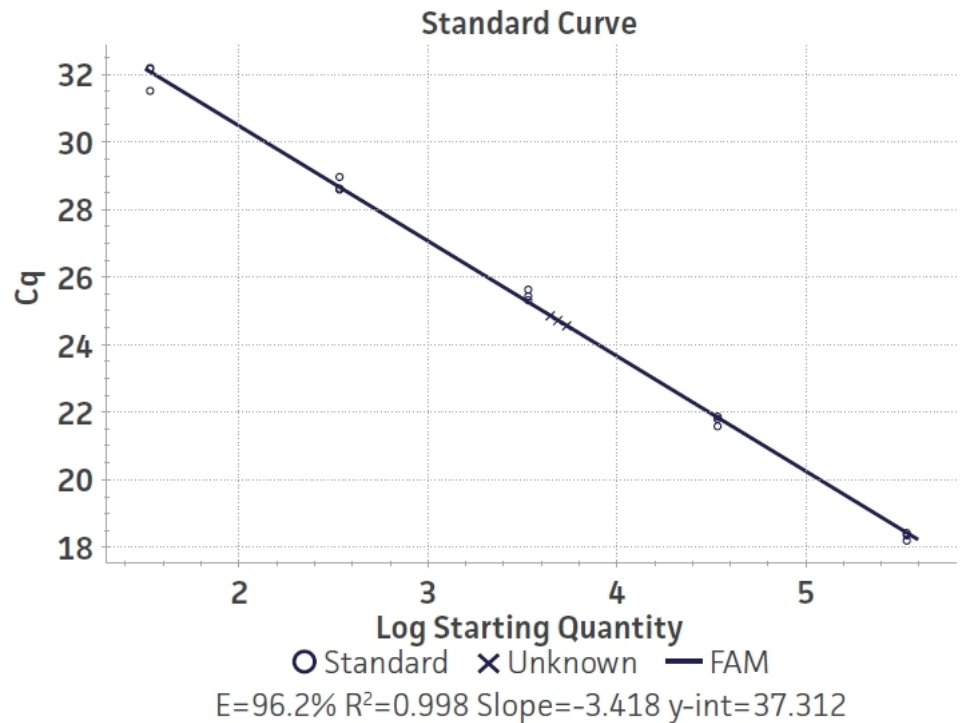
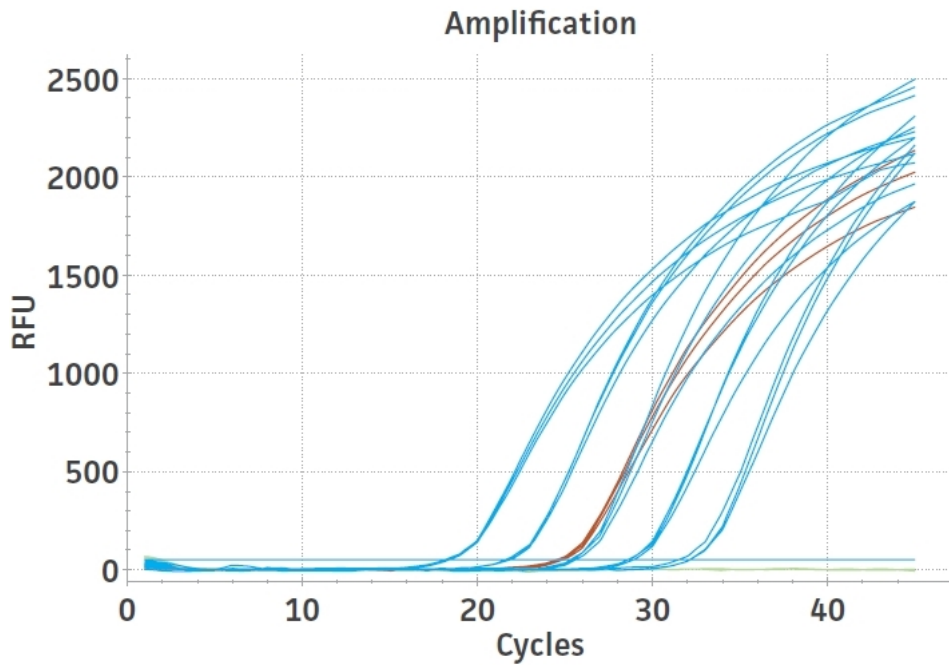
- **VR-3232SD™**
- **Hepadnaviridae, Orthohepadnavirus**
- **DNA construct [includes DNASTABLE® (Biomatrix)]**
- **Portions of precore, core, P, S, and X regions**

Hepatitis C virus

- **VR-3233SD™**
- **Flaviviridae, Hepacivirus**
- **RNA construct [includes RNASTABLE® (Biomatrix)]**
- **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



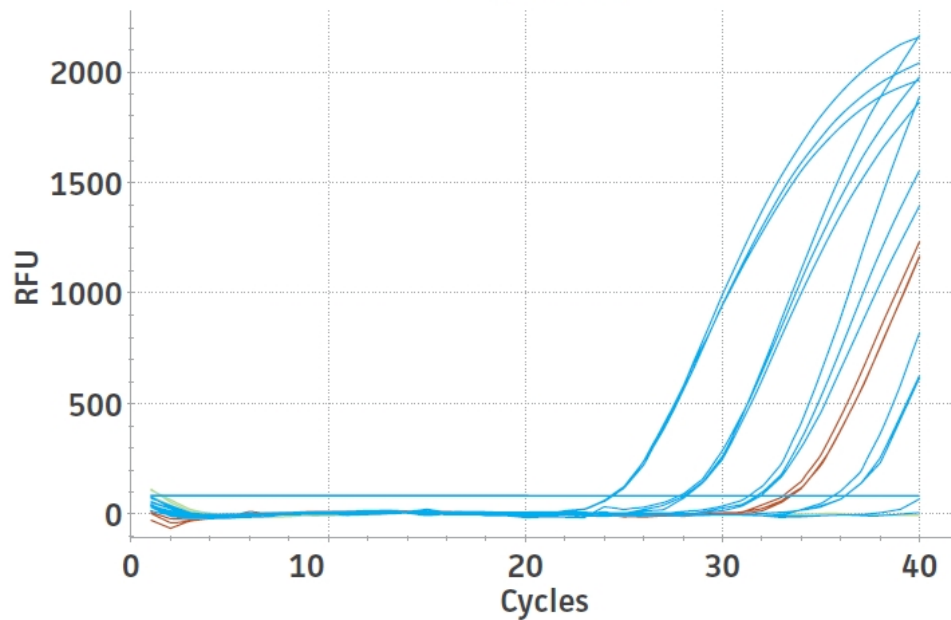
Blue = VR-3232SD™

Red = NIBSC code 10/264 (3rd WHO international working reagent for HBV)

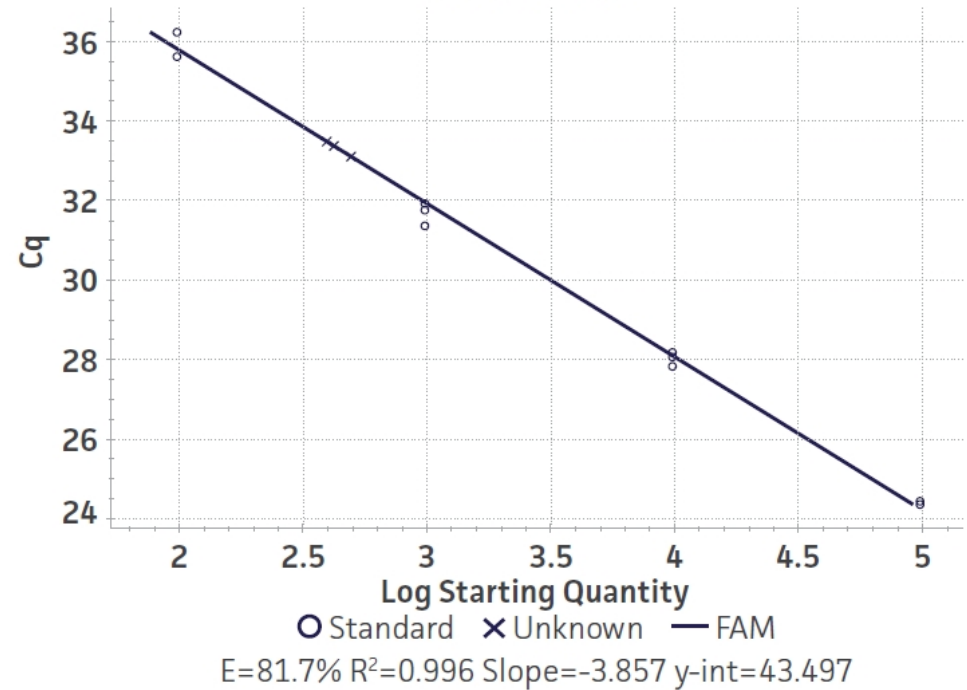
Sun S, *et al.* Development of a new duplex real-time polymerase chain reaction assay for hepatitis B viral DNA detection. *Virology*. J. 8: 227, 2011. PubMed: 21569595

Hepatitis C virus

Amplification



Standard Curve



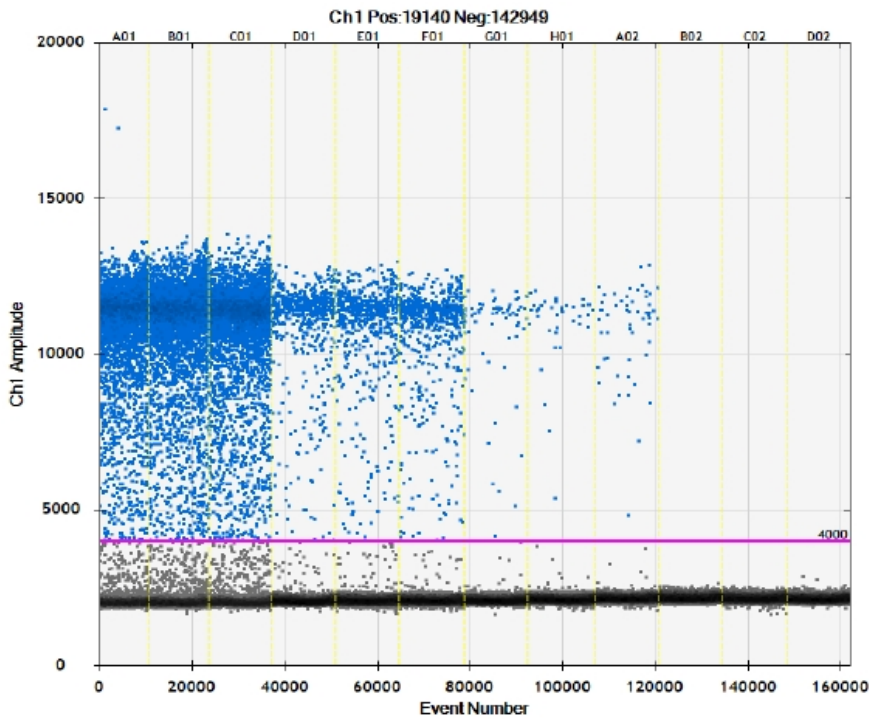
Blue = VR-3233SD™

Red = NIBSC code 06/102 (4th WHO international standard for HCV)

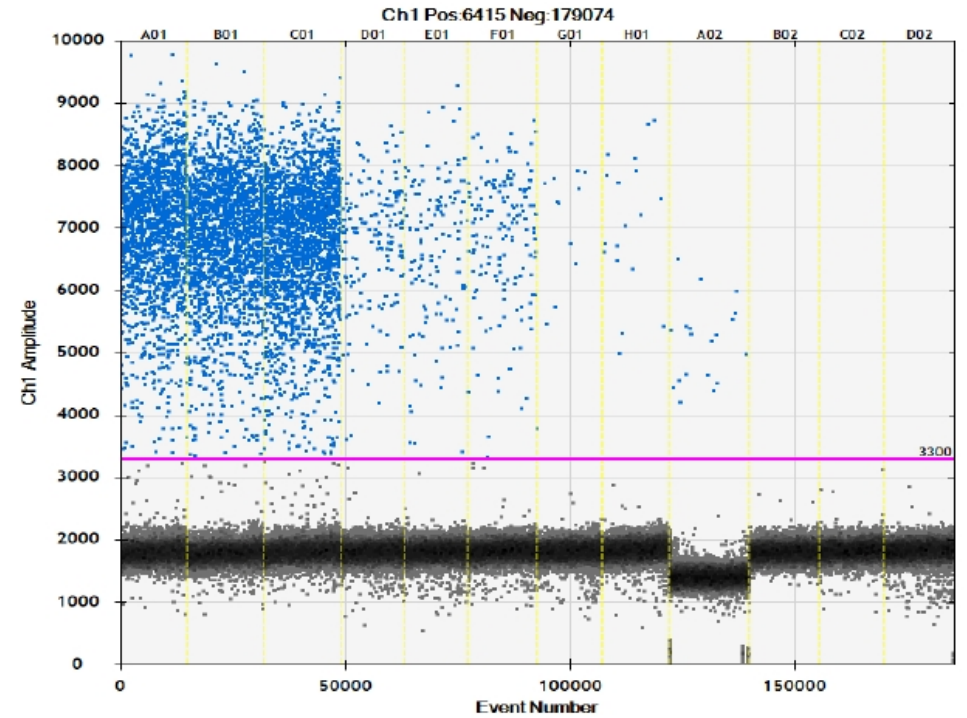
Lee SC, *et al.* Improved version 2.0 qualitative and quantitative AMPLICOR reverse transcription-PCR tests for hepatitis C virus RNA: calibration to international units, enhanced genotype reactivity, and performance characteristics. *J. Clin. Microbiol.* 38(11): 4171-4179, 2000. PubMed: 11060086

Hepatitis viruses – ddPCR™

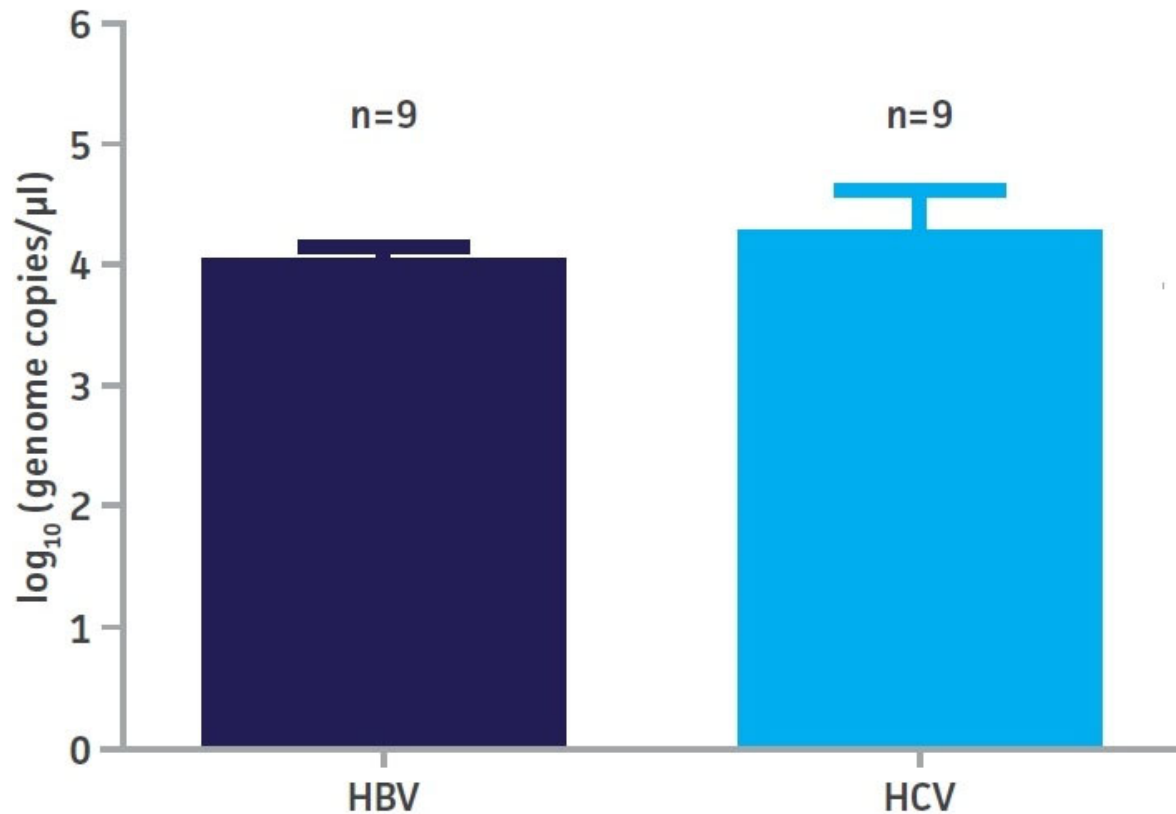
VR-3232SD™ (HBV synthetic standard)



VR-3233SD™ (HCV synthetic standard)

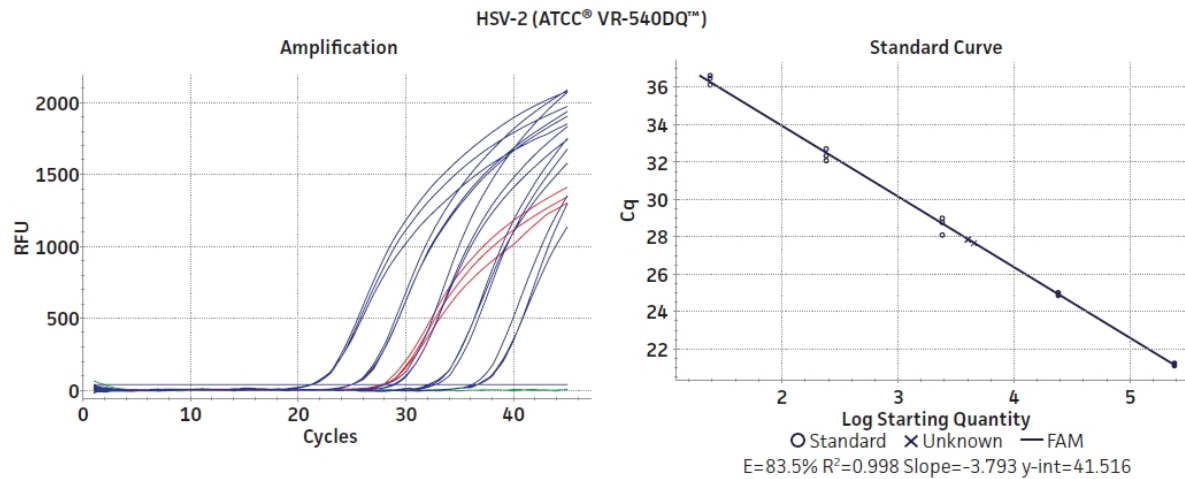
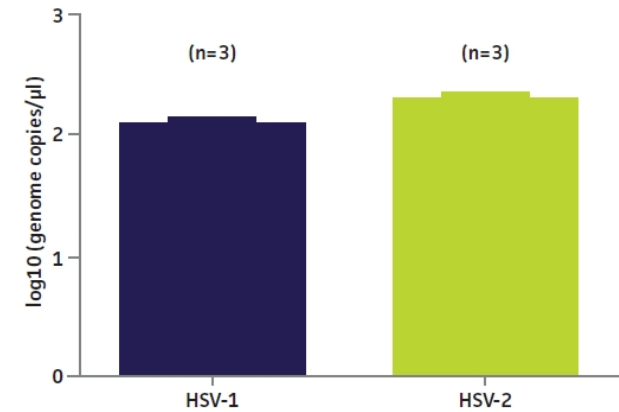
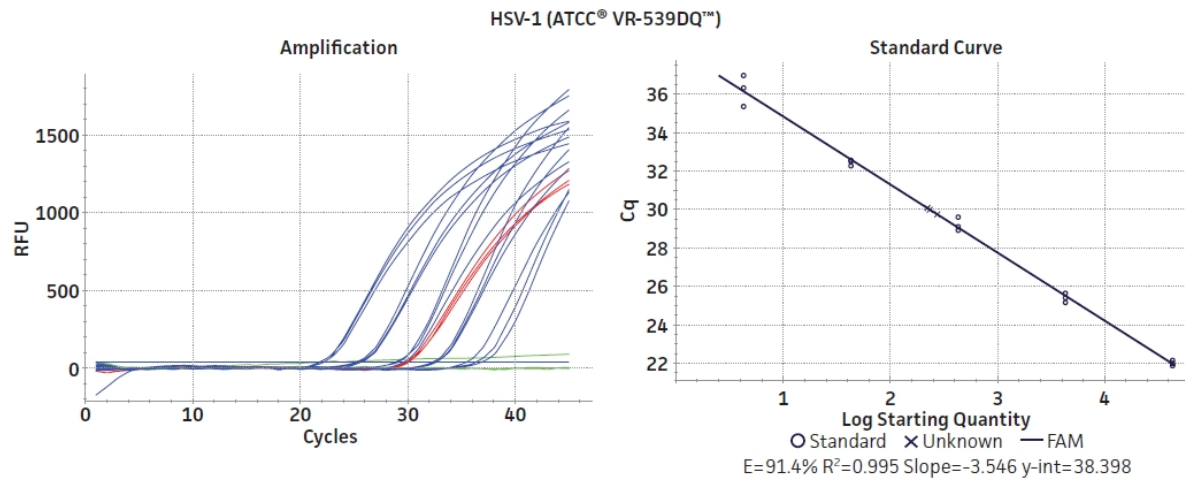


Quantitation of NIBSC Hepatitis standards



- As determined by the WHO:
 - HBV standard = 8.5×10^5 IU/mL
 - HCV standard = 2.6×10^5 IU/mL
- qRT-PCR and qPCR quantitation at ATCC:
 - HBV: 9.7×10^6 genome copies/mL
 - HCV: 1.6×10^7 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

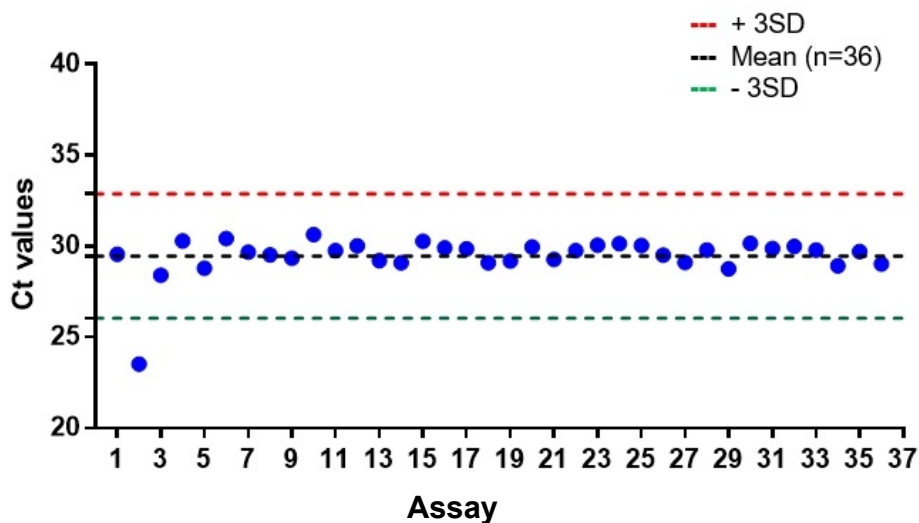
Human herpes viruses



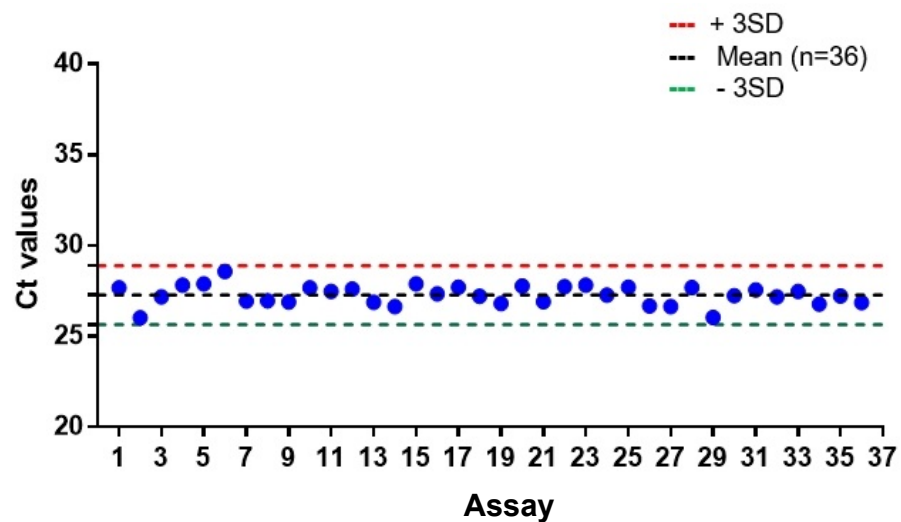
Ryncarz AJ, *et al.* Development of a high-throughput quantitative assay for detecting herpes simplex virus DNA in clinical samples. J Clin Microbiol 37(6): 1941-1947, 1999. Pubmed: 10325351

Human herpes viruses

HSV-1 (ATCC® VR-539D™)



HSV-2 (ATCC® VR-540D™)



	Average Ct	Standard Deviation	Coefficient of Variation
HSV-1 (ATCC® VR-539D™)	29.46	1.14	3.9%
HSV-2 (ATCC® VR-540D™)	27.27	0.54	2.0%

Other application data and posters

Computational Design of a Synthetic Molecular Standard for Human Parechovirus 3

ATCC Poster # 10

Britany Tang, B.S., Michael Geimer, M.S., Maria Mayda, Ph.D., and Dev Mittar, Ph.D.
ATCC, Manassas, VA 20110

Synthetic Human Parechovirus 3 RNA (ATCC® VR-32605™)

Advantages	Applications
<ul style="list-style-type: none"> Manufactured and authenticated with ISO 15485:2016 compliance BSL-1 reagent No shipping Quantities Stabilized 	<ul style="list-style-type: none"> Generation of a standard curve for quantitative RT-PCR

Development and Evaluation of Quantitative Synthetic and Genomic Molecular Standards for Zika

Helen Christina, M.S., Sujatha Rashid, Ph.D., Dev Mittar, Ph.D.
ATCC, Manassas, VA

Background and Introduction
Early detection of the Zika virus (ZIKV), an emerging mosquito-borne pathogen, is infected people is of paramount importance for patient management and for outbreak control. Currently, one of the most reliable molecular-based methods used for viral detection during outbreaks is RT-PCR. However, the use of genomic RNA standards for quantitative RT-PCR assays is limited. In this study, we designed and synthesized a synthetic ZIKV RNA standard for use in quantitative RT-PCR assays. The synthetic RNA standard was used to generate a standard curve for quantitative RT-PCR assays. The synthetic RNA standard was used to generate a standard curve for quantitative RT-PCR assays. The synthetic RNA standard was used to generate a standard curve for quantitative RT-PCR assays.

Development of Synthetic Molecular Standards for Dengue Virus

Shamalia Ashraf, Melissa Wilson, Afshin Sohrabi, Stephen King, Brian Chase, Dev Mittar, Kurt Langenbach and Andrew G. Carvithon
Poster # 1272

Background & Introduction
Dengue fever is an acute illness caused by any one of four serotypes (1-4) of genetically related dengue viruses (DENV), with an estimated 500 million cases reported annually. Currently, quantitative RT-PCR (qRT-PCR) is the preferred method for the detection and quantification of DENV in clinical diagnosis and epidemiological surveillance. The accuracy of a qRT-PCR assay relies on the generation of a standard curve using a positive control with a known viral genome concentration. Native DENV RNA can be used as a standard for these assays; however, the full-length dengue viral RNA is on the Government Control List and requires a permit from the US Department of Commerce for international shipment. To make DENV RNA standards more accessible, ATCC has developed four synthetic molecular standards that represent DENV serotypes 1-4. Each standard contains short fragments from the capsid, membrane, and envelope genes of the DENV genome, as well as unique regions encoding the primer sequences from commercial qRT-PCR assays. Including the DENV-1 4 Real-Time RT-PCR Assay developed by the CDC. The synthetic RNA standards were synthesized by Original Clone™ PCR in order to package precise copies of RNA. Synthetic genes are inserted into a plasmid vector. A transcription start site was added to the synthetic RNA preparation. As compared to native RNA, these synthetic standards are easier to use as controls for qRT-PCR assays, which less variability, have a longer shelf life, eliminate the need to culture viruses and can be used under BSL-1 conditions. Further, this synthetic quantitative RNA approach can be extended to other pathogenic viruses which are unculturable or need to be grown in a high-containment facility.

ATCC Design Strategy

Generation of Standard Curves from DENV Synthetic Molecular Standards

Method/Assay	Slope	Y-Intercept	R-squared
qRT-PCR Assay	-3.344	-0.977	0.942
qRT-PCR Assay	-3.358	-0.938	0.938
Published Assay	-3.352	-0.937	0.938

Quantification of Native DENV-1, 2 and 4 Using Synthetic Molecular Standards

References

- CDC. DENV-1-4 Real-Time RT-PCR Assay for Detection and Serotype Identification of Dengue Virus. Instructions for Use Package Insert. <http://www.cdc.gov/dpdx/resources/dengue/rtPCR/assay.html>
- Hagman et al. Single-reaction, multiplex, real-time RT-PCR for the detection, quantitation, and serotyping of dengue viruses. *PLoS Negl Trop Dis* 7(4): e2116, 2013.

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- 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

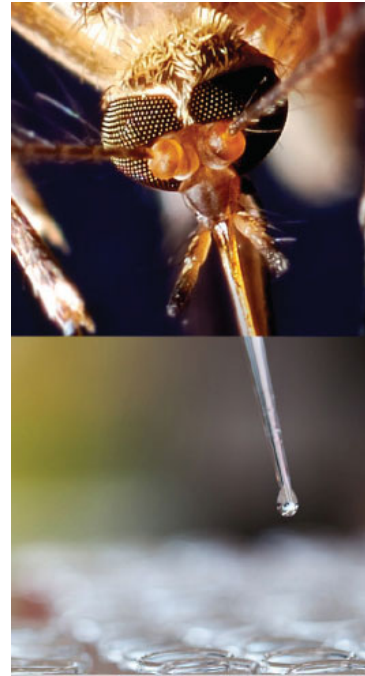
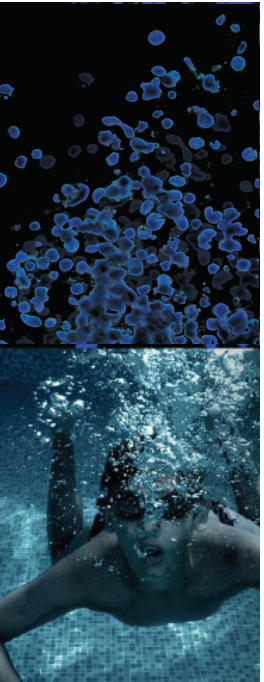
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- **On the edge of the bubble: Use of exosomes as reference materials in biomedical research** October 31, 12:00 ET

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