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

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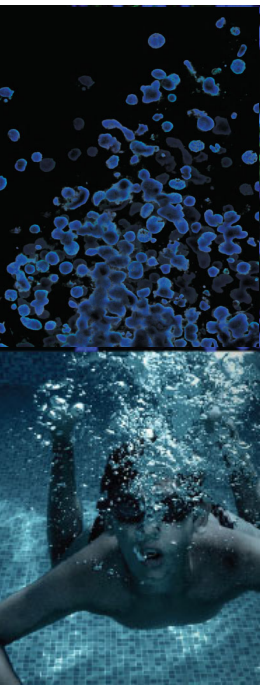
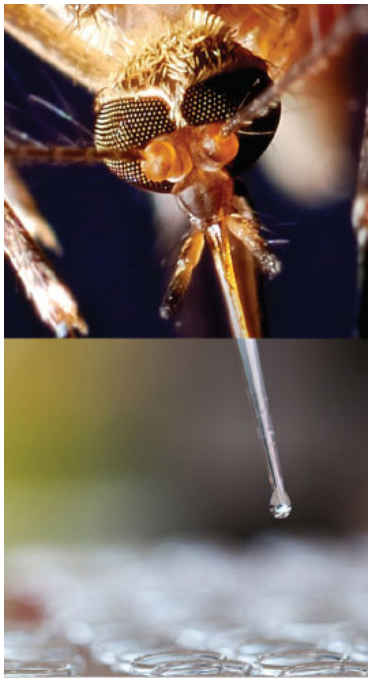
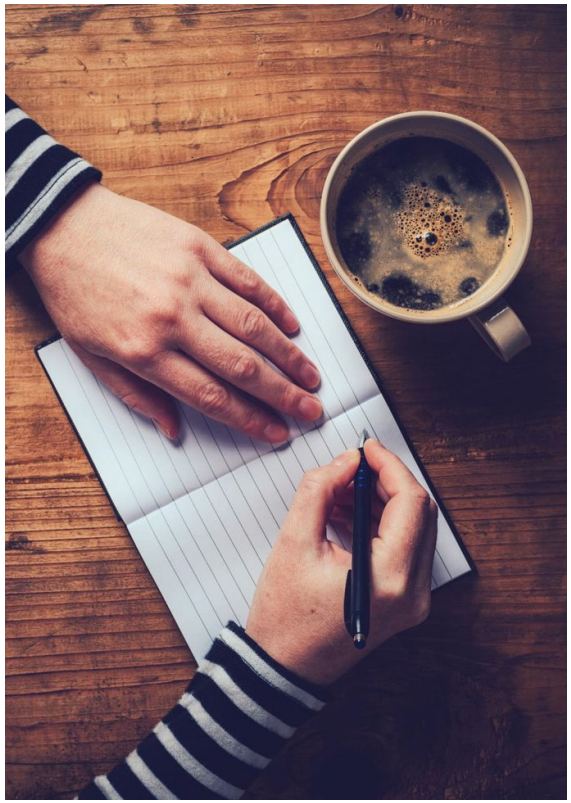
Credible Leads to Incredible™





Agenda

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

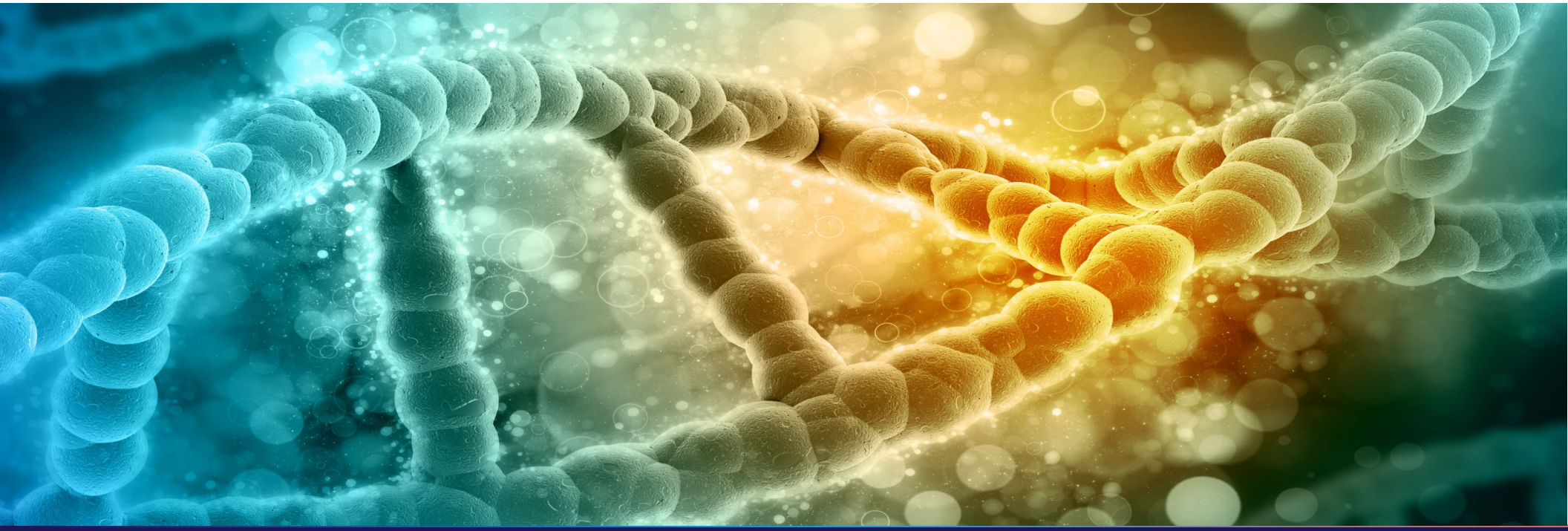


About ATCC

- 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

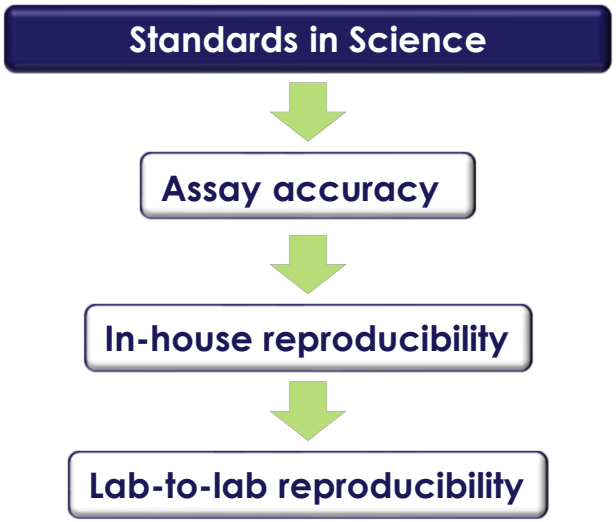
- ATCC collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Growing portfolio of products and services
- Sales and distribution in 150 countries, 15 international distributors
- Talented team of 450+ employees, over one-third with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

ATCC®

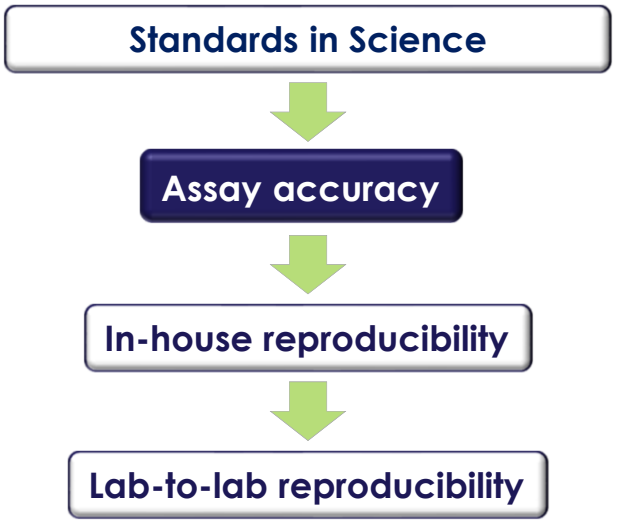


Molecular Standards

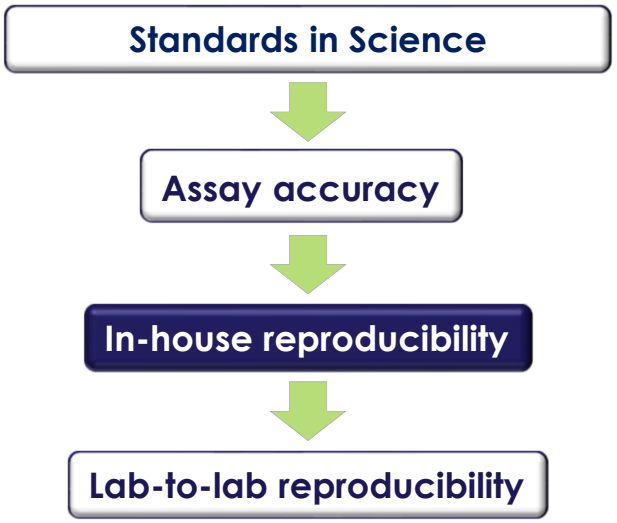
ATCC molecular standards



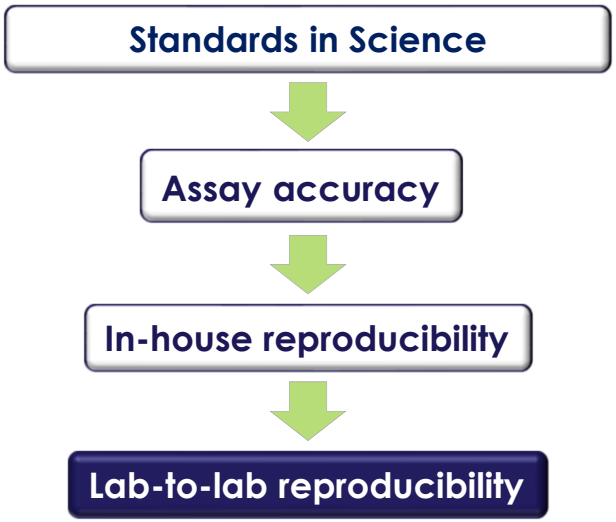
ATCC molecular standards



ATCC molecular standards



ATCC molecular standards



Project history

A culture collection provides good value for research.

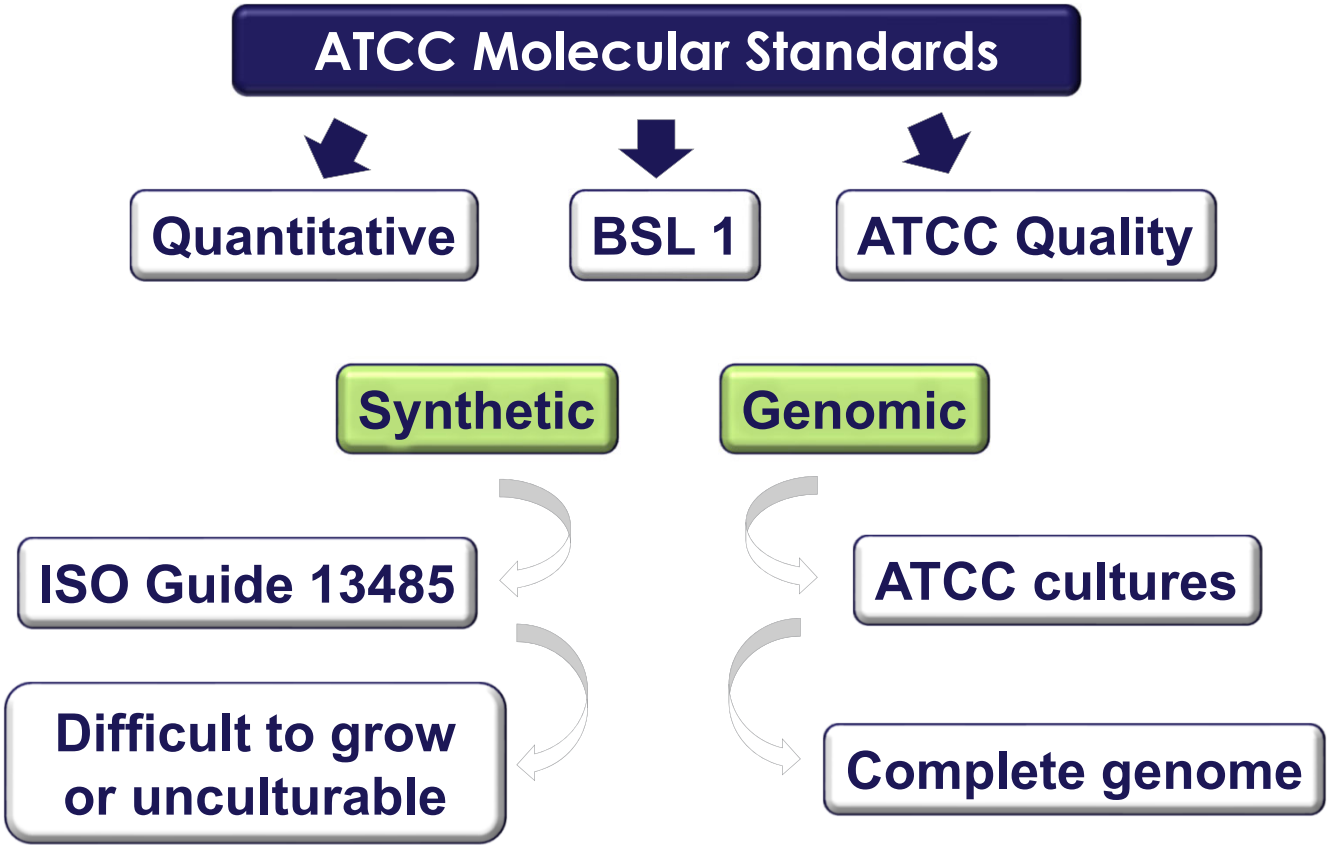
A standards organization with a culture collection provides even more!

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.

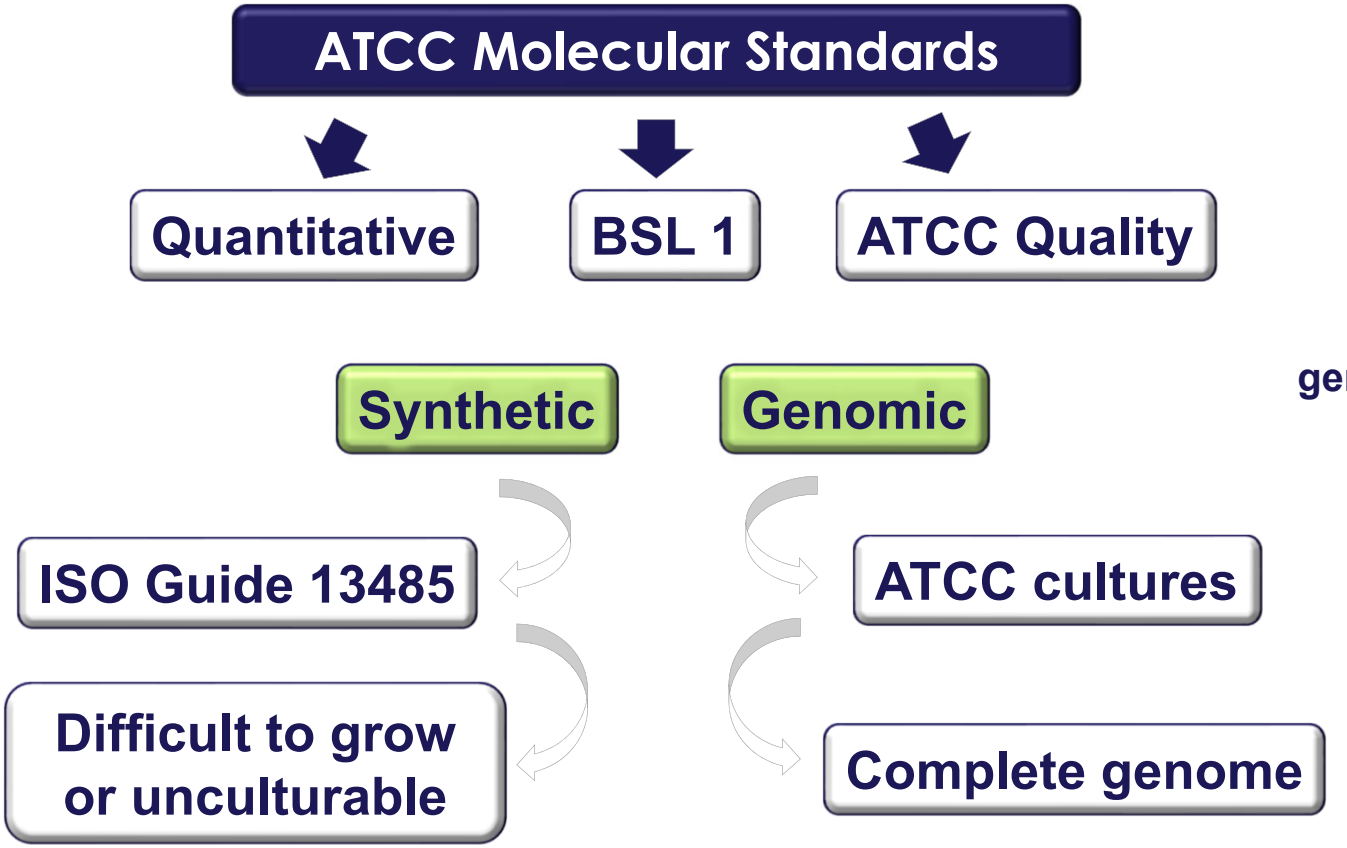
Those standards launched in May of 2013:

- 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



genomes.atcc.org

Specifications

Synthetic Standards

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

Genomic Standards

Authentication	Amplicon sequencing
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
Fill Volume	100 µL per vial
Format	Frozen
Certifications	ISO 9001, ISO 17025

ATCC®

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

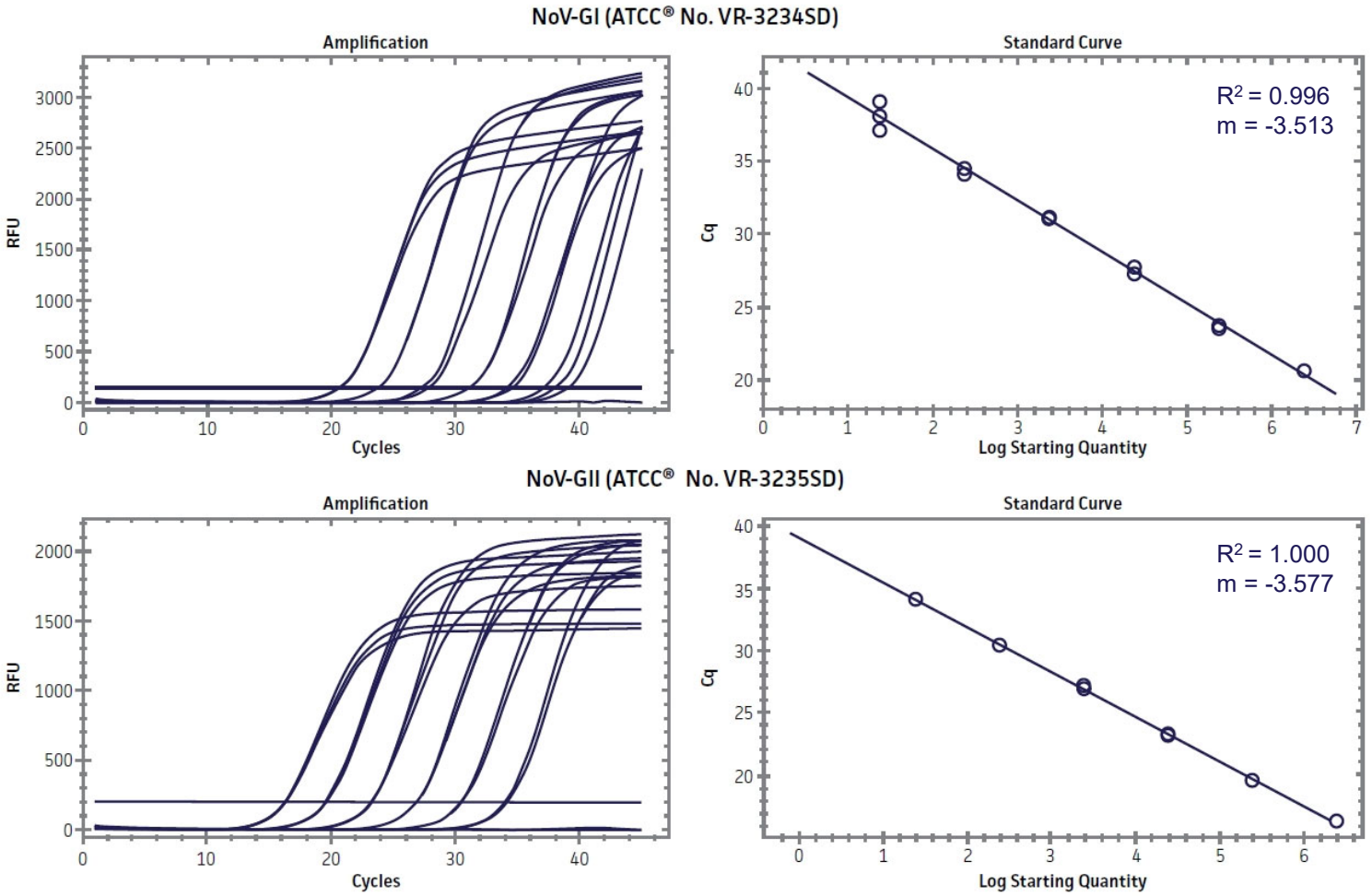
Added ISO 13485 compliance

Versatility

Added RdRp fragments to construct

ATCC

Validating the next generation of standards

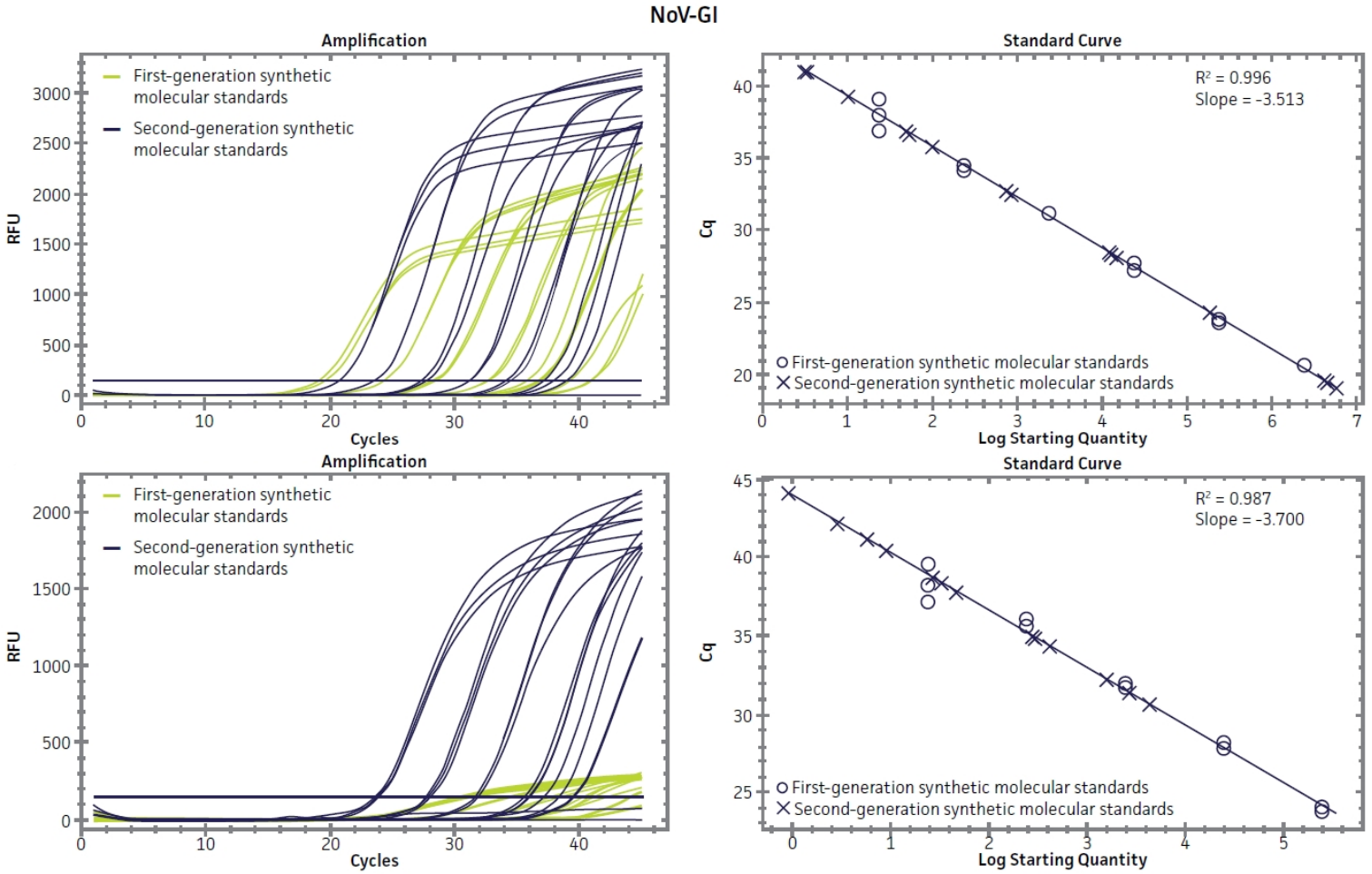


Pictured: Standard curves generated with CaliciNet primer and probe set.

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

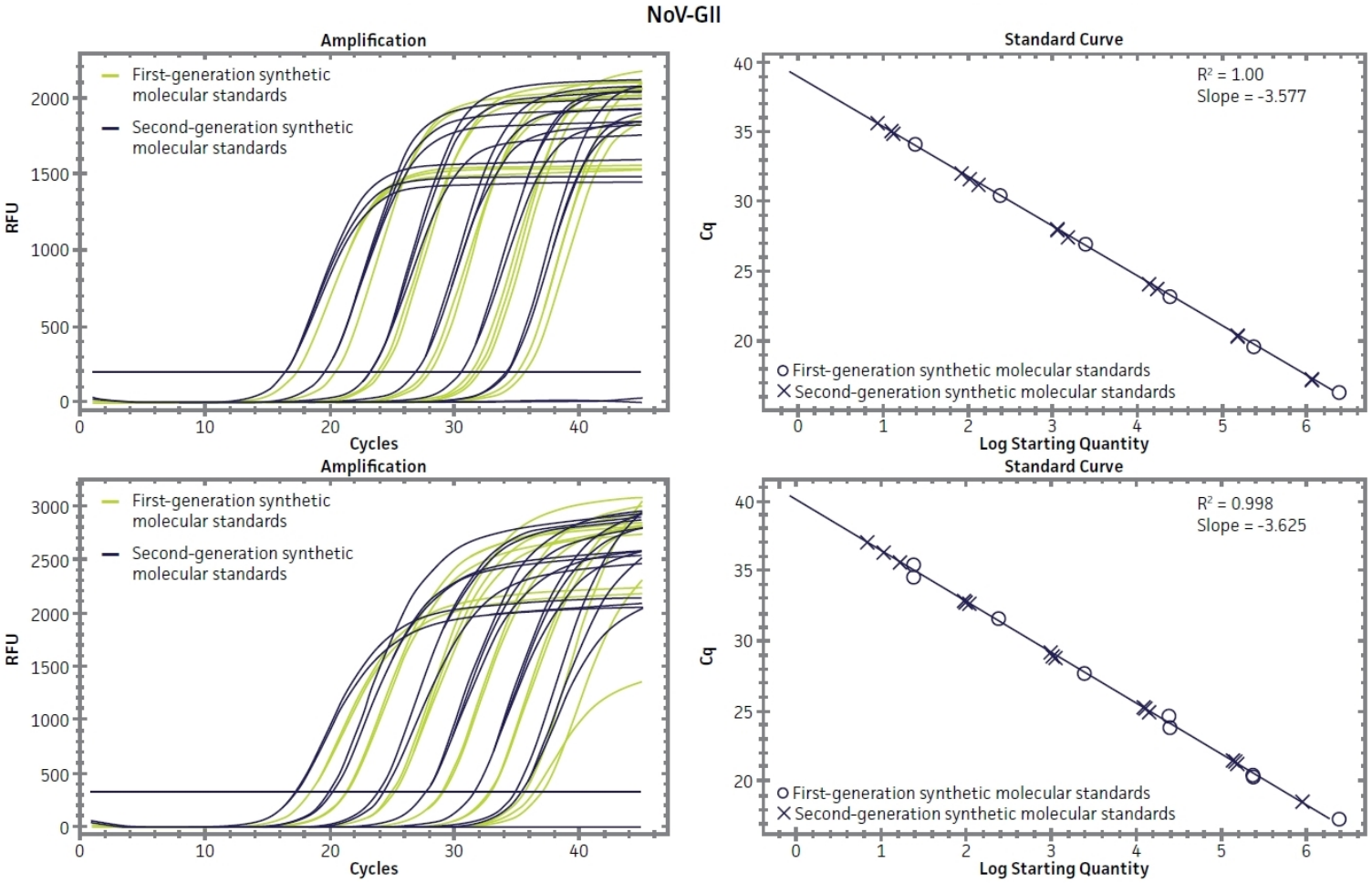
Old vs. new standards, part 1



CaliciNet primers & probe

ECS working group primers & probe

Old vs. new standards, part 2

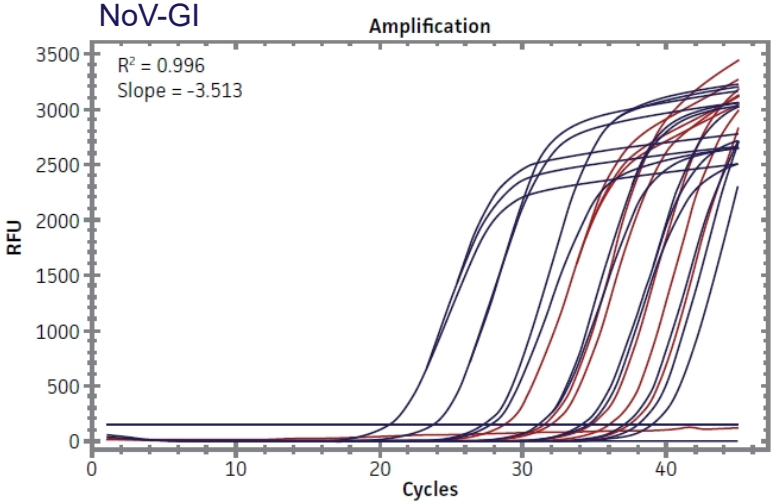


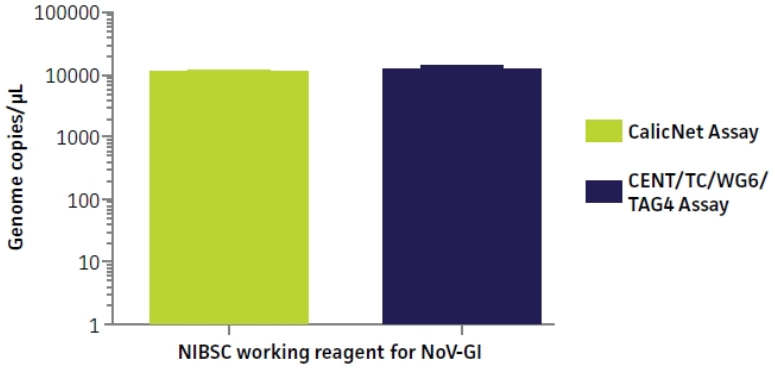
CaliciNet
primers & probe

ECS
primers & probe

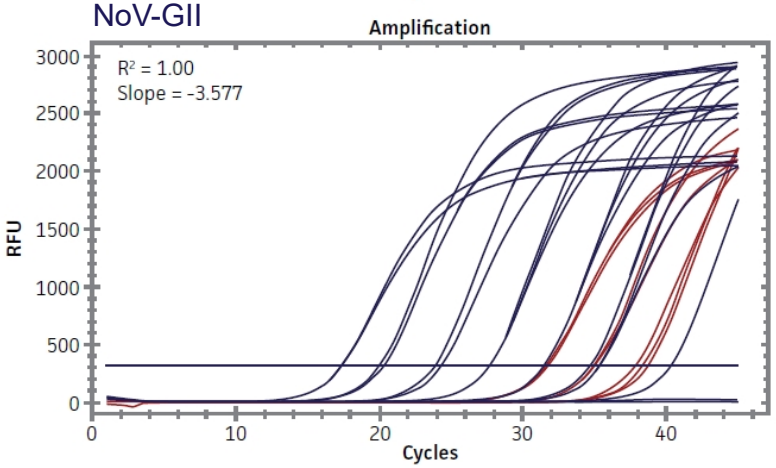
A test drive with NIBSC working reagents

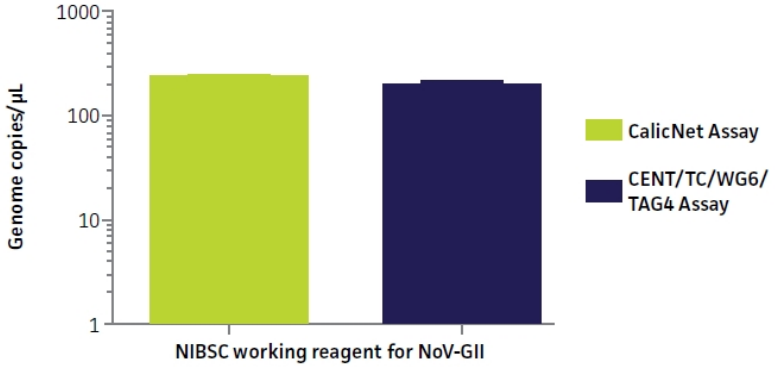
NoV-GI



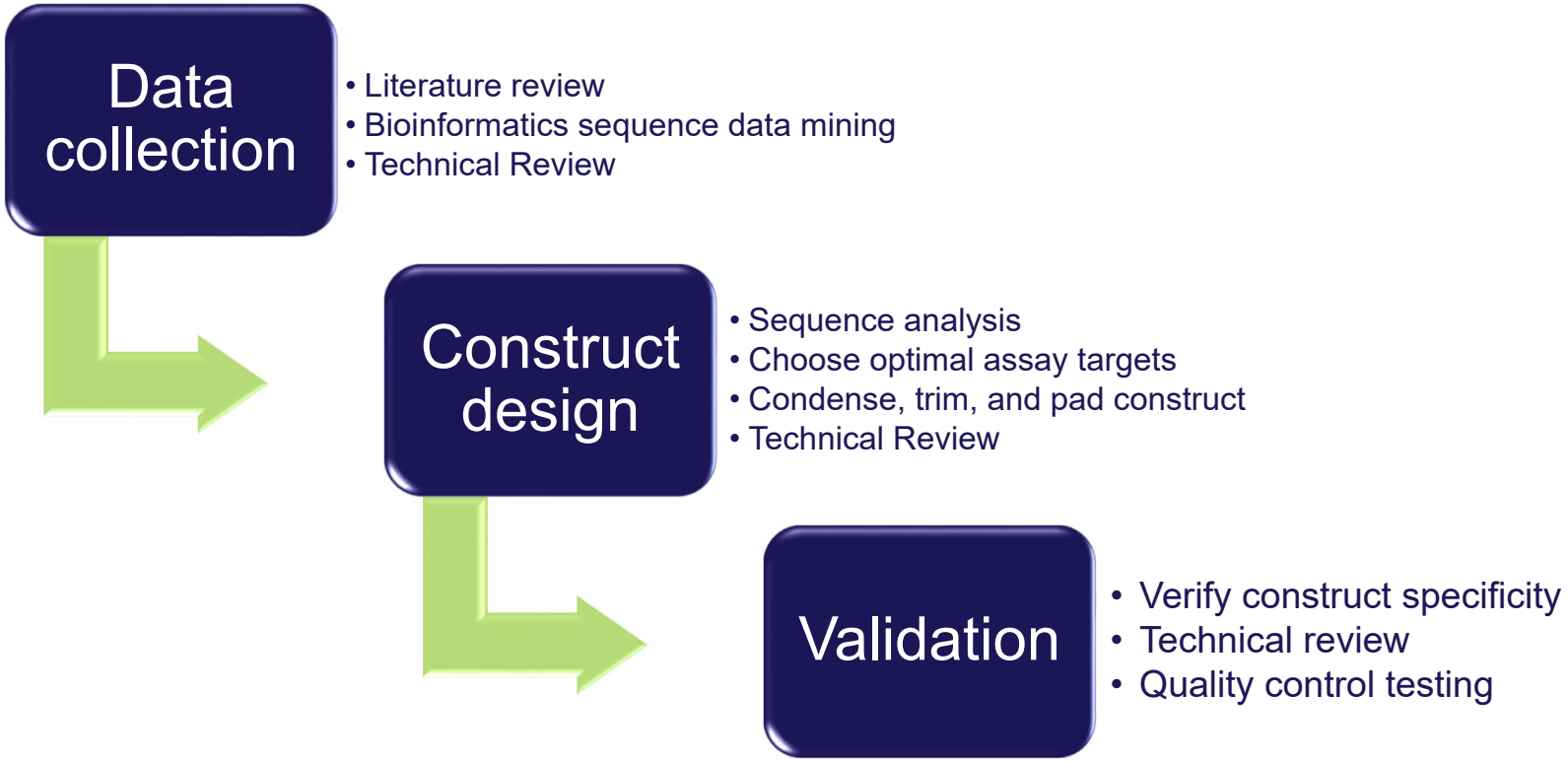


NoV-GII





Design approach – synthetic standards



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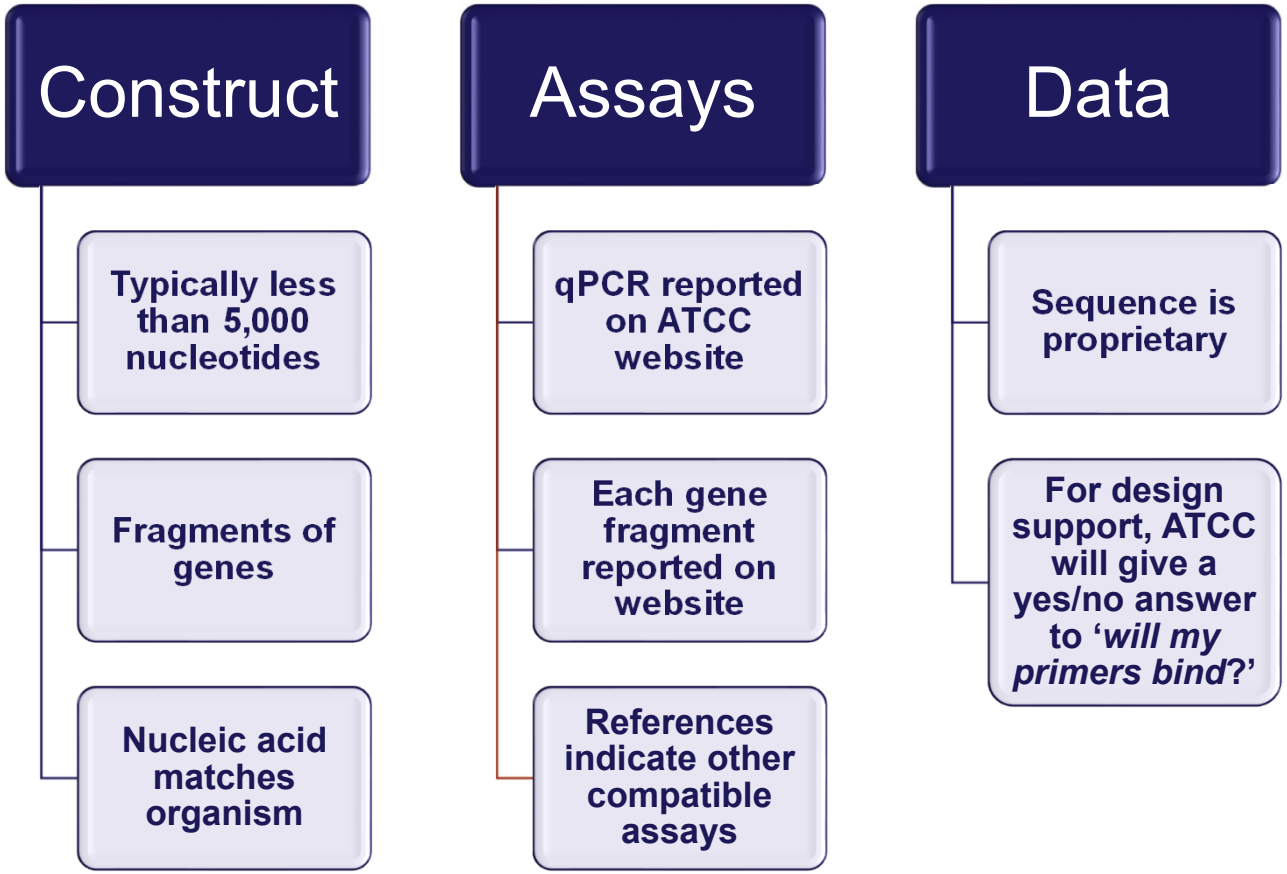
Design approach – synthetic standards

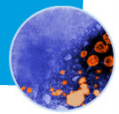


ATCC's Molecular Standards

Pathogen standards

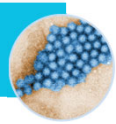
www.atcc.org/MolecularStandards

Blood-borne disease



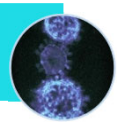
- BK virus
- Hepatitis B virus
- Hepatitis C virus
- Epstein-Barr virus
- Human immunodeficiency virus 1
- Human T-cell leukemia virus
- Human cytomegalovirus
- Varicella-zoster virus
- *Neisseria meningitides*
- *Plasmodium malariae*
- *Human parechovirus 3*

Gastro-Intestinal disease



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

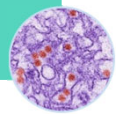


- Human bocavirus
- Human coronavirus HKU1
- Human coronavirus NL63
- Human coronavirus 229E
- Middle East respiratory syndrome coronavirus
- Human metapneumovirus
- *Bordetella pertussis*
- *Mycobacterium africanum*
- *Mycobacterium bovis*
- *Mycobacterium talmoniae*
- *Mycobacterium microti*
- *Mycobacterium pinnipedii*
- *Mycobacterium tuberculosis*
- *Streptococcus pneumonia*
- 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 influenza*
- *Streptococcus pneumoniae*

Sexually transmitted infections

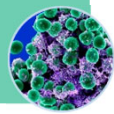
- Hepatitis B virus
- Human immunodeficiency virus 1
- Human papillomavirus 16
- Human papillomavirus 18
- Human papillomavirus 31
- Human T-cell leukemia virus 2
- *Treponema pallidum*
- *Chlamydia trachomatis* serovar I
- *Chlamydia trachomatis* serovar II
- *Chlamydia trachomatis* serovar III
- Human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

Vector-borne disease



- 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

Epidermal & Nosocomial disease



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

ATCC

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*

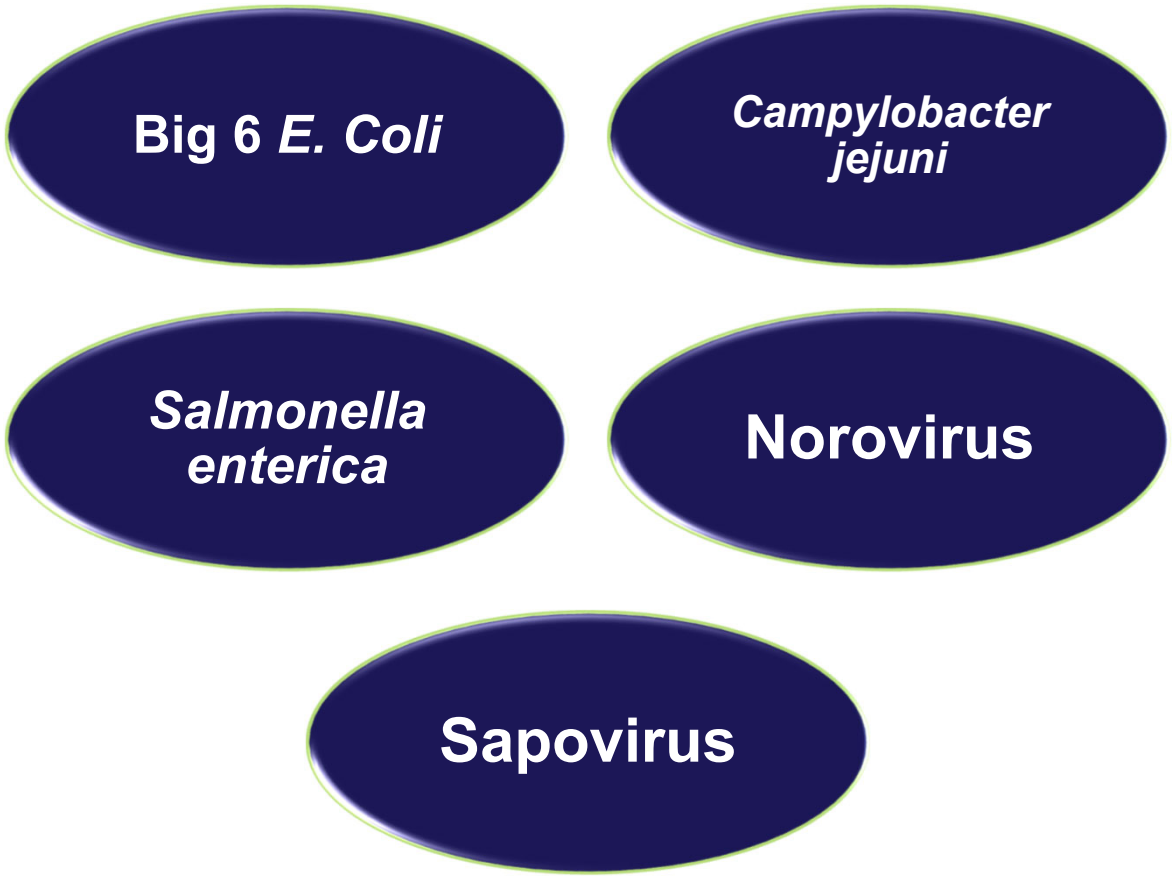
ATCC

Water safety standards

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

ATCC

Food safety standards



Designation	ATCC Item Number
Norovirus GI	VR-3234SD
Norovirus GII	VR-3235SD
Murine norovirus	VR-2355SD
E. coli O145	BAA-2192DQ
E. coli O45:H2	BAA-2193DQ
E. coli O26:H11	BAA-2196DQ
E. coli O103:H11	BAA-2215DQ
E. coli O121:H19	BAA-2219DQ
E. coli O111	BAA-2440DQ
C. jejuni	700819DQ
S. enterica	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 *Trichomonas vaginalis*	Causative agent of trichomoniasis.
VR-1360DQ	Quantitative Genomic DNA from *Chlamydophila pneumoniae*	A causative agent of pneumonia.
27853DQ	Quantitative Genomic DNA from *Pseudomonas aeruginosa*	Quality control strain for numerous applications. Opportunistic pathogen.
15311DQ	Quantitative Genomic DNA from *Bordetella parapertussis*	Causative agent of whooping cough.

ATCC

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 *Proteus vulgaris*
VR-1826DQ	Quantitative Genomic RNA from Enterovirus D68
MYA-2876DQ	Quantitative Genomic DNA from *Candida albicans*
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 *Saccharomyces cerevisiae*

ATCC®

Validation of Molecular Standards

ATCC®

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**

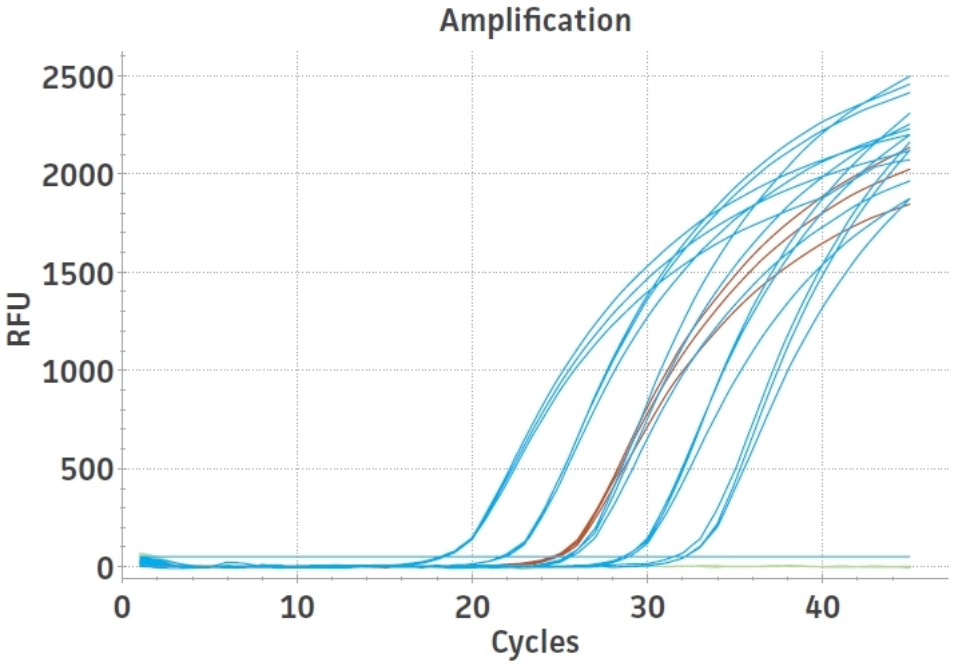
Hepatitis C virus

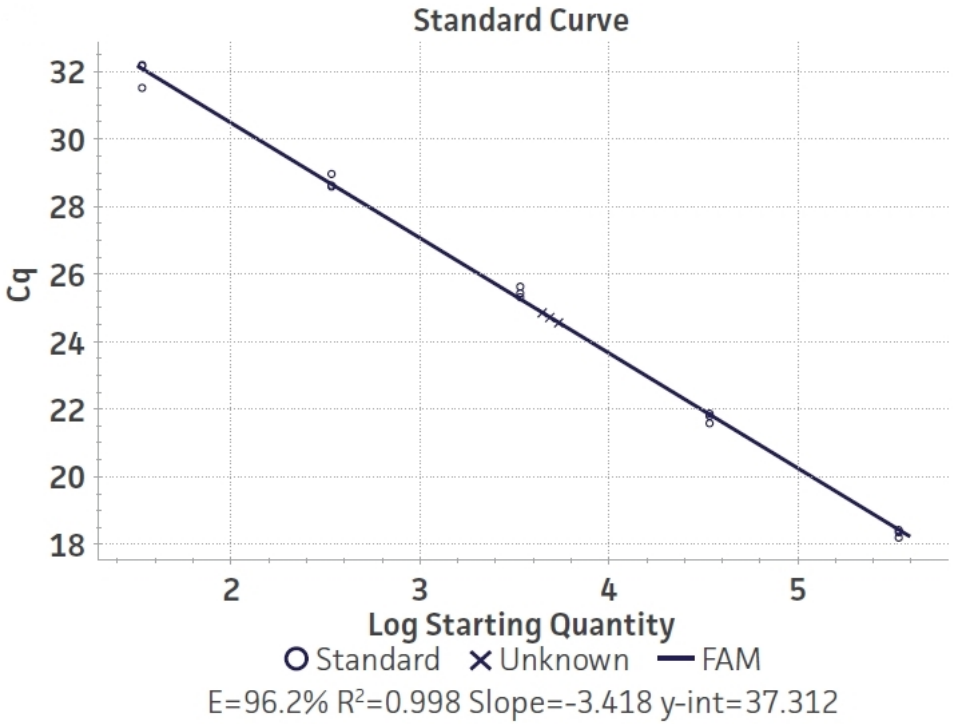
- **VR-3233SD™**
- **Flaviviridae, Hepacivirus**
- **RNA construct [includes RNAstable® (Biomatrica)]**
- **Portions of 5' UTR, and X-tail region (3' UTR)**

- 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.

ATCC

Hepatitis B virus



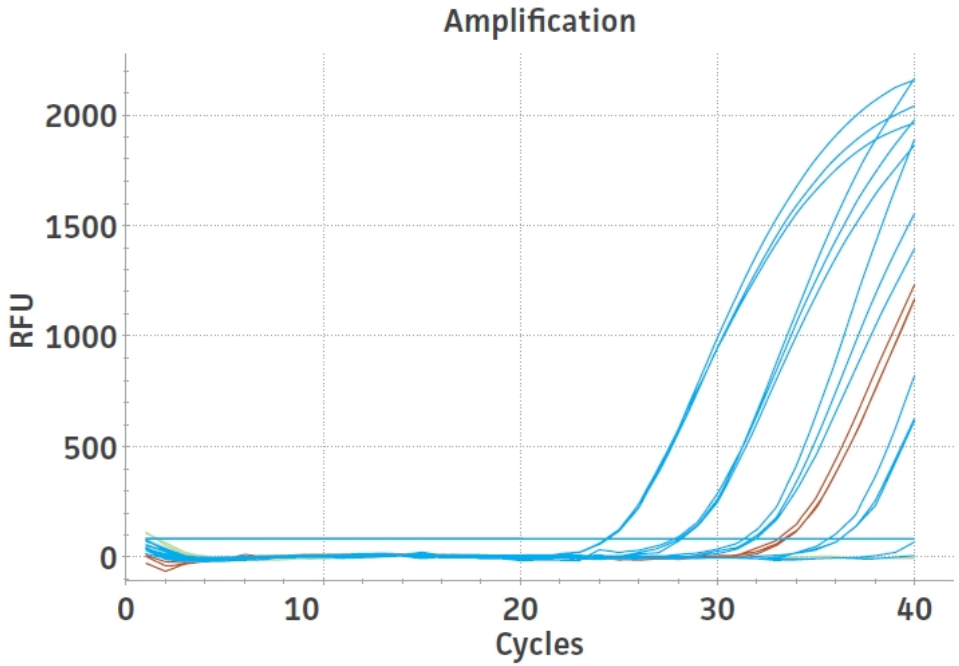


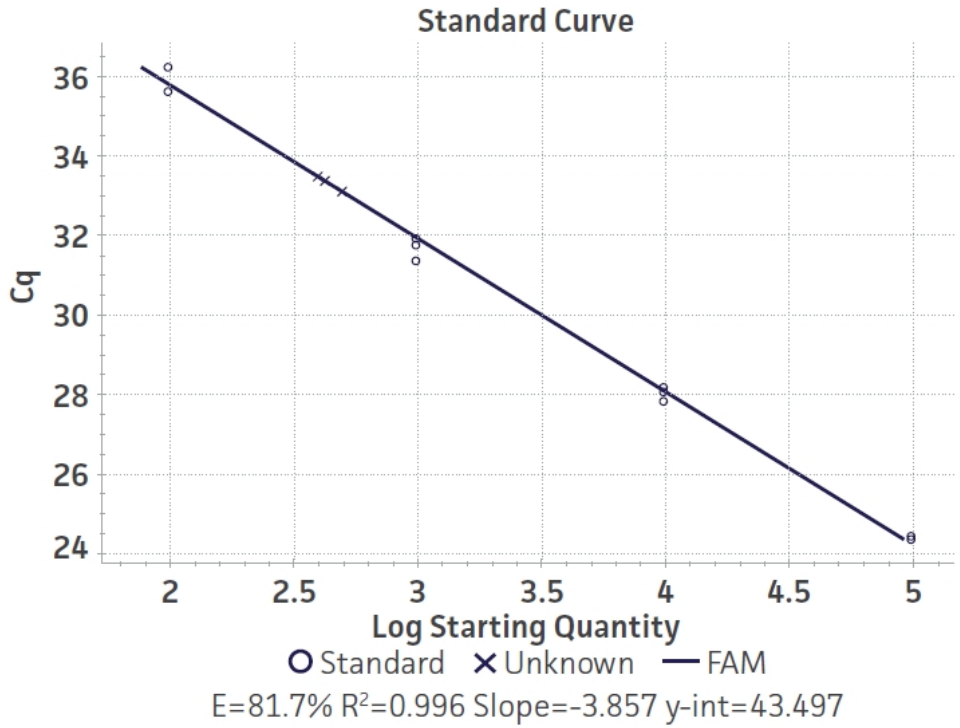
Blue = VR-3232SD™

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. Virol. J. 8: 227, 2011. PubMed: 21569595

Hepatitis C virus





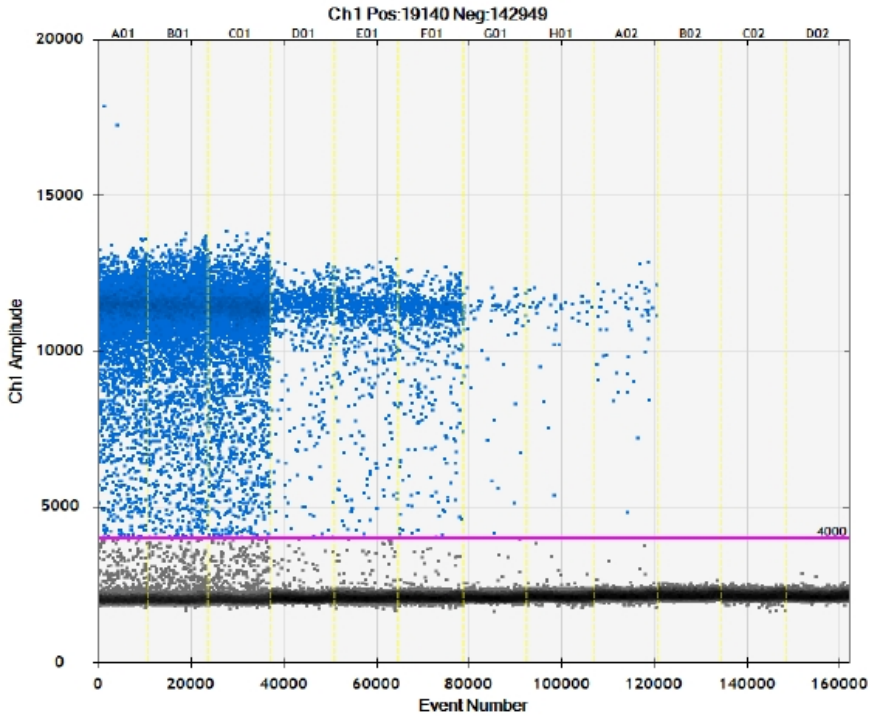
Blue = VR-3233SD™

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

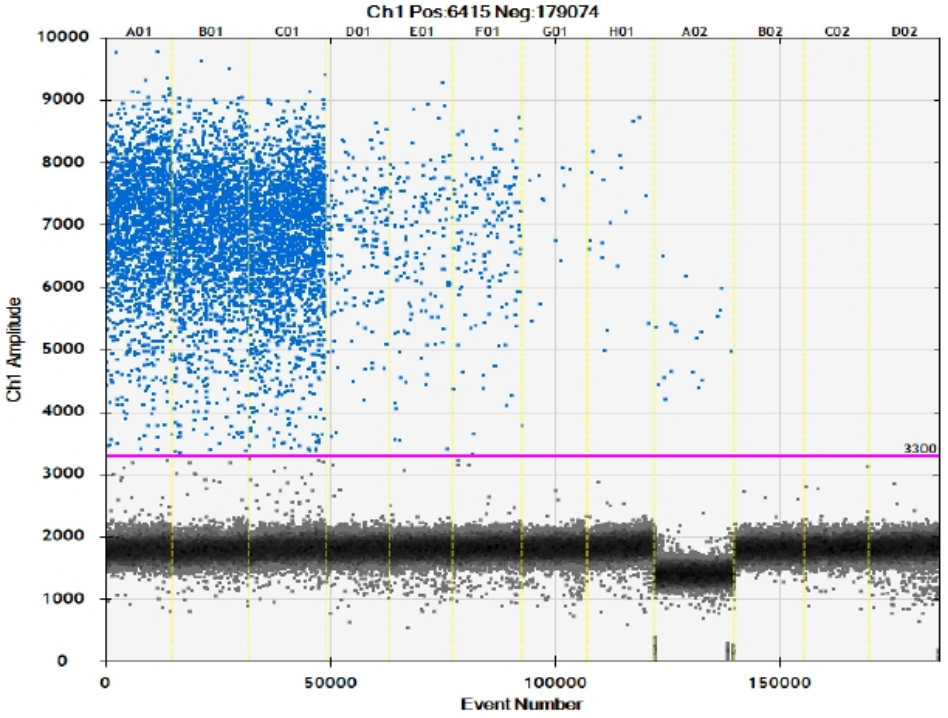
Lee SC, *et al*. Improved version 2.0 qualitative and quantitative AMPLICOR reverse transcription-PCR tests for hepatitis C virus RNA: calibration to international units, enhanced genotype reactivity, and performance characteristics. J. Clin. Microbiol. 38(11): 4171-4179, 2000. PubMed: 11060086

Hepatitis viruses – ddPCR™

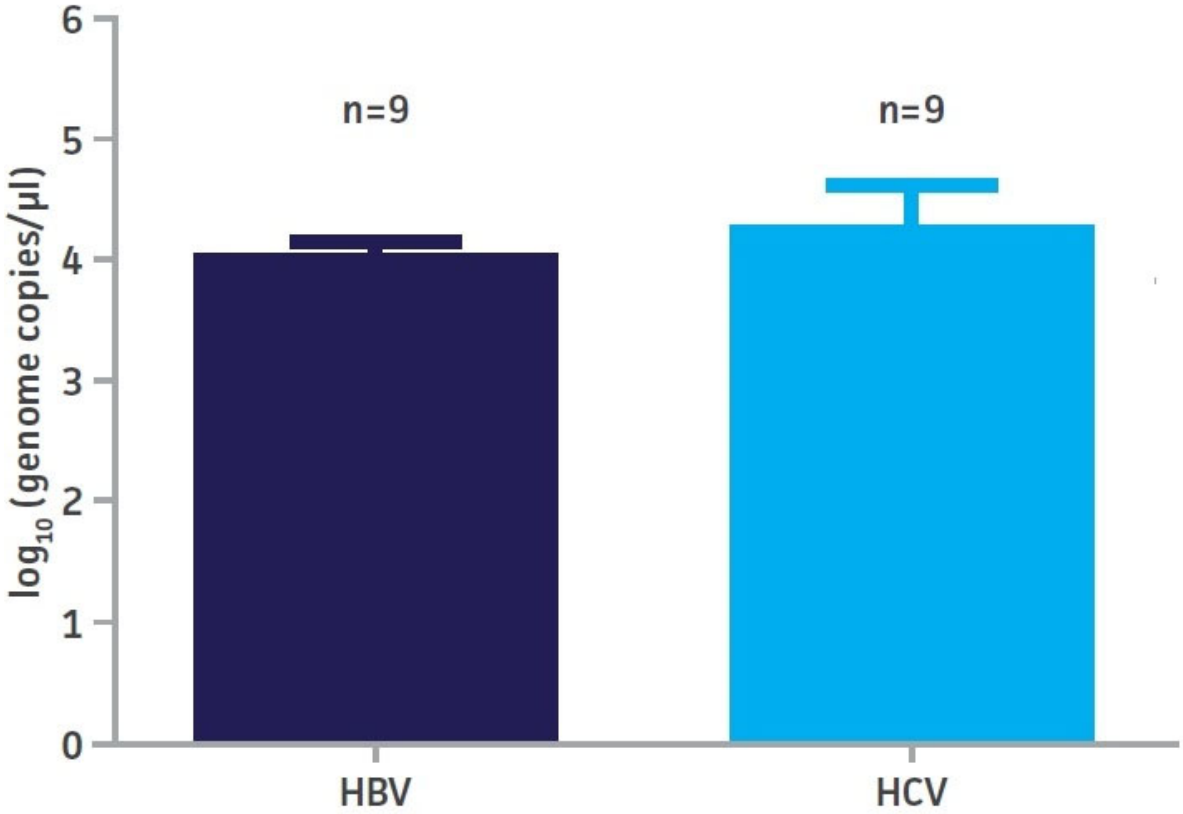
VR-3232SD™ (HBV synthetic standard)



VR-3233SD™ (HCV synthetic standard)



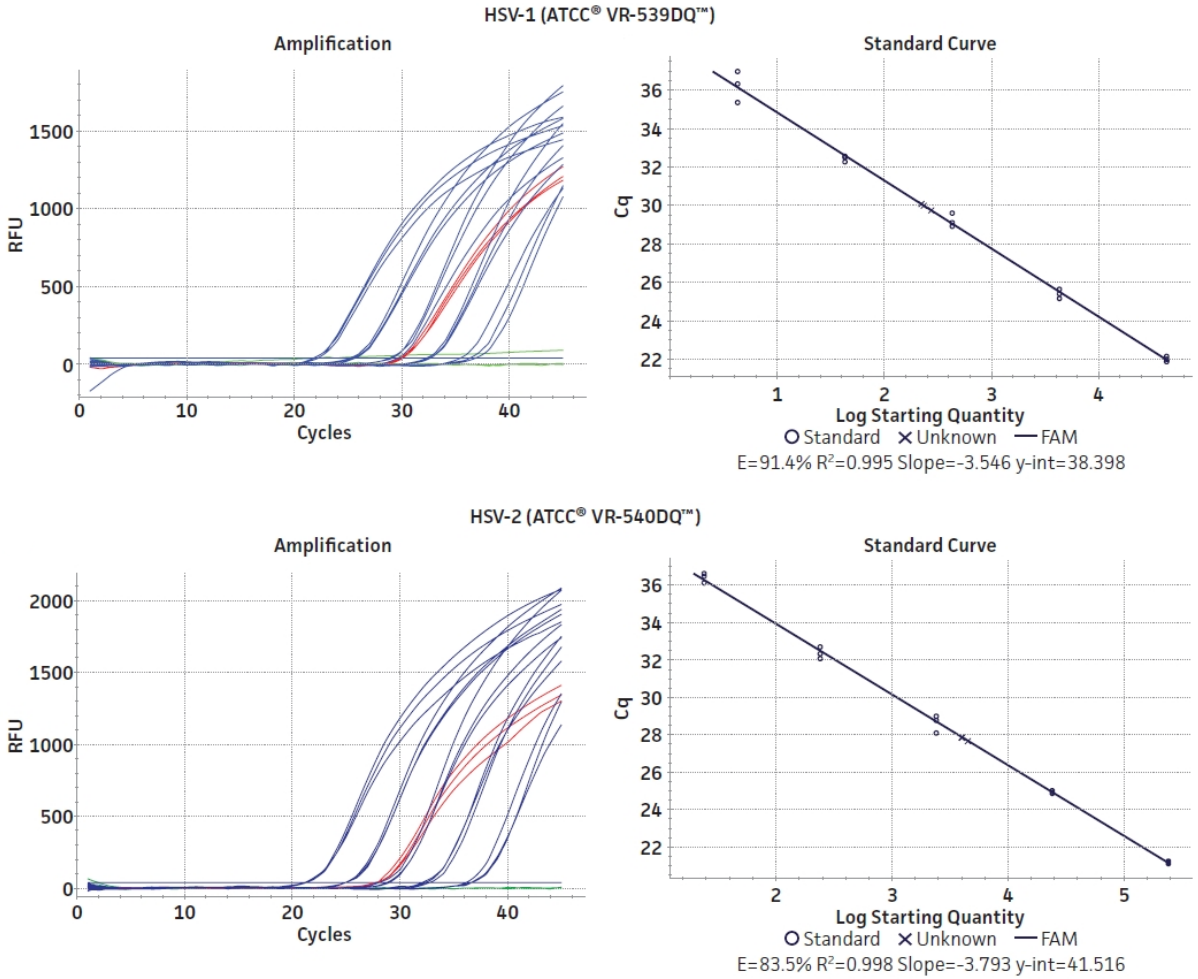
Quantitation of NIBSC Hepatitis standards

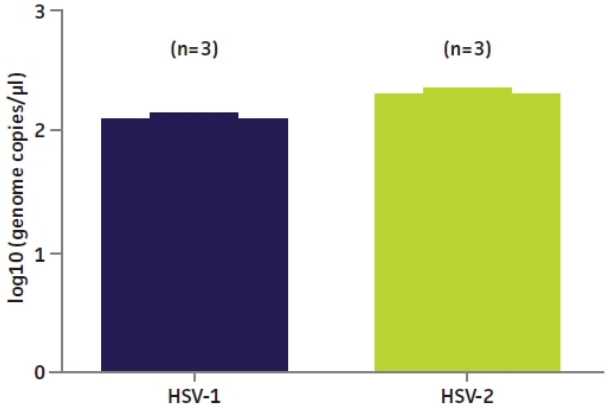


- As determined by the WHO:
  - HBV standard = 8.5 x 10^5 IU/mL
  - HCV standard = 2.6 x 10^5 IU/mL
- qRT-PCR and qPCR quantitation at ATCC:
  - HBV: 9.7 x 10^6 genome copies/mL
  - HCV: 1.6 x 10^7 genome copies/mL
- Conversion ratio as quantified at ATCC:
  - HBV: 1 IU/mL = 11.4 genome copies
  - HCV: 1 IU/mL = 61.5 genome copies

ATCC

Human herpes viruses

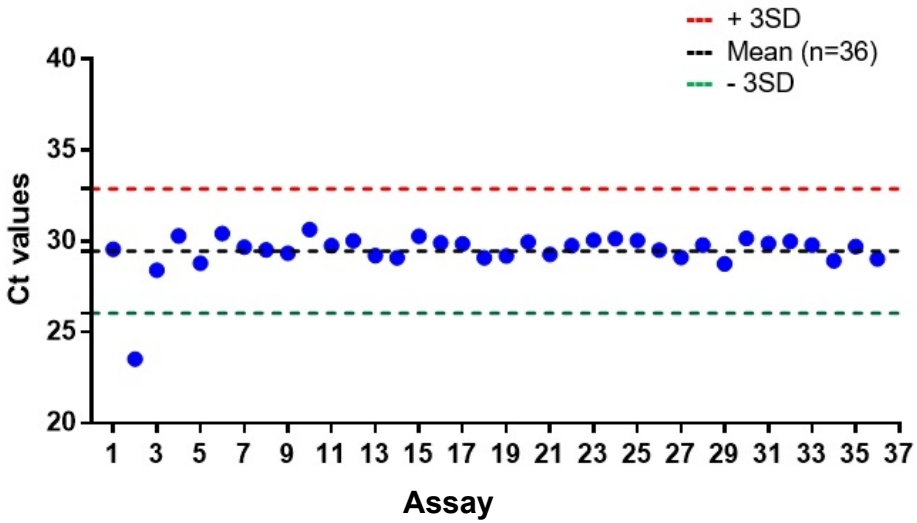




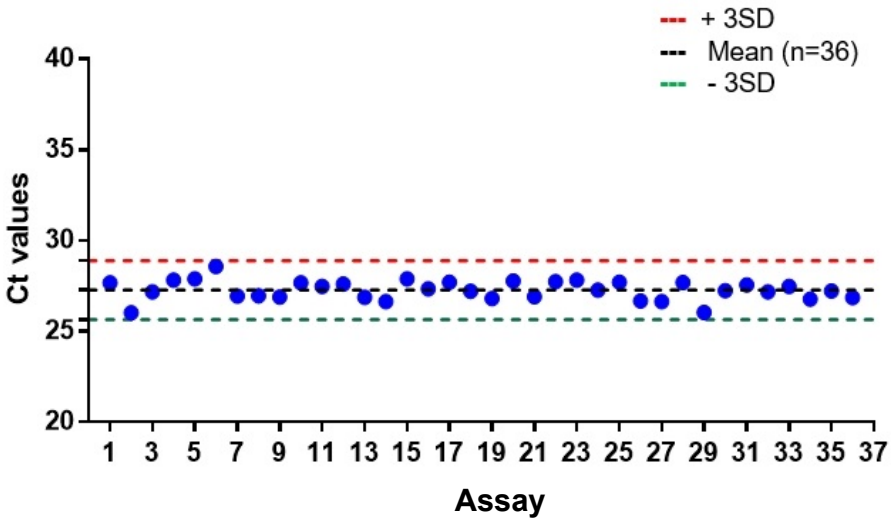
Ryncarz AJ, *et al.* Development of a high-throughput quantitative assay for detecting herpes simplex virus DNA in clinical samples. J Clin Microbiol 37(6): 1941-1947, 1999. Pubmed: 10325351

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

- 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!**

ATCC

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

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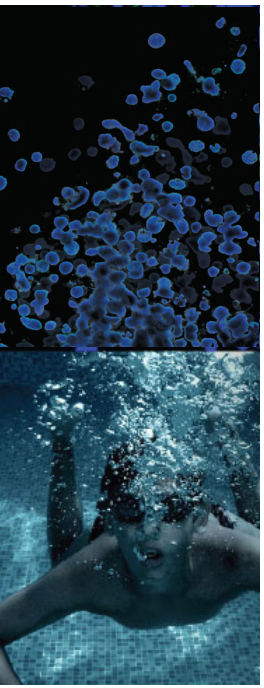
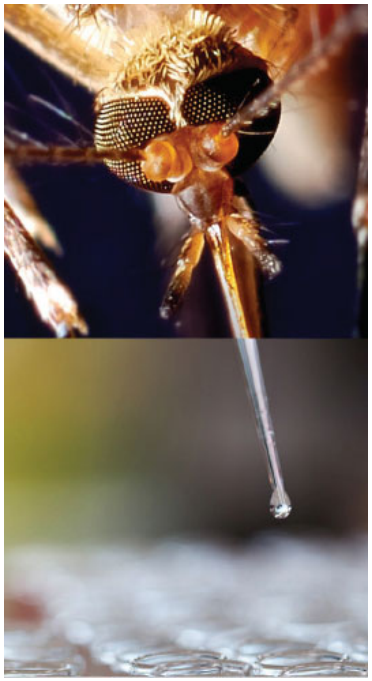


Questions?

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