

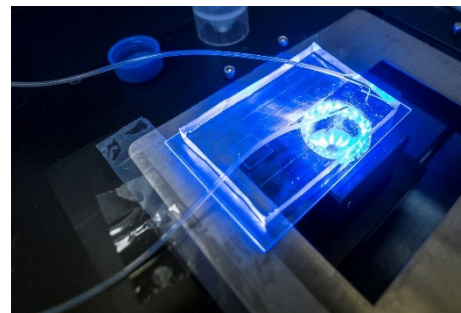
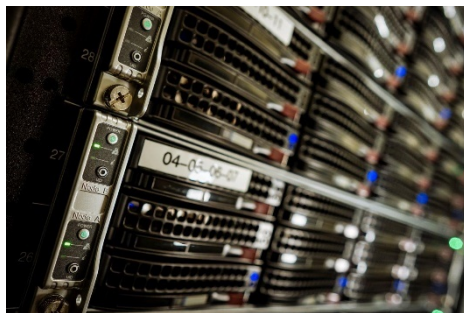
Overview of NIST Viral Standards

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Research Biologist

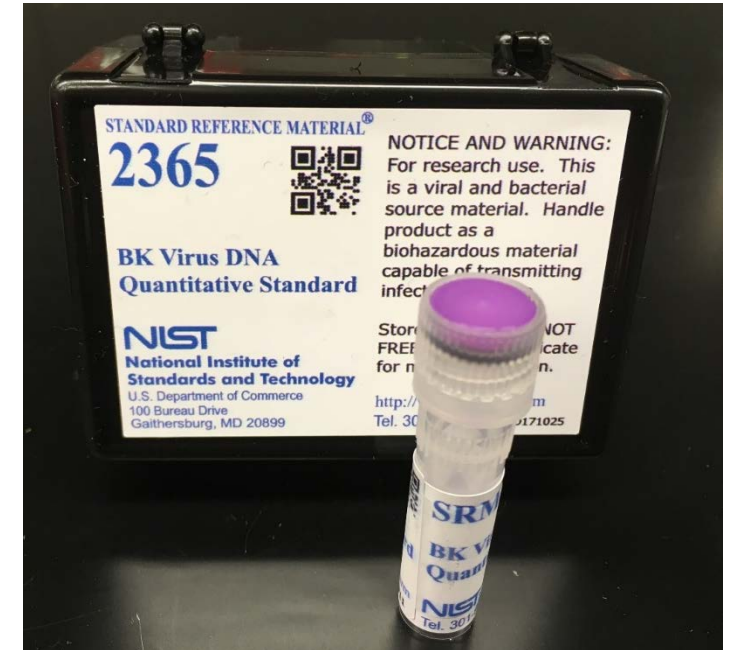
Applied Genetics Group

National Institute of Standards and Technology



NIST

- National Metrological Institute (NMI)
- Founded in 1901
- ~3500 employees
- 2 campuses (Gaithersburg, MD and Boulder, CO)



- Five Nobel prize winners
- NIST supplies over 1,300 Standard Reference Materials (SRMs) for industry, academia, and government use in calibration of measurements

NIST Strategic Priorities



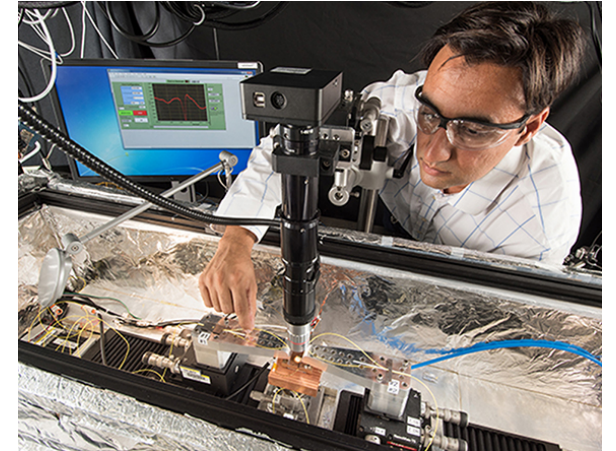
Artificial Intelligence



Bioeconomy



Internet of Things



Quantum Science

Role of Standards

- To help develop accurate methods of analysis
- To *calibrate measurement systems* used to facilitate exchange of goods, institute quality control, determine performance characteristics, or measure a property at the state-of-the-art limit
- To ensure the long-term adequacy and integrity of measurement *quality assurance programs*

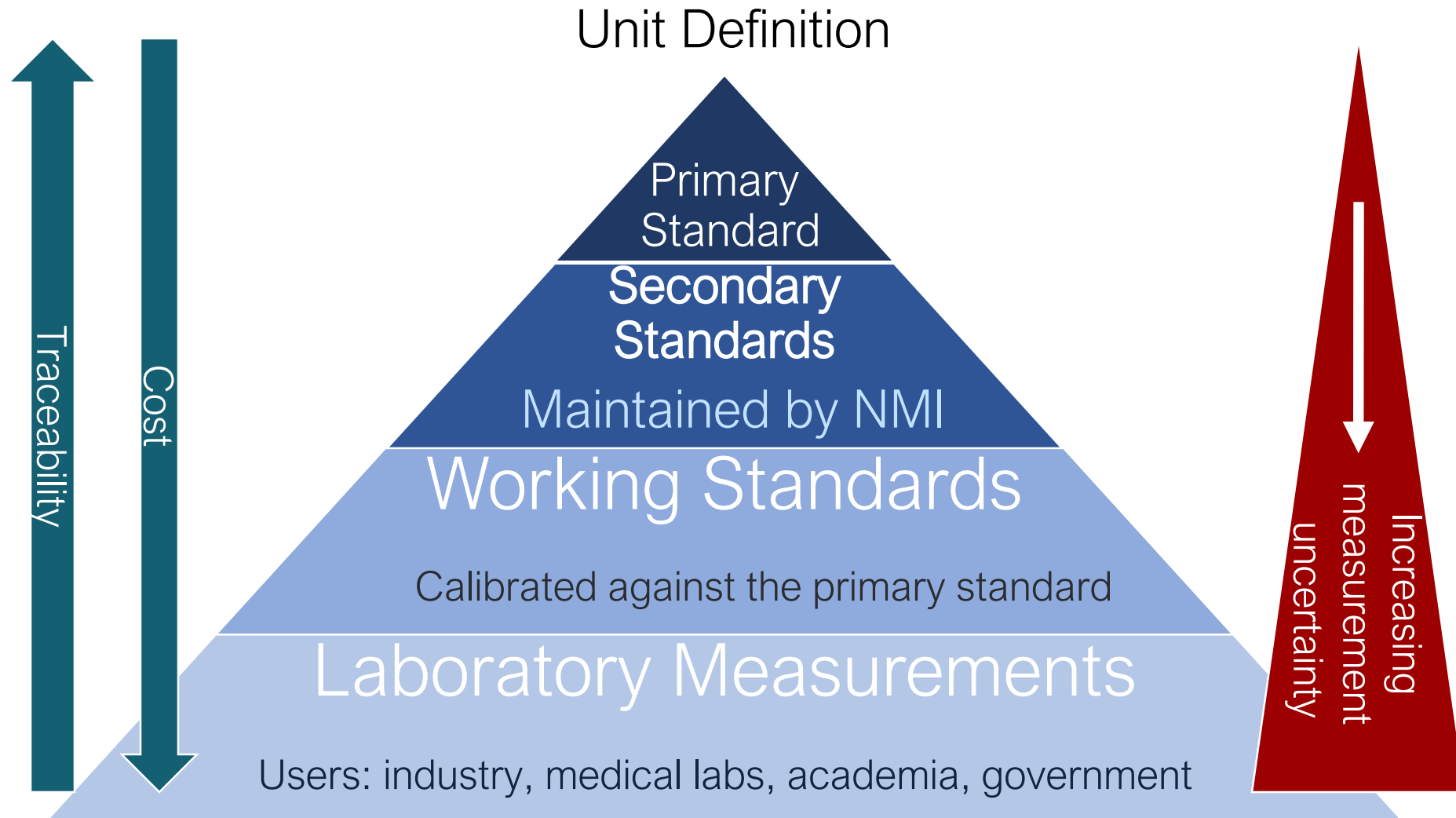
Reference Material (RM)

- A RM is a material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process
- RM is a generic term
- The properties of an RM can be quantitative or qualitative (e.g. identity of substances or species)

Certified Reference Material (CRM)

- NIST name for a CRM is: Standard Reference Material (SRM™)
- *A CRM is a reference material characterized by a **metrologically valid procedure** for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its **associated uncertainty**, and a statement of **metrological traceability***

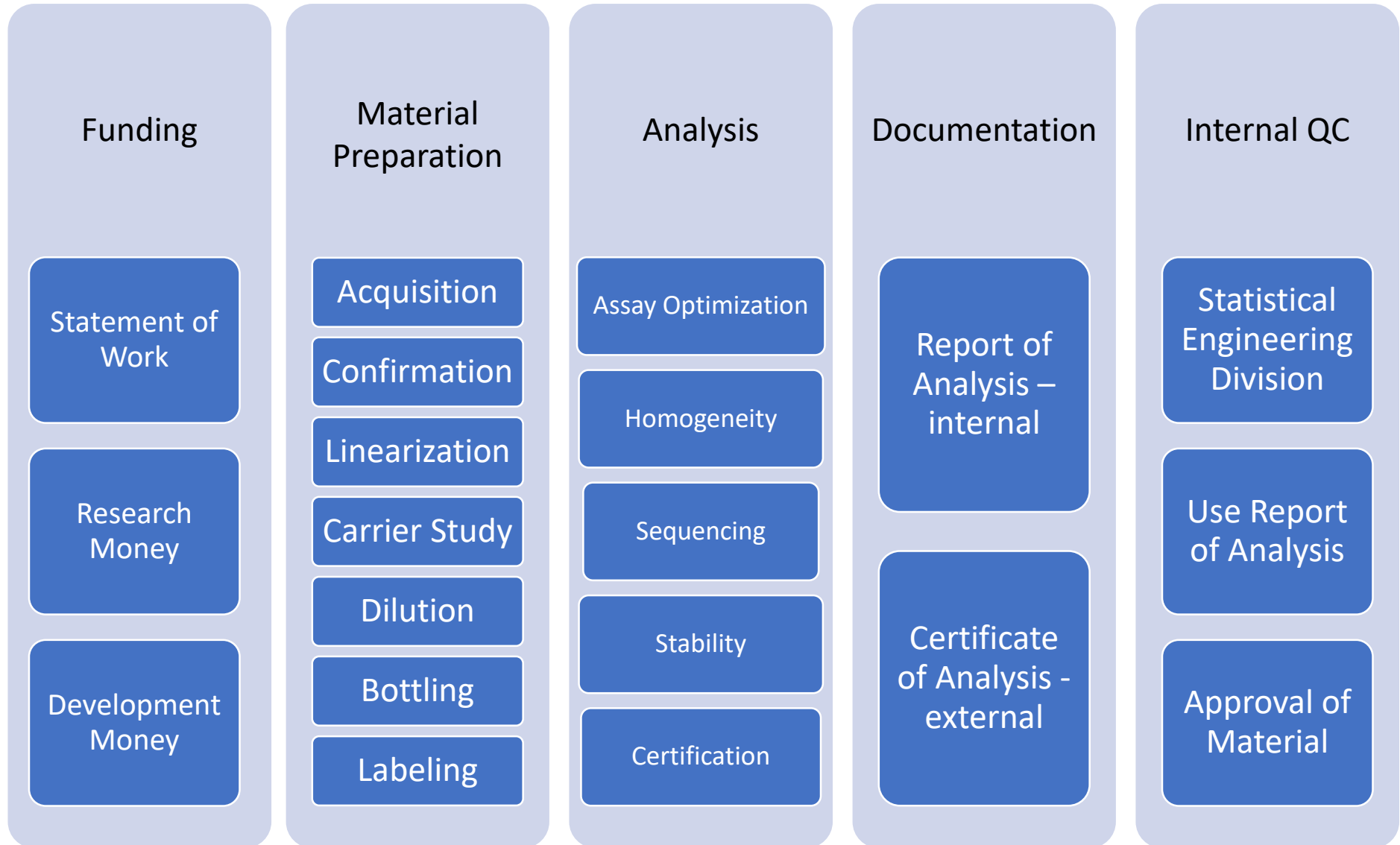
Metrological Traceability



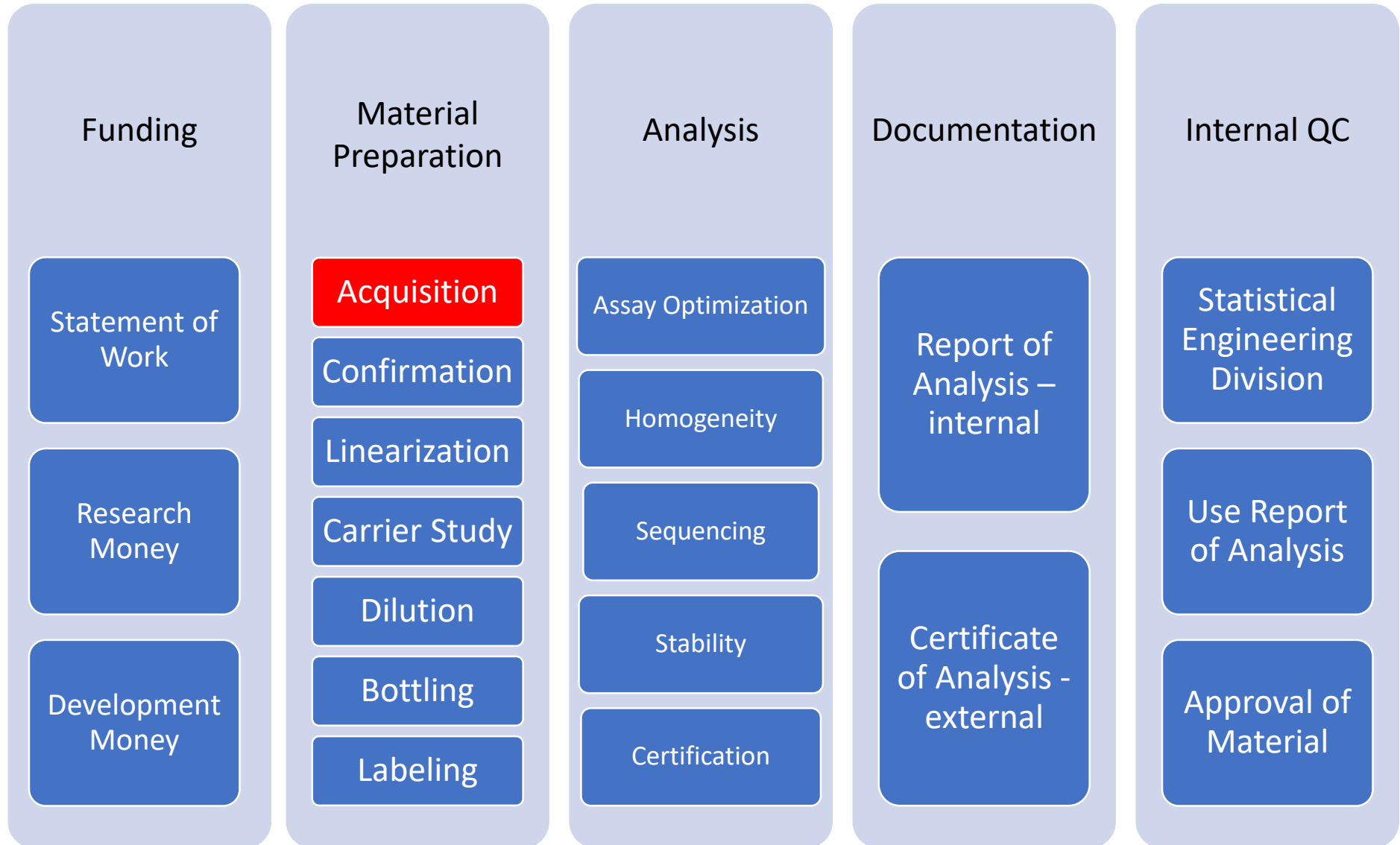
SRM vs RM

	SRM	RM
Homogeneous	✓	✓
Stable	✓	✓
At Least One Certified Value	✓	-
Complete Uncertainty Analysis	✓	-
Highest Confidence Material	✓	-

SRM Process



SRM Process



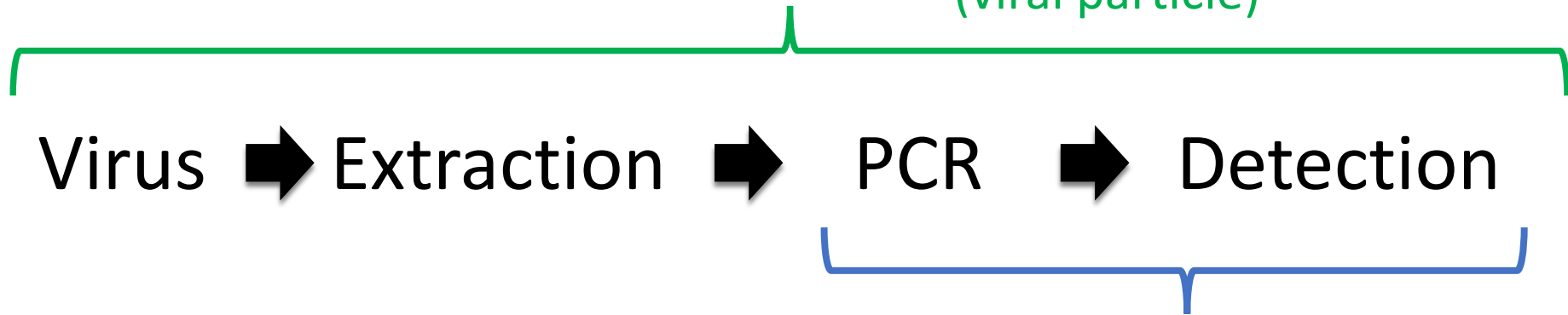
Acquisition of Materials

- Acquisition of fit for purpose material is often a significant delay in the production of an SRM or RM
 - Material can be donated by a company
 - Material can be purchased from a company (if company will allow us to resell the material)
 - Material can be made at NIST

Process Control vs Quantitation Control

WHO Viral International Standards

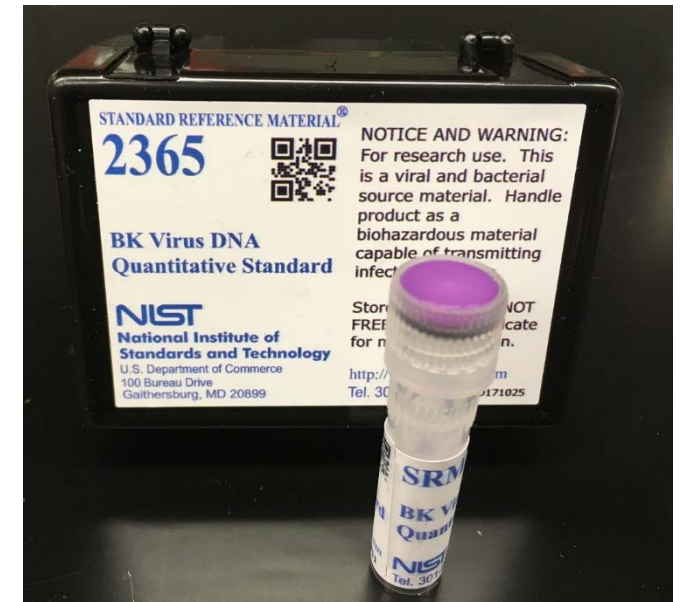
(viral particle)



NIST Viral SRMs (viral DNA)

Current NIST Viral Standards

- Cytomegalovirus (CMV; SRM 2366a)
 - CMV sequence encoded in a BAC; propagated in E. coli and purified
- BK Virus (BKV; SRM 2365)
 - Synthetic BKV DNA sequence in a plasmid; propagated in E. coli, purified and linearized
- JC Virus (Coming Soon)



Digital PCR

- Droplet digital (ddPCR) used for copy number determination
- Does not require an external calibrant
- Bio-Rad QX200 instrument

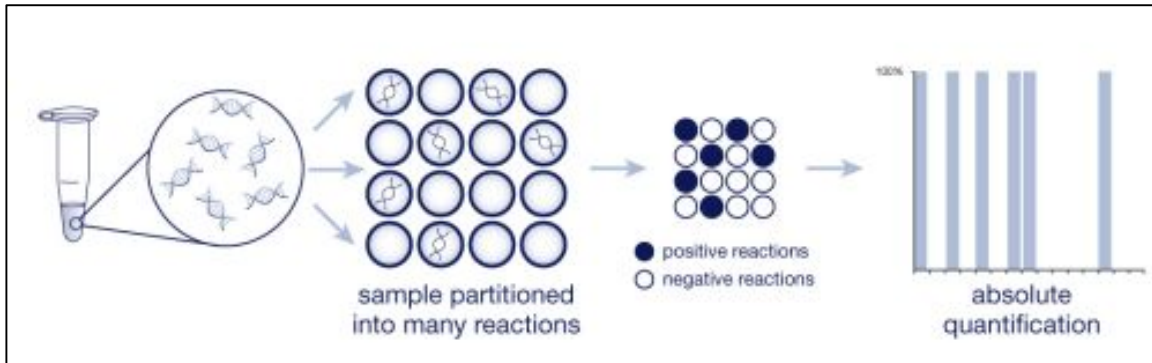


Image credit: <http://digital-pcr.gene-quantification.info/>



Image credit: <http://bio-rad.com/>

$$\lambda = -\ln(\text{Fraction Negative})$$

$$\text{Copies}/\mu\text{L} = \lambda / (\text{Droplet Volume in } \mu\text{L})$$

Production of the BK Virus SRM

BK Virus

- Polyomavirus
- Double stranded, circular DNA virus
- Most infections are mild or asymptomatic
- But can be dangerous if the patient is immunocompromised
 - BKV can cause BKV nephritis in renal transplant recipients
 - Levels of BKV must be carefully monitored and, if BKV levels are increasing, the dose of immunosuppressants must be reduced

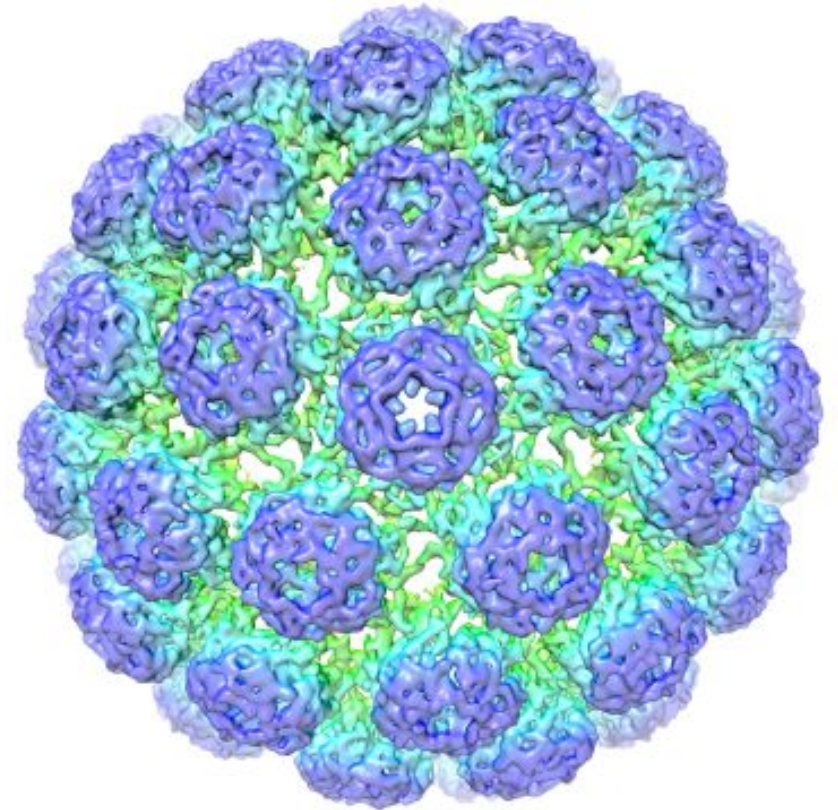
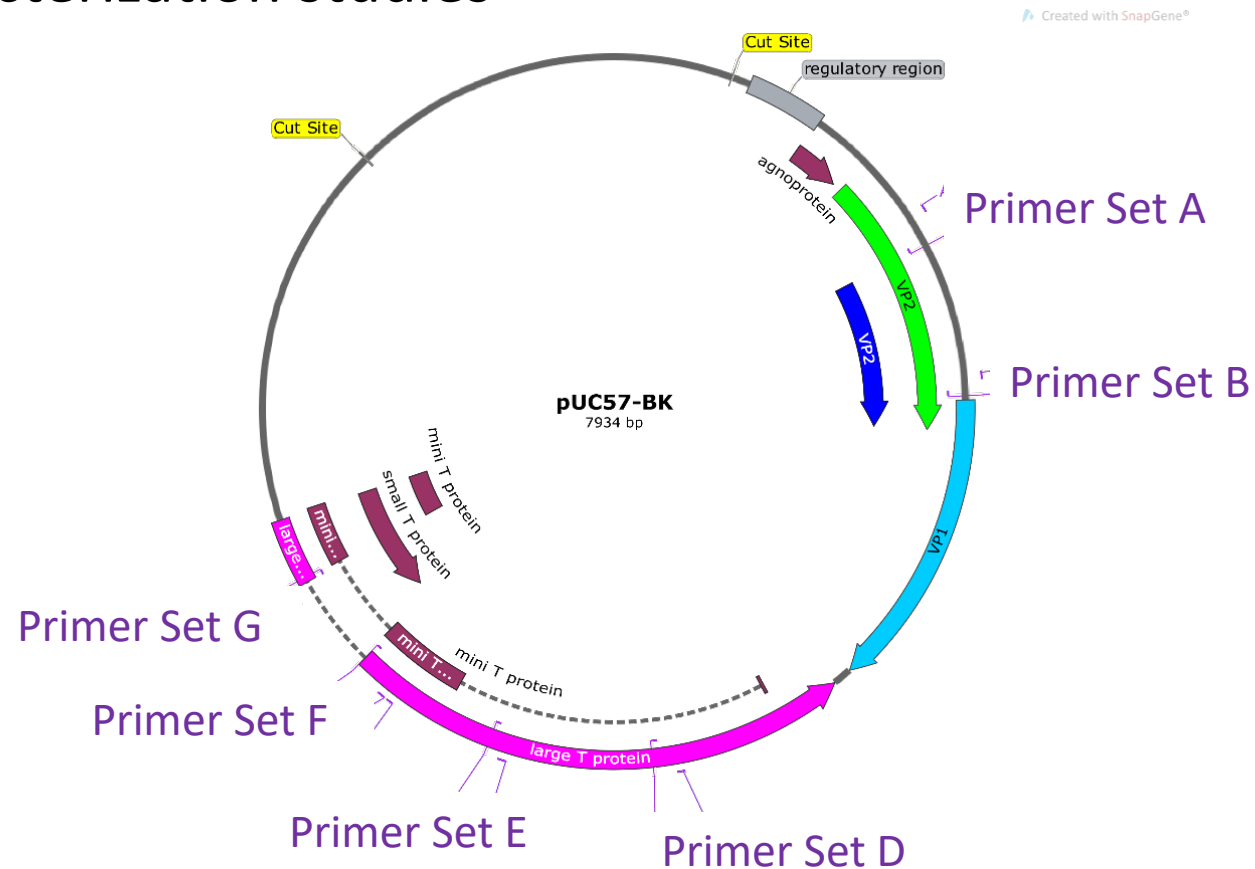


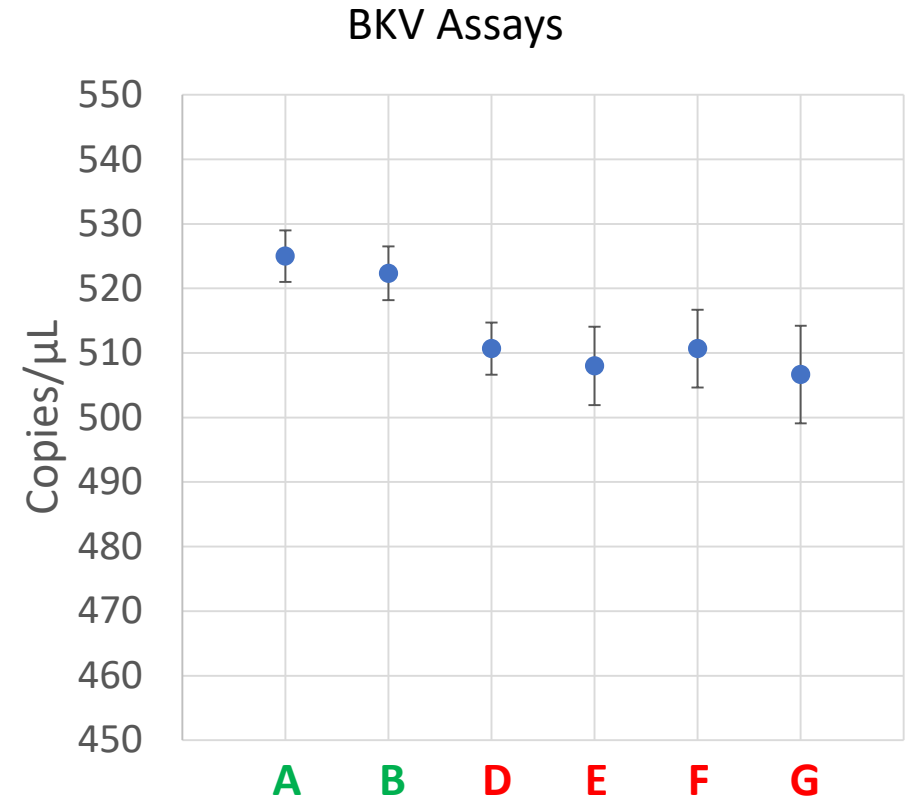
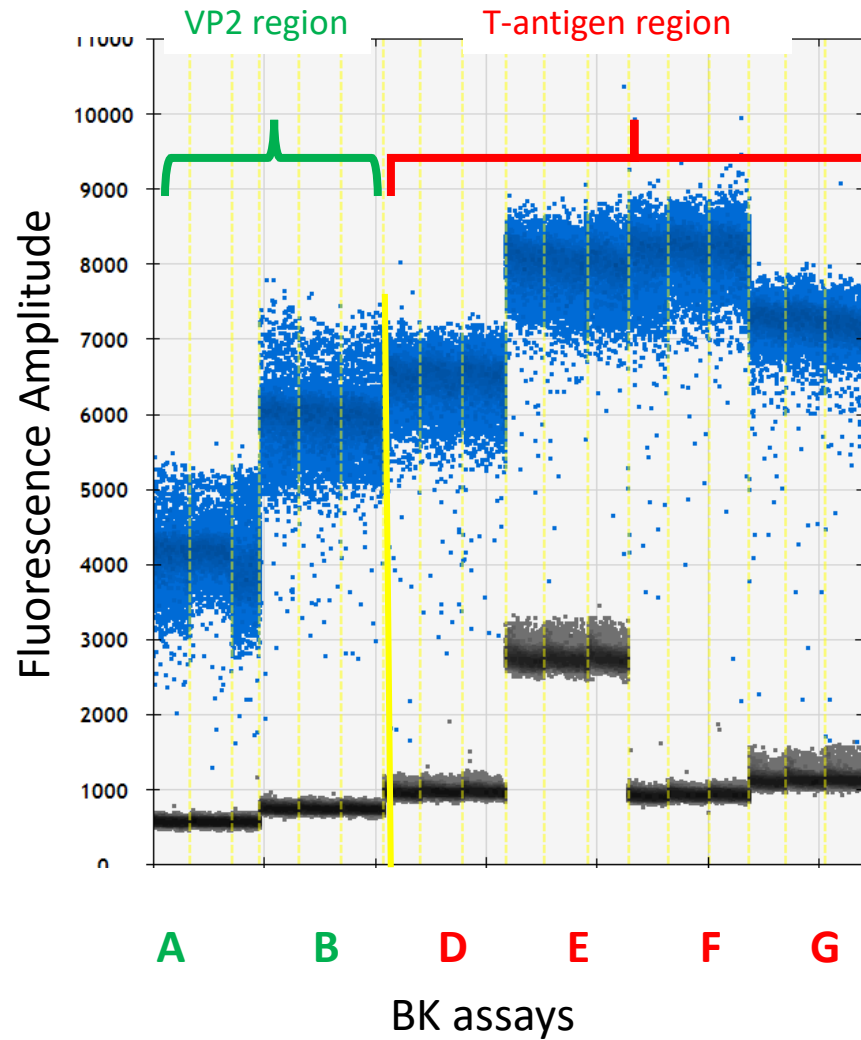
Image credit:
<http://www.kidneyresearchuk.org/>

BK Assay Design

- Designed and validated 6 dPCR assays for BK for use in homogeneity and stability characterization studies

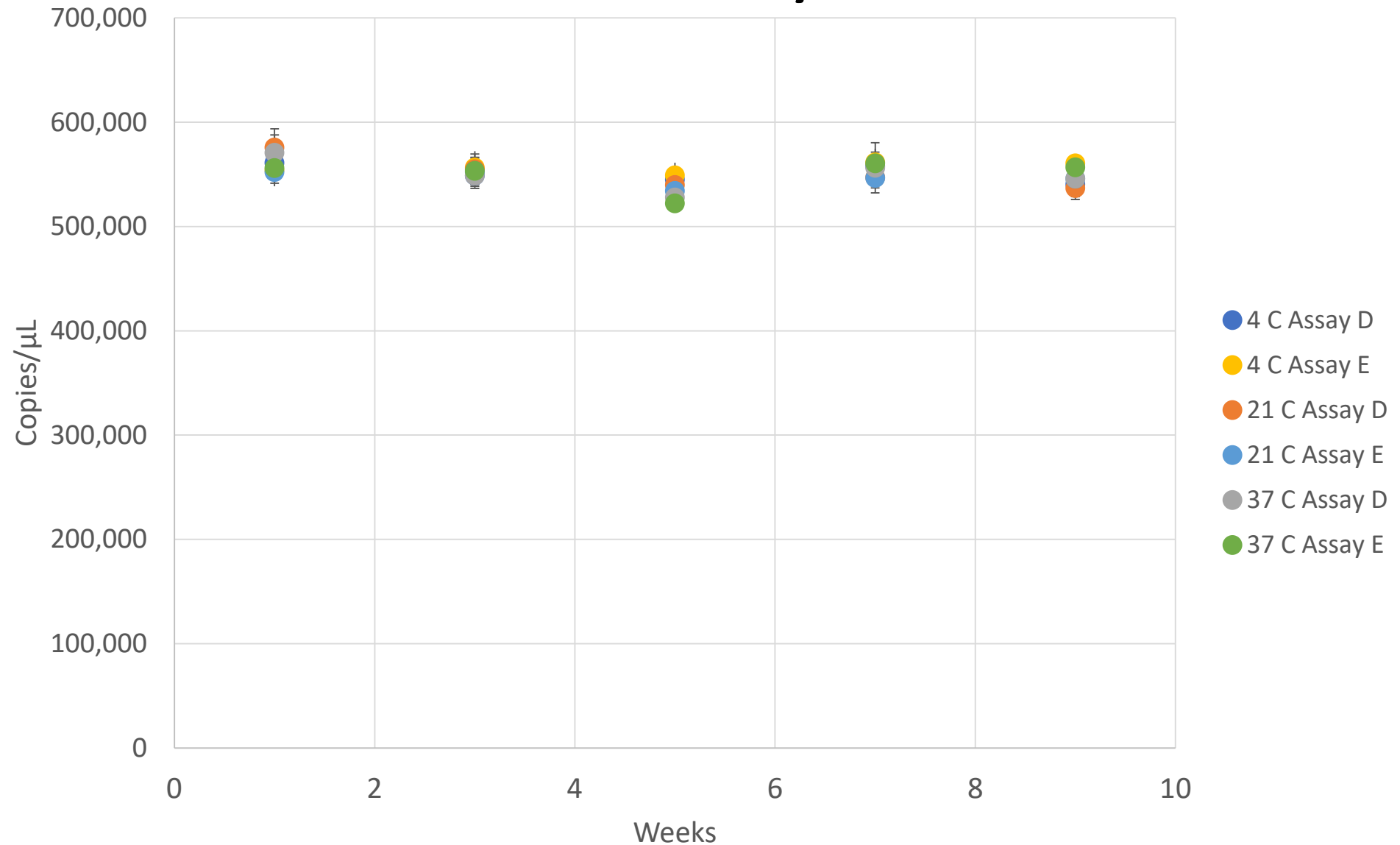


ddPCR assays for NIST BKV



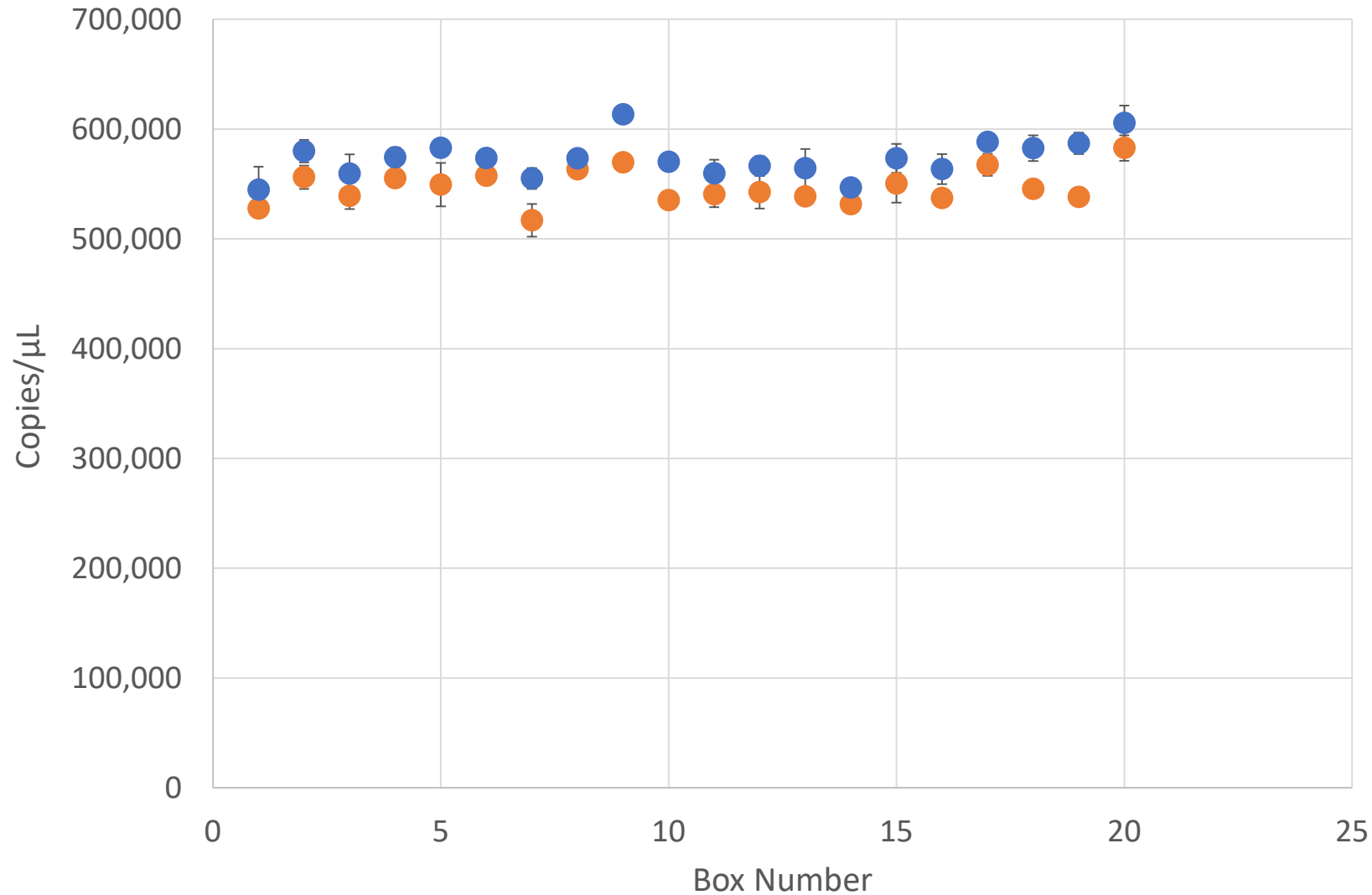
CV across all 6 assays is 4.4%

Stability



50 ng/μL tRNA added for stability

Homogeneity

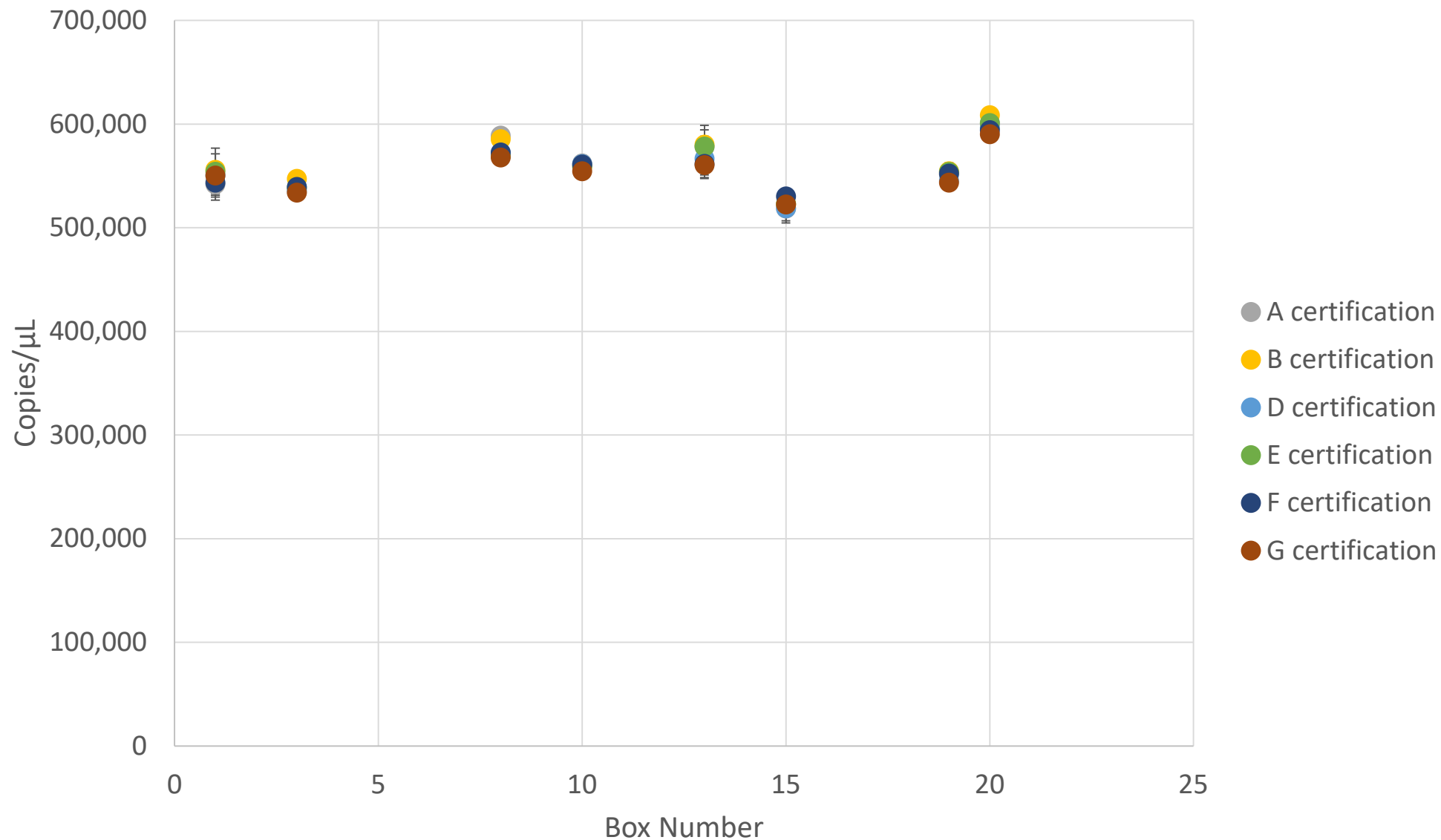


Copies/mL
Average = 8.76Log_{10}
Std Dev = 0.02Log_{10}

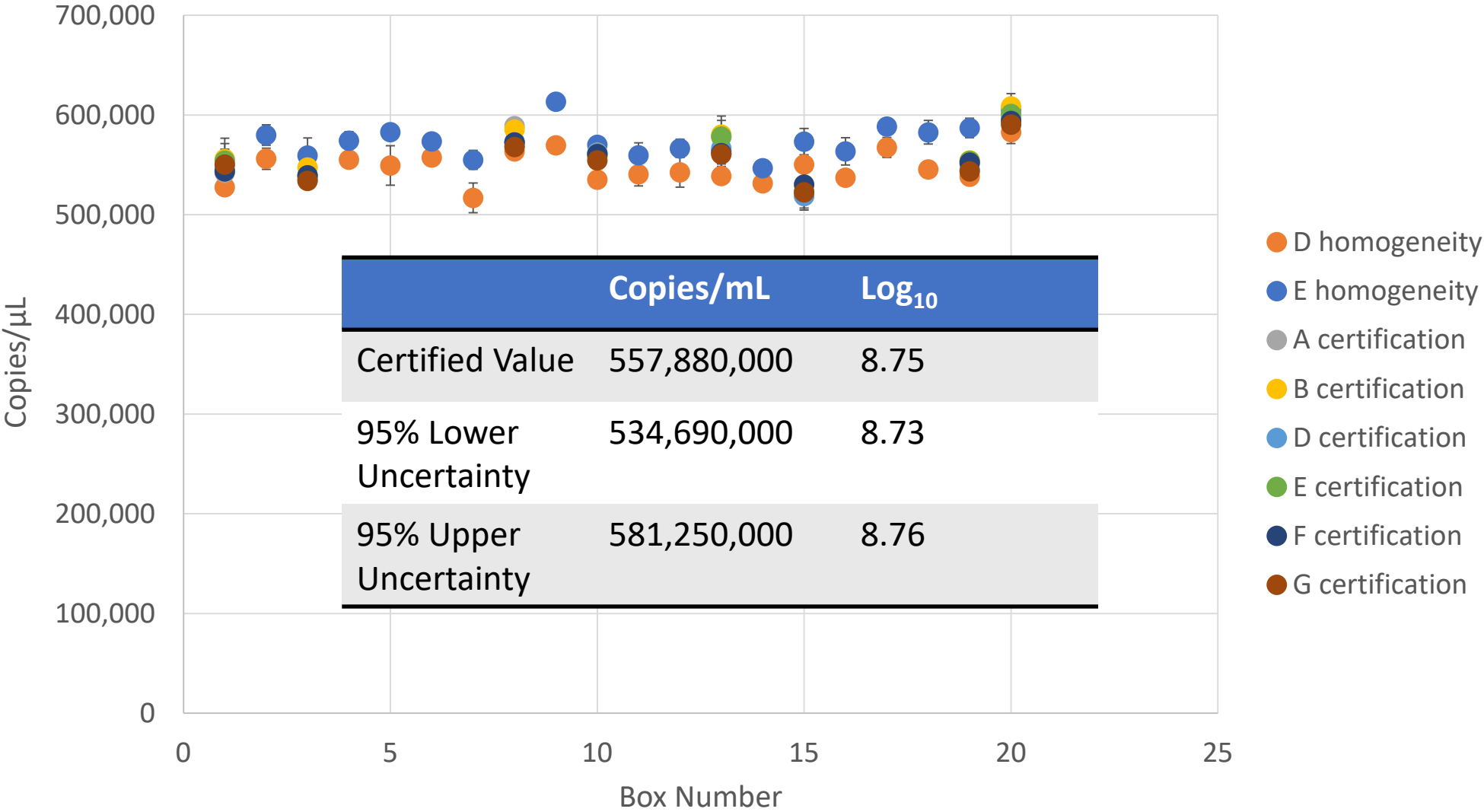
● D homogeneity
● E homogeneity

2000 units; 20 tubes sampled for homogeneity

Certification



Homogeneity/Certification



SRM 2365




NIST Special Publication 260-191

Certification of Standard Reference Material® 2365 BK Virus DNA Quantitative Standard

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Blaza Toman
Peter M. Vallone



<https://doi.org/10.6028/NIST.SP.260-191>

 National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2365

BK Virus DNA Quantitative Standard

This Standard Reference Material (SRM) is intended for use in the value assignment of BK virus deoxyribonucleic acid (DNA) quantitation materials, primarily those used for quantitative polymerase chain reaction (qPCR). SRM 2365 consists of a well-characterized, linearized plasmid, containing BK virus DNA solubilized in 10 mmol/L 2-amino-2-(hydroxymethyl)-1,3 propanediol hydrochloride (Tris HCl) and 1 mmol/L ethylenediaminetetraacetic acid disodium salt (disodium EDTA) pH 8.0 buffer (TE), with 50 ng/μL yeast tRNA added to ensure stability. A unit of the SRM consists of one 0.5 mL tube containing approximately 110 μL of DNA solution. The tube is labeled and is sealed with a screw cap.

Certified Values: Certified values are provided in Table 1. A NIST certified value is a value for which NIST has the highest confidence in that all known or suspected sources of bias have been accounted for. The copy number values are metrologically traceable to the natural units count 1 and ratio 1 and International System of Units (SI) derived units of volume.

Table 1. Certified Value for SRM 2365

Analyte	Certified Value (copies/μL)	95% Probability Uncertainty Interval (copies/μL)	Standard Uncertainty, $u(X)$ (copies/μL)	Effective Coefficient of Variation, $CV=100 \times u(X)/X$
BK Virus DNA copy number	558,000	534,000 to 582,000	12,000	2.2%

Expiration of Certification: The certification of SRM 2365 is valid, within the stated measurement uncertainties, until 15 July 2023, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Storage and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Coordination of the technical measurements and analysis leading to the certification was under the direction of M. Cleveland of the NIST Biomolecular Measurement Division.

Statistical consultation for this SRM was provided by B. Toman of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

Michael J. Tarlov, Chief
Biomolecular Measurement Division

Steven J. Choquette, Director
Office of Reference Materials

Gaithersburg, MD 20899
Certificate Issue Date: 11 September 2018

Research Grade Material (RGM)

	SRM	RM	RGM
Homogeneous	✓	✓	✓
Stable	✓	✓	?
At Least One Certified Value	✓	-	-
Complete Uncertainty Analysis	✓	-	-
Highest Confidence Material	✓	-	-

SRMs and RMs take 3+ years to release for sale; NIST is investigating releasing a new class of materials referred to as “research grade materials”

Research Grade Materials

- Homogeneous
- Sufficiently stable for interlaboratory studies
- Not *necessarily* stable for long periods of time
- Can be useful in benchmarking measurements in rapidly emerging fields to begin to sort out measurement comparability problems
- Can be produced faster than RM/SRM
- RGM are meant for the “learning phase” whereas SRMs/RMs are meant for the regulatory phase

Points to Consider

- What is needed?
- How much material is needed?
- What concentration should it be?
- Type of material (naked DNA, viral particles, synthetic virus, other)
- Other factors?

Acknowledgements

- Arifa Khan – Conference Co-Chair, CBER/FDA
- Peter Vallone – Leader, Applied Genetics Group
- Erica Romsos – Applied Genetics Group
- Margaret Kline –Applied Genetics Group
- Jason Kralj – Complex Microbial Systems Group
- Kelley Rogers – Technical Program Manager for NIIMBL

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