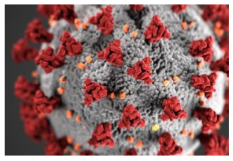
How Translational Science can help now and prepare us for the next one?

John J. Sninsky, PhD

JCTLM
The Joint Initiative for Metrology in Biology

Coronavirus Standards Working Group

A COVID-19 Diagnostic Standards Development Partnership



June 26, 2020

Reminder of Naming Convention

COVID-19 (the disease) and SARS-CoV-2 (the virus)

Diagnostics

We were forewarned, we did not listen; we were tested and we ignored;
let's learn lessons for the future

Gates *NEJM* (2015).
Gates NEJM (2018).
Gates NEJM (2020).

Pandemic Impressions

Impressed by

- Speed of virus sequence & direct assay development
- Speed of Emergency Use Authorization
- Early availability of viral reference material
- Timely revoking of EUAs for poor performance tests (serology)
- Rapid sharing of data & preprints of papers

Discouraged by

- Despite forewarnings with SARS and MERS, unprepared for COVID-19 epidemic/pandemic
- Lack of appreciation of critical early role of diagnostic tests, contact tracing and isolation to control pandemic
- Lack of analytical standardization (requirement for viral isolates); overlooked PCR lessons learned in past
- Lack of appreciation of critical diagnostic test statistical metrics prior to allowing EUAs
- Overlooked bias and chance in early studies

Mixed Review of Pandemic Response

SARS-CoV-2 Test Availability: Sequence reported in early January

- Emergency use regulatory authority finalized in 2017 permitting rapid availability of tests
  - Section 564 of the FD&C Act was amended by the Project Bioshield Act of 2004 and was further amended by the Pandemic and All-Hazards Preparedness Reauthorization Act of 2013 (PAHPRA), the 21st Century Cures Act of 2016, with final FDA EUA guidance **January 2017**
- Authorization does not require usual extensive validation due to urgency of a pandemic
  - Informative online posting of authorization decision as well as description of test and links to instructions for use
  - FDA extended EUA to Laboratory Developed Tests (LDTs) developed by CLIA laboratories (**March 31, 2020**).
  - Agency can periodically review performance of tests enabling revisions and can revoke tests should performance prove unacceptable
- Commercial In-process control SARS-CoV-2 reference material available **February 29** (SeraCare)
  - National and International efforts are ongoing; Stanford Coronavirus Standards Working Group initiated **March 27,2020**
- LabCorp launched SARS-CoV-2 test **March 5 (2020)**.
- First IVD test authorized **March 12, 2020** (Roche)

https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization#covidinvitrodev
https://jimb.stanford.edu/covid-19-standards

Adopting a New Perspective

Past

Vaccine

↓

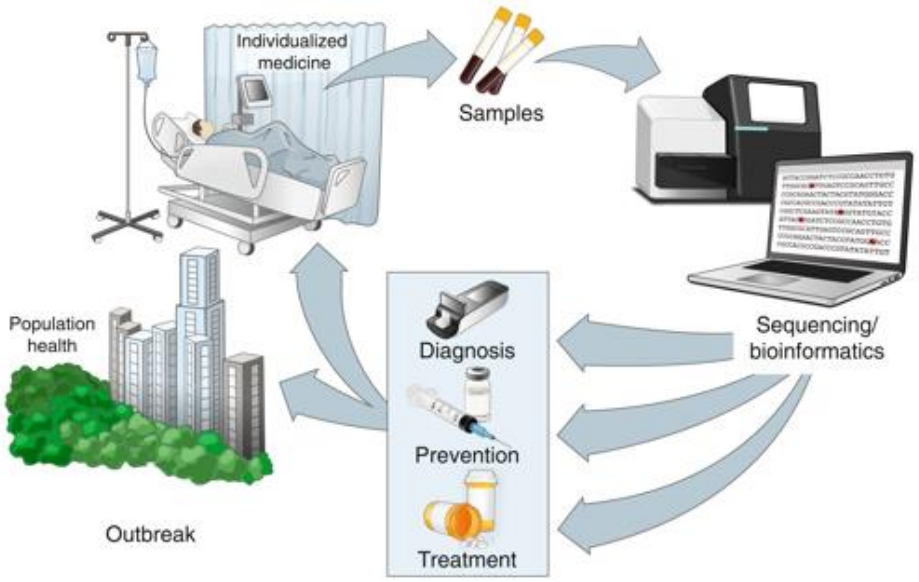
Treatment

↓

Diagnostics

Future

Diagnostics = Treatment = Vaccine



Diagnostics needs to be equally prioritized relative to vaccine and treatment efforts because detection plays a role earlier in the pandemic and requires shorter time frame to develop.

COVID-19 Diagnostic Test Rollout

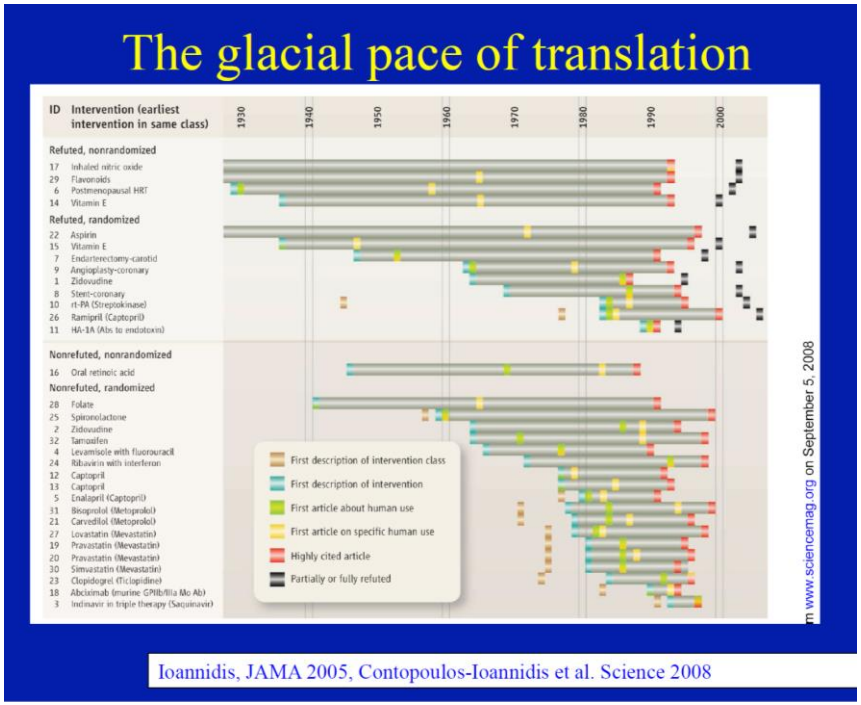
"Testing availability remains a signature failure of the battle against coronavirus in the US."

Dr. Cham UCSF Benihoff
Children's Hospital (Oakland)

Past innovation permitted us to respond rapidly (2 months) with Diagnostics but tests lacked standardization and we were unable to scale.

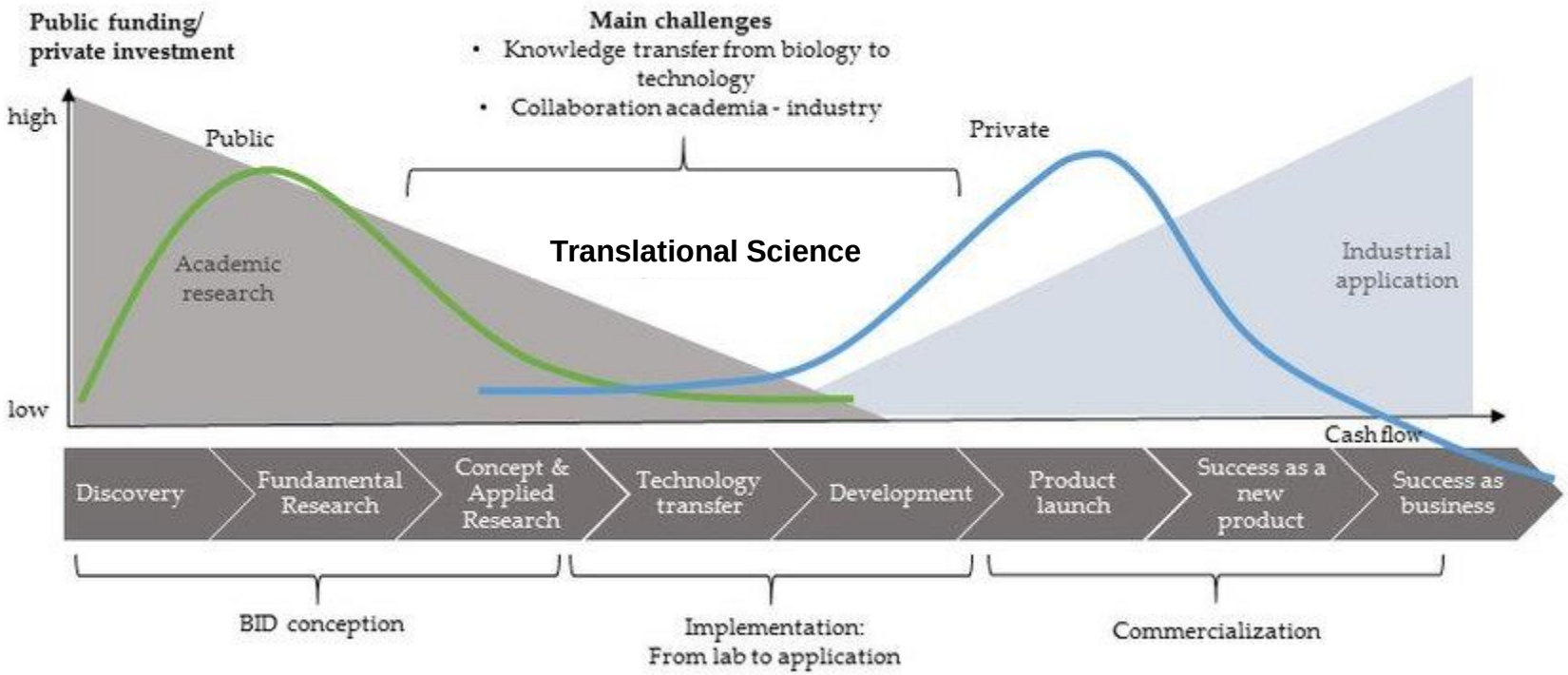
Setting the Stage for Translational Medicine

- Institute of Medicine reports ~20 year lag from innovation to patient impact in 1999
- NIH Director Zerhouni includes importance to advance science to patient care in 2003 *Science* NIH roadmap
- Contopoulos-Ioannidis *et al. Science* 2008 report on life cycle time lag of medical interventions
- NIH Director Collins comments on the importance of re-engineering translation in *Science* in 2011
- FDA Commissioner Hamburg highlighted delay in bringing innovation to patients in 2011
- NIH created new institute in 2012 - National Center to Advance Translational Science (NCATS)



A pandemic requires we respond in weeks not years

Translational Science: Critical Bridge



Research-grade tests → Clinical grade-tests

Near term value to patient

Value to Science and
longterm value to patient

Ineffective Translational infrastructure shown in high relief

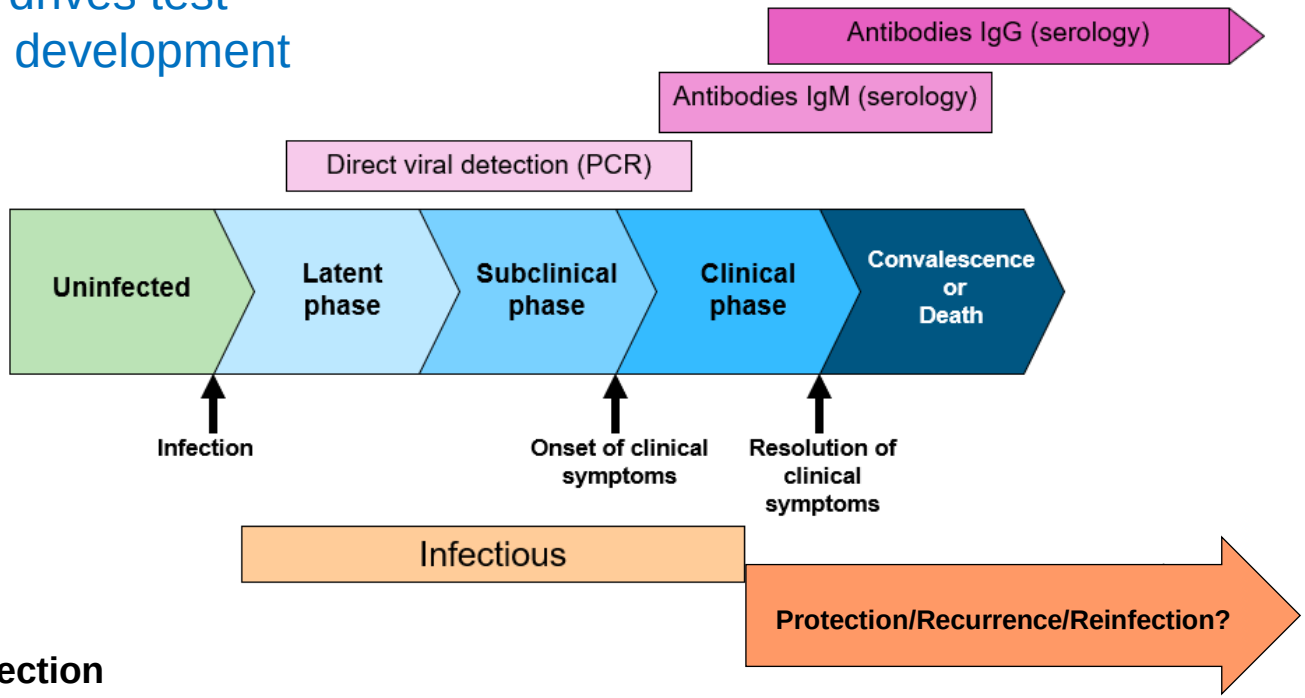
False positives because knowledge not 'carried over': CDC missteps

- Forewarned about consequences of highly sensitive PCR procedure
  - Kwok and Higuchi *Nature* (1989).
- Initial recommendations on laboratory measures and physical containment
- Molecular procedures to address PCR carryover introduced
  - Longo *et al. Gene* (1990).
  - Cimino *et al. Nucleic Acids Res* (1990).
  - Lo *et al.* Incorporation of biotinylated UTP (1990).
    Chapter 14 in *PCR Protocols*
  - Persing and Cimino (1993) *Diagnostic Molecular Microbiology*
- Reminded of critical issue of stray PCR products and errant positive controls
  - Mifflin (1997)
  - Aslanzadeh *Annals Clin Lab Sci* (2004).
  - Borst *et al. Eur J Clin Micro Infec Dis* (2004).
  - Hu Intech (2016).

Thanks to Tom White for his suggestions

Natural History of Viral Infection

Intended use drives test selection and development



- **Direct detection**
  - Diagnosis (to distinguish pandemic pathogens from others; screen contacts; isolate true cases)
- **Serology**
  - Screening of contacts (to identify source, types and dynamics of transmissions; quarantine positives; release negatives)
  - Identify true prevalence of infection in community; duration of immunity; vaccine response; release asymptomatic & recovered people from quarantine)

https://www.finddx.org/covid-19/sarscov2-eval-molecular/
https://www.finddx.org/covid-19/sarscov2-eval-immuno/

Carter *et al. ACS Central Science* (2020).

Steps in Diagnostic Test Development

- **Analytical Validation** refers to how well the test predicts the presence or absence of a biomarker. In other words, can the test accurately detect whether a specific biomarker is present or absent?

  Clinical-grade assays and software are critical, not research-grade versions
  3 Rs of AV: repeatability, reproducibility and robustness
  Follow professional society guidelines (e.g. CLSI, FDA, WHO, FIND, AMP, etc.)

- **Clinical Validation** refers to how well the biomarker being analyzed is related to the presence, absence, or risk of a specific disease.

  The limited availability of characterized clinical samples in a pandemic compromises the rigor of clinical validation

- **Clinical Utility** refers to whether the biomarker can provide clinically relevant information about diagnosis, treatment, management, or prevention of a disease that will be helpful to a patient, healthcare provider, or family member.

  In a pandemic, the clinical usefulness or actionability varies with direct detection of virus in those infected and detection of antibodies in those previously infected

http://ghr.nlm.nih.gov/handbook/testing/validtest
http://www.cdc.gov/genomics/gtesting/ACCE

Virus Reference Materials are Critical

- Quality reference materials are critical for the rigorous development, optimization and monitoring of analytical validity
- The features of different reference materials need to be considered
- National and International reference materials (WHO, FIND, NIBSC, *etc.*) will take longer to develop than commercial reference materials
- Commercial reference materials should be used to independently review performance and for comparative diagnostic test studies in advance of national and international reference materials

National and International standardization efforts initiated

Stanford Coronavirus Standards Working Group
https://jimb.stanford.edu/covid-19-standards
https://poeli.gitlab.io/collated_vendor_info/

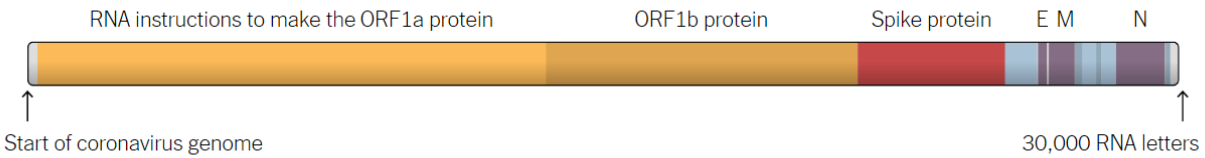
	Nucleic Acid	Bacteriophage (MS2) [1,4,5]	Eukaryotic virus (Alphaviruses) [2]	Liposomes [6]	Coronavirus	Comment
Time to develop	√	√	√	√	√	Timely availability is critical
Particle structure	√	√	√	√	√	Enveloped particle may be more representative of coronavirus
Pre-analytical process control [7]	√	√	√	√	√	If process control not included, result potentially less accurate
Size of insert	√	√	√	√	√	Accommodate multiple regions of virus and tests that target different regions
Scale	√	√	√	√	√	Complexity and cost to increase batch size
Safety	√	√	√	√	√	Infectivity limits availability
Storage	√	√	√	√	√	Minimizes batch differences
Qualitative reference	√	√	√	√	√	Less complex characterization
Quantitative calibrator	√	√	√	√	√	Increased complexity and cost to develop and characterize amounts
Proficiency program [3]	√	√	√	√	√	Integrates features above

SARS-CoV-2 reference material was available at end of February

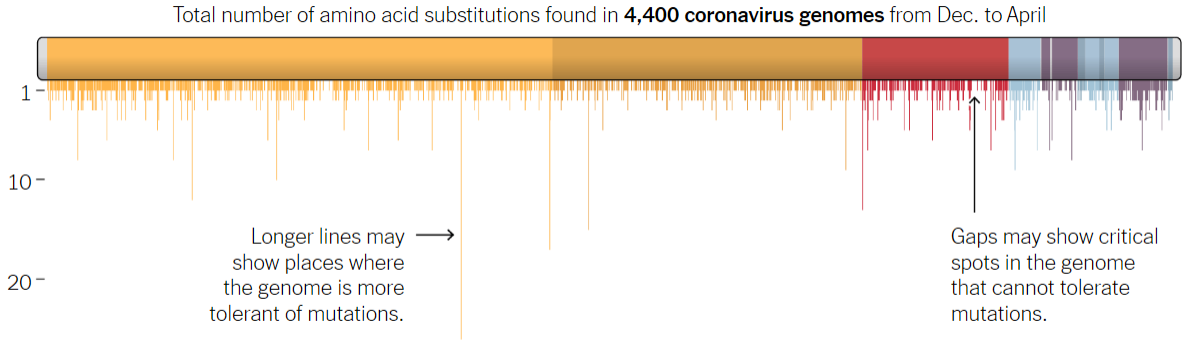
1. Pasloske et al. J Clin Micro (1998).
2. Schlesinger Adv Virus Res (2000).
3. Holden *et al. Expert Rev Mol Diag* (2011).
4. Karimi *et al. Adv Drug Dev Rev* (2016)
5. Pumpens *et al. Intervirology* (2016).
6. Barba *et al. Pharmaceutics* (2019).
7. Geeurickx and Hendrix *Mol Aspects Med* (2020).

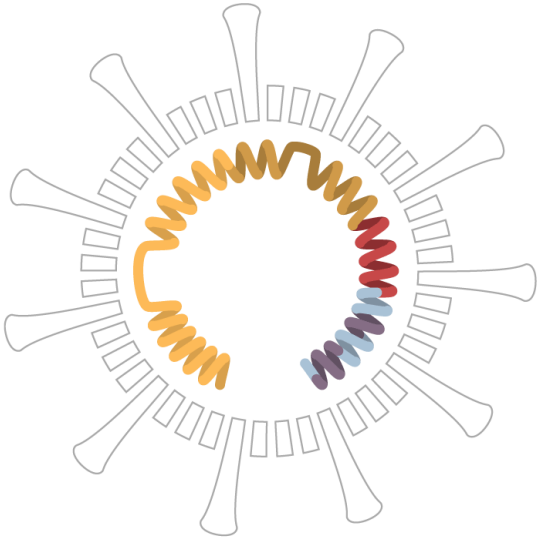
Thanks to Andrew Anfora and David Merriam (SeraCare) for discussions

Direct Viral Detection



Likely conserved regions known in virus group





Viral genome
Viral antigens

Corum and Zimmer New York Times April 30, 2020

https://www.finddx.org/covid-19/sarscov2-eval-molecular/

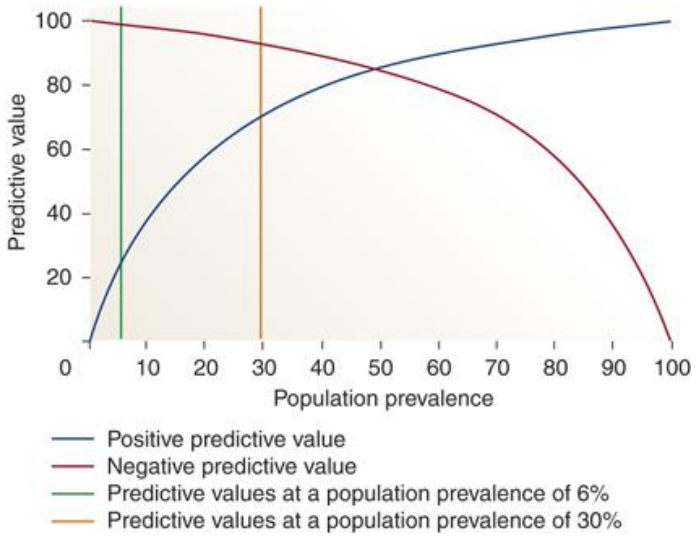
Common Statistical Missteps in a Pandemic

- Use of sensitivity and specificity instead of negative (NPV) and positive predictive values (PPV)
- Use of prevalence in sample set to calculate NPV and PPV instead of intended use population/community
- Use of prevalence in geographical region instead of likely risk group
- Use of 100% values: minimally the number of samples should be noted or 100% not used at all
- Contextual perspective not provided (stage of disease)
- Confidence intervals or coefficient of variation ranges not reported
- Early reporting has missing data, not accurate and likely compromised by bias and chance

Diagnostic Test Metrics: tests vary considerably

		Condition (as determined by "gold standard")	
		Condition positive	Condition negative
Test outcome	Test outcome positive	True positive	False positive (Type I error)
	Test outcome negative	False negative (Type II error)	True negative
		Sensitivity = Σ True positive/ Σ Condition positive	Specificity = Σ True negative/ Σ Condition negative

	Positive predictive value = Σ True positive/ Σ Test outcome positive
	Negative predictive value = Σ True negative/ Σ Test outcome negative



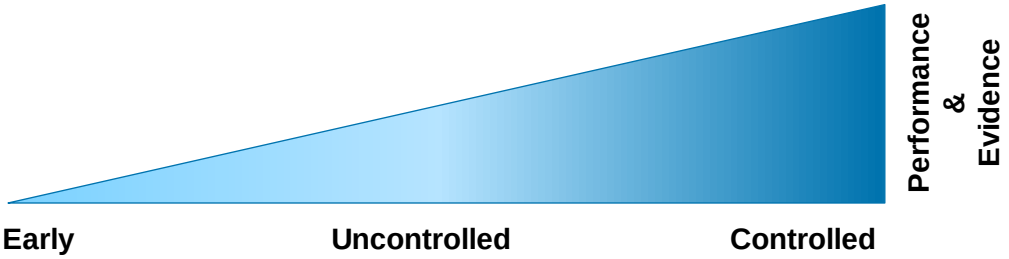
Disease Prevalence in the Intended Test Population	Probability of having the Disease if you have a Positive Result
0.1%	1.9%
1%	16%
10%	68%
20%	83%
50%	95%

Assumes a 95% sensitive and 95% specific test

Cautionary note that prevalence of intended use testing may vary from sample set tested and prevalence may vary with risk group separate from geographical region

Lo, Kaplan and Kirk *Nature Reviews Nephrology* 10, 215 (2014).

Fit-for-Purpose Diagnostic Test Performance



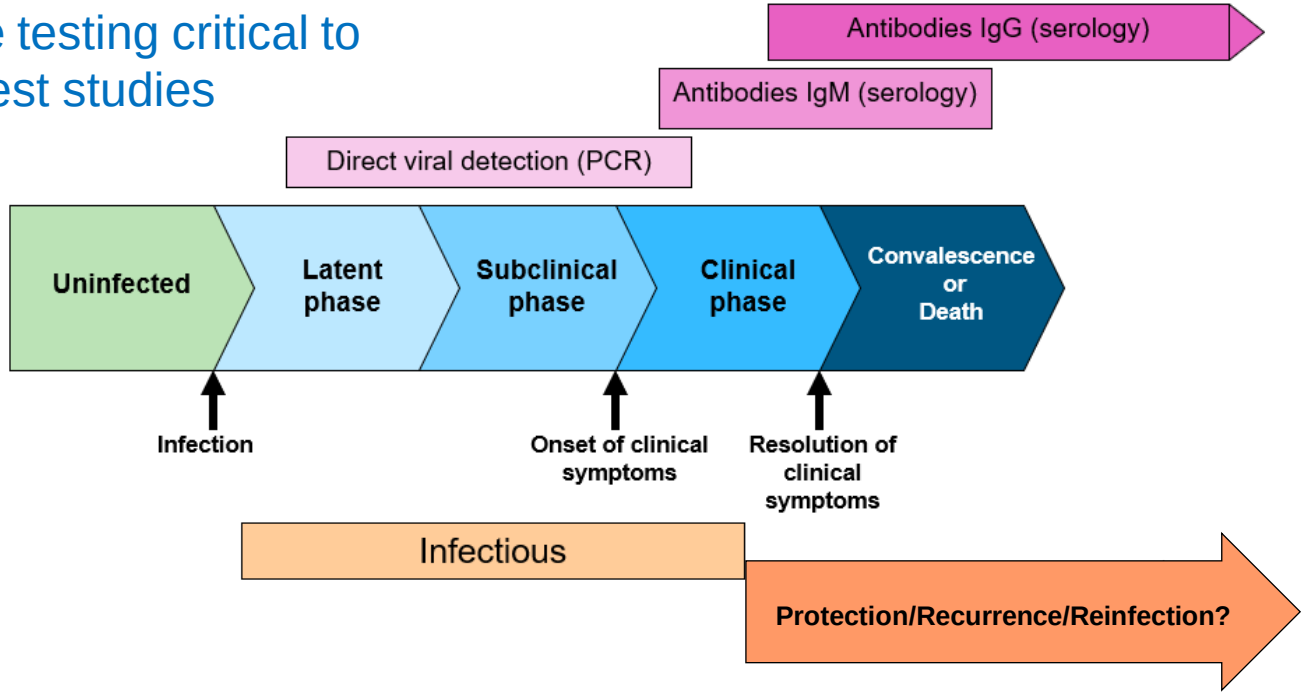
Prior to outbreak	Acute stage	Late stage	Resolution
To allow surveillance for initial detection of outbreak	To verify cases and determine the extent of the outbreak To identify cases, allowing isolation, hygiene measures and treatment to interrupt transmission	For continued surveillance to detect and anticipate further spread of disease To confirm the efficacy of the measures implemented, and the decrease in number of cases	To confirm that there are no further cases and declare the end of the outbreak To allow continued surveillance for future outbreaks

FIND stage description

- Benefit-to-risk performance of diagnostic tests varies across pandemic stages
- Direct pathogen detection has earlier value relative to exposure (serology)
- Critical that <u>independent</u> performance evaluations of authorized tests are timely
- In some cases no test may be better than using a poorly performing test

Test Performance Varies with Stage of Disease

Paired sample testing critical to comparative test studies



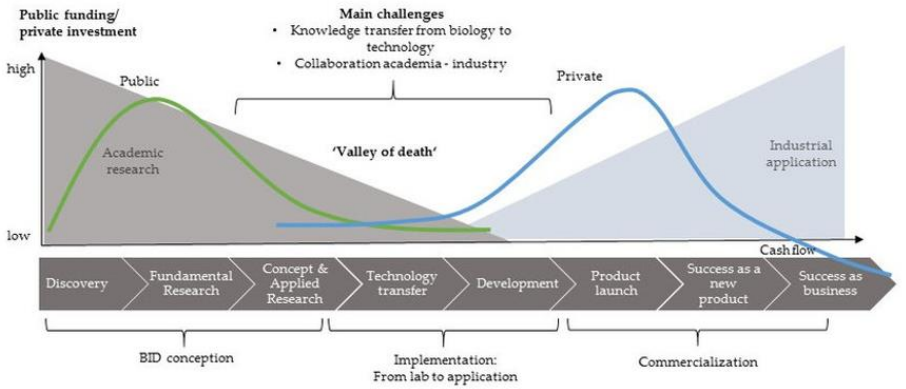
Direct Detection	-	+/-	+	+	+/-	-	-	-	-
Serology IgM	-	-	-	-	+/-	+	+	+/-	-
Serology IgG	-	-	-	-		+/-	+	+	+ (?)

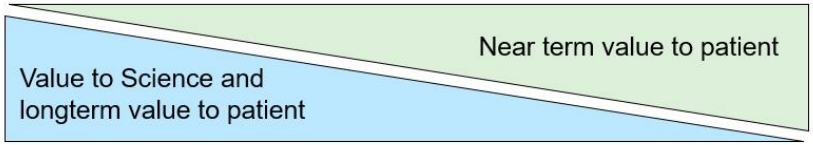
https://www.finddx.org/covid-19/sarscov2-eval-molecular/
https://www.finddx.org/covid-19/sarscov2-eval-immuno/

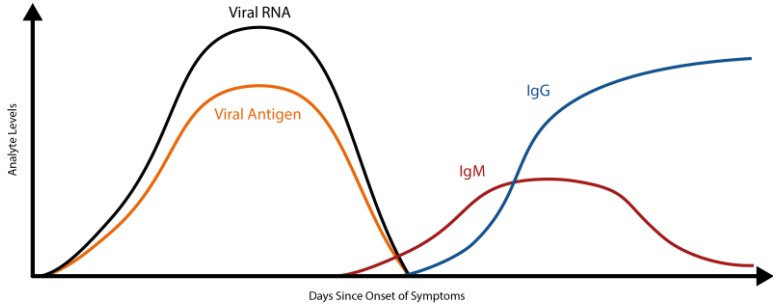
Carter *et al. ACS central science* (2020).

Lessons (to be) Learned from COVID-19

- Establish thorough understanding of Translational Science for ‘clinical-grade’ diagnostic test development
- Review safety measures for pathogen type
- Review inactivation recommendations for pathogen type
- Review diagnostic metrics and what they mean (predictive values critical, not sensitivity and specificity)
- Follow industry best practices (e.g. CLSI, FDA, FIND, AMP, etc.)



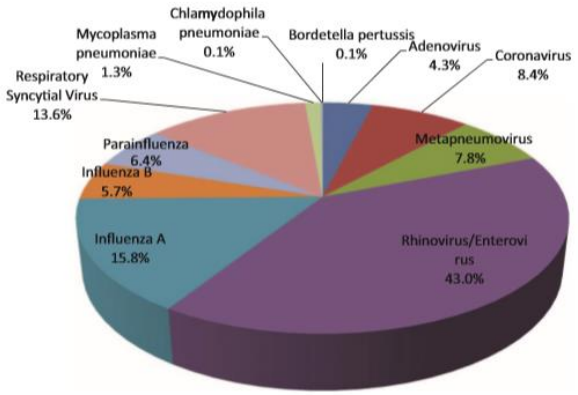




Amen *et al. Nature Biotech* (2020): SARS-CoV-2 ‘pop-up lab’.

Lessons (to be) Learned from COVID-19

- Deploy mature technologies since new technologies harbor substantial unpredictable risk
- Prioritize highly characterized general-purpose reagents already manufactured under GMP/GLP/ISO
- Anticipate, as much as possible, supply chain bottlenecks
- Beyond pandemic pathogen, consider ‘collateral’ testing
  - Multiplex respiratory pathogen tests
  - ‘Cytokine release syndrome’ (storm) diagnostic tests
- Transparency of efforts to encourage collaboration
- Collaborative effort to ensure rapid development and minimize redundancy



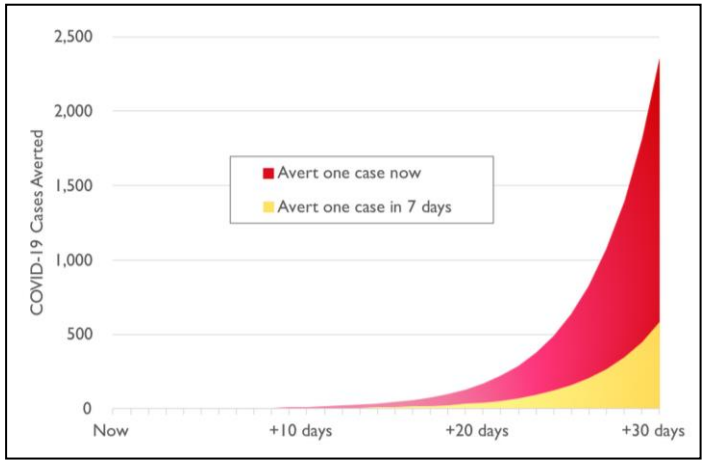
During the pandemic, I have been struck by

- Critical role of direct pathogen detection tests early in a pandemic
- Continued absence of standardization for diagnostic tests with attendant confusion on comparative test performance
- Extremely limited availability of well-characterized samples for clinical validation
- Nucleic acid extraction providing a normalization for diagnostic tests
- Even though national and international efforts are advancing for standards, by definition, these efforts, though critically important and admirable take a long time to develop
- Availability of commercial reference material was available early in the pandemic (indeed, in advance of EUA decisions on tests).

Integrating these challenges, shortfalls and opportunities leads me to propose **an EUA for reference material** for a future pandemic. The reference materials would be synthetic as well as available both alone and combined with an expected background of non-target nucleic acid as well as encapsulated in virus-like particle to be used as a in-process reference. Of course, a group steeped in metrology and diagnostic experience would need to serve as an independent advocate and recommend the engineering of and criteria and features for such a reference material.

Early Analytical Reference Material

- **Advantages**
  - Can be used early in an epidemic/pandemic
  - Can be used if virus can not be cultured
  - Facilitate test development & EUA review
  - Permits performance evaluations of tests
  - Minimizes numerous controls being synthesized in large quantitaties at multiple locations
  - Can build in ‘watermarks’ to discern from natural virus sequence
  - Pre-analytical variation partially addressed due to nucleic acid extraction
- **Disadvantages**
  - Does not account for variation due to pre-analytical collection
  - Care to ensure that different and most likely regions of pathogen genome are included
  - Only serves as a reference material, but may be confused with a ‘standard’;

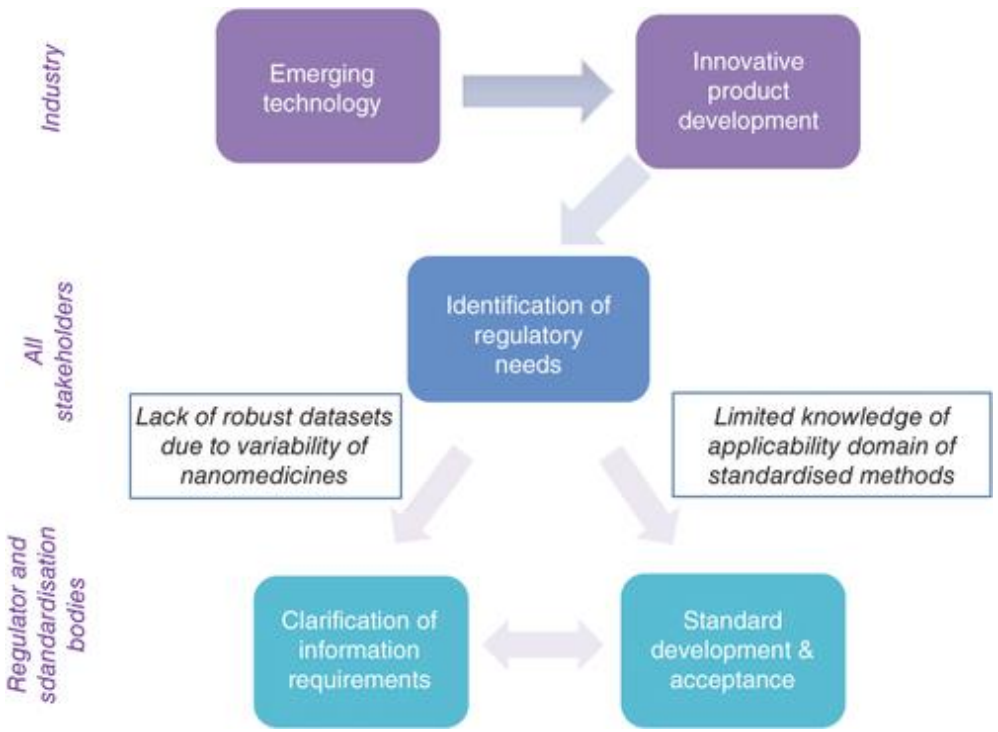


Not a ‘standard’, but an early valuable ‘reference’ material

Thanks to Tom White for his suggestions

Mapping Standards Against Regulatory Needs

- Exciting potential for ‘nanodiagnostic’ reference material
- Promote intensive knowledge exchange among all stakeholders
- Early days of standards development for nanomedicines and nanodiagnostics
- Benefit from knowledge of key physiochemical properties
- Guidance needed for comparability of methods



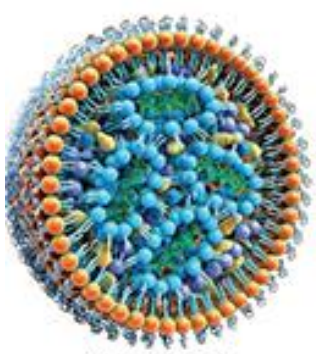
Standardization initiatives for liquid biopsy: JIMB, NIBSC, EDRN, ERCC, BloodPAC, etc.

Freeman & Inglese *Can Res* (2014).
Geeurickx & Hendrix *Mol Aspects Med* (2020).
Halamoda-Kenzaoui *et al. WIREx Nanomed Nanobiotech* (2019).
Zhang et al. Int J Nanomedicine (2017).

RNA Reference Materials

Synergy in therapeutic and diagnostic reference RNA standards efforts

- Standardize “clinical-grade” chemical and synthetic approaches
  - Enzyme assay characterization
  - Explore validation of unconventional nucleotides
- Standardize RNase inhibitor
- Explore, standardize and validate delivery systems
- Characterize and implement plasma-mimics (synthetic plasma)
- Identify scalable manufacturing solutions

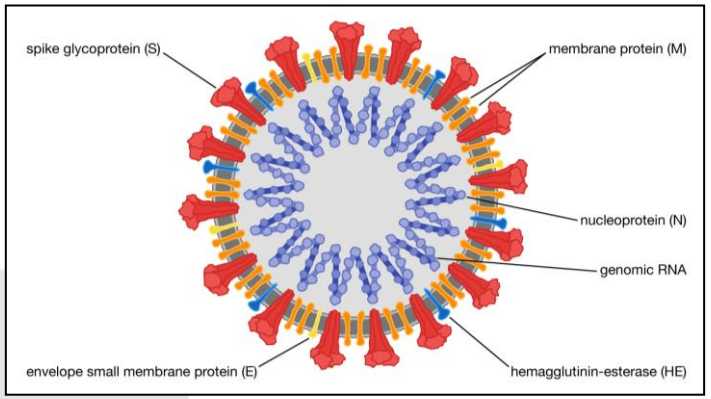


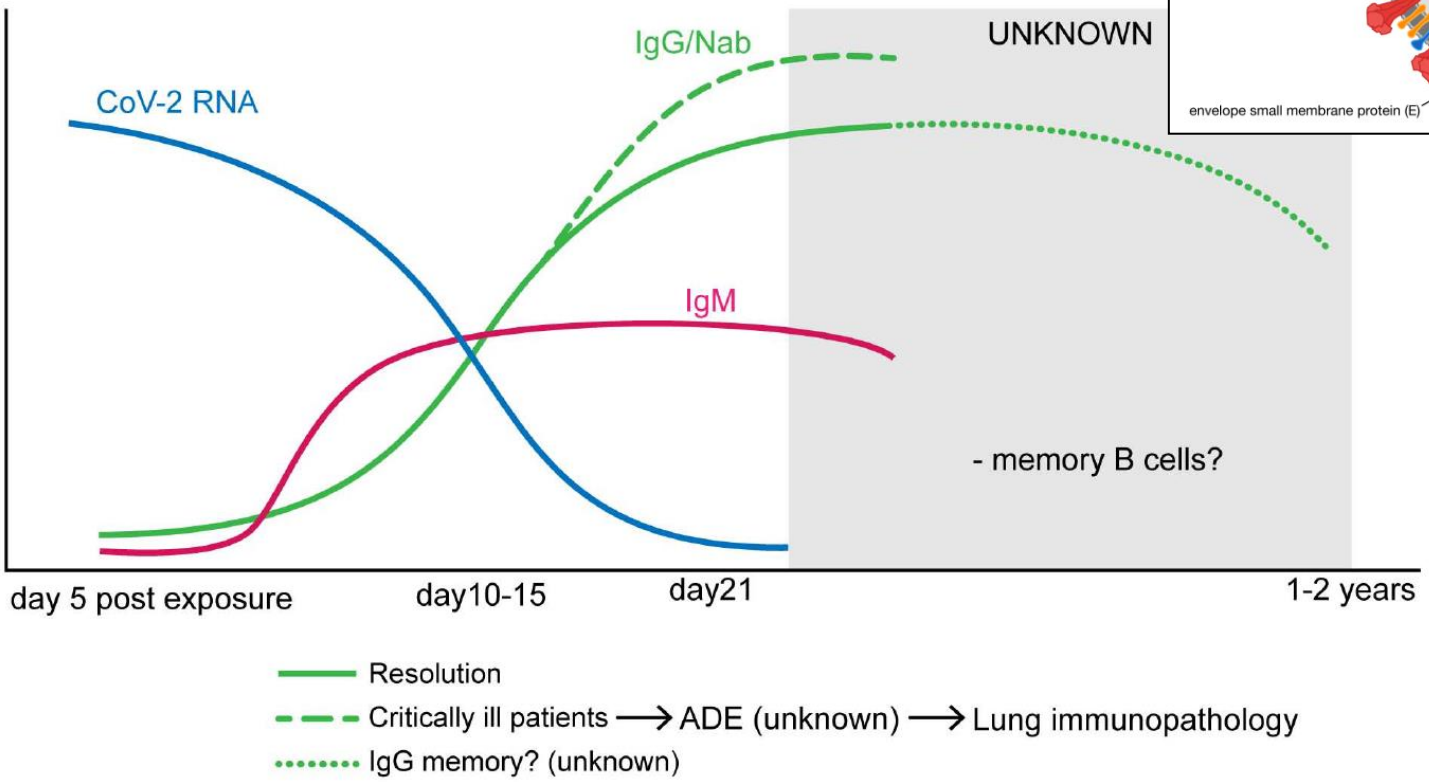
Barba *et al. Pharmaceu* (2019).
Agrahari *et al. Trends in Biotech* (2019).
Geeurickx *et al. Mol Aspects Med* (2020).
Shin *et al. Advan Therap* (2018).
https://www.nist.gov/news-events/news/2016/04/newly-patented-nist-technique-creates-precisely-sized-nanocontainers-useful

We were forewarned, we did not listen; we were tested and we ignored; let’s learn lessons for the future

Diagnostics

Viral Exposure: serology (antibodies)

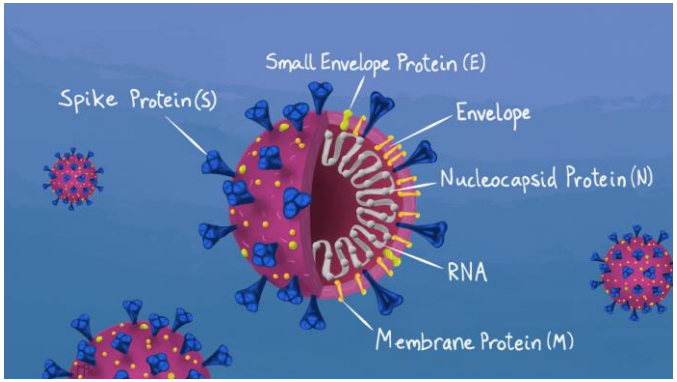


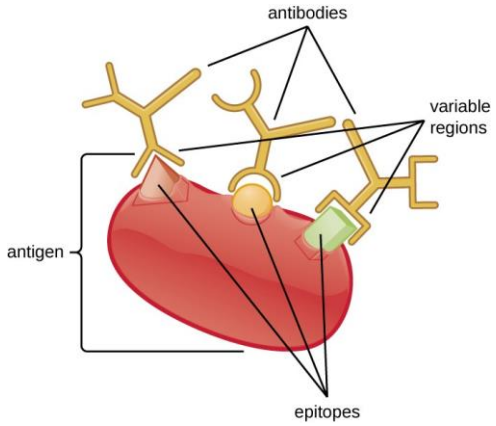


Vabret *et al. Immunity* (2020).
https://www.finddx.org/covid-19/sarscov2-eval-immuno/

Serology Reference Materials

- Characterized and documented sera from uninfected and infected individuals
- Well-characterized samples from individuals infected with similar viruses or likely viruses for intended use
- Longitudinal panels from acute through convalescent stages of infection to assess development of type and titer of antibodies as well as antigens and epitopes recognized
- Well-characterized antigen sources
  - cultured virus, individual recombinant viral proteins, pseudo virus to test for neutralization, separate IgA, IgM, IgG, Western blot filters, etc.
- Pseudovirus neutralization for functional read out





Prior to exposure	Exposure	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 24	Day 27	Day 30	Day 33	Day 36

Case for widely available ‘reagents’ that can be used for multiple purposes

- Synthetic templates/targets of known concentration that are used by LDT and IVD tests developers for LOD and LLOD measurements by all labs seeking an EUA
- Panels of non-infective whole virus or bacterial nucleic acid templates for the relevant specificity tests by all labs seeking an EUA
- Panels of non-infective clinical specimens from individuals with confirmed infections with the new pandemic agent
- Panels of non-infective clinical specimens from individuals without confirmed infections with the new pandemic agent
- Blind panels of positive and negative non-infective clinical specimens to be sued for required comparative performance evaluations of commercialized tests.

Tom White, pers commun