Demystifying the Development and Implementation of Molecular Tests in a Clinical Laboratory

The Simple, Sensible, Salient & Still Spell Binding Seven Questions About Laboratory Developed Tests

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Learning Objectives

- Describe different types of in-vitro diagnostic tests
- Identify differences between laboratory developed tests (LDT) and In-Vitro Diagnostic (IVD) tests
- List the benefits of running IVDs and LDTS
- Explain how LDTs and IVD tests are designed and regulated in the US

Agenda

1. Where do we start: Basics of Diagnostic Test Terminology
2. What is a Laboratory Developed Test (LDT) and an In-Vitro Diagnostic (IVD)?
3. Why use LDTs? Why use IVDs?
4. Who uses LDTs? Who uses IVDs?
5. When can I develop these tests?
6. How do LDTs compare to IVDs?
7. Can a lab protect their Intellectual Property?

Diagnostics Test Terminology

In Vivo

- Physiology
  EKG / BP
- Imaging
  X-Ray / CT
  MRI / PET
- Technology
  DRIVEN BY PHYSICIANS

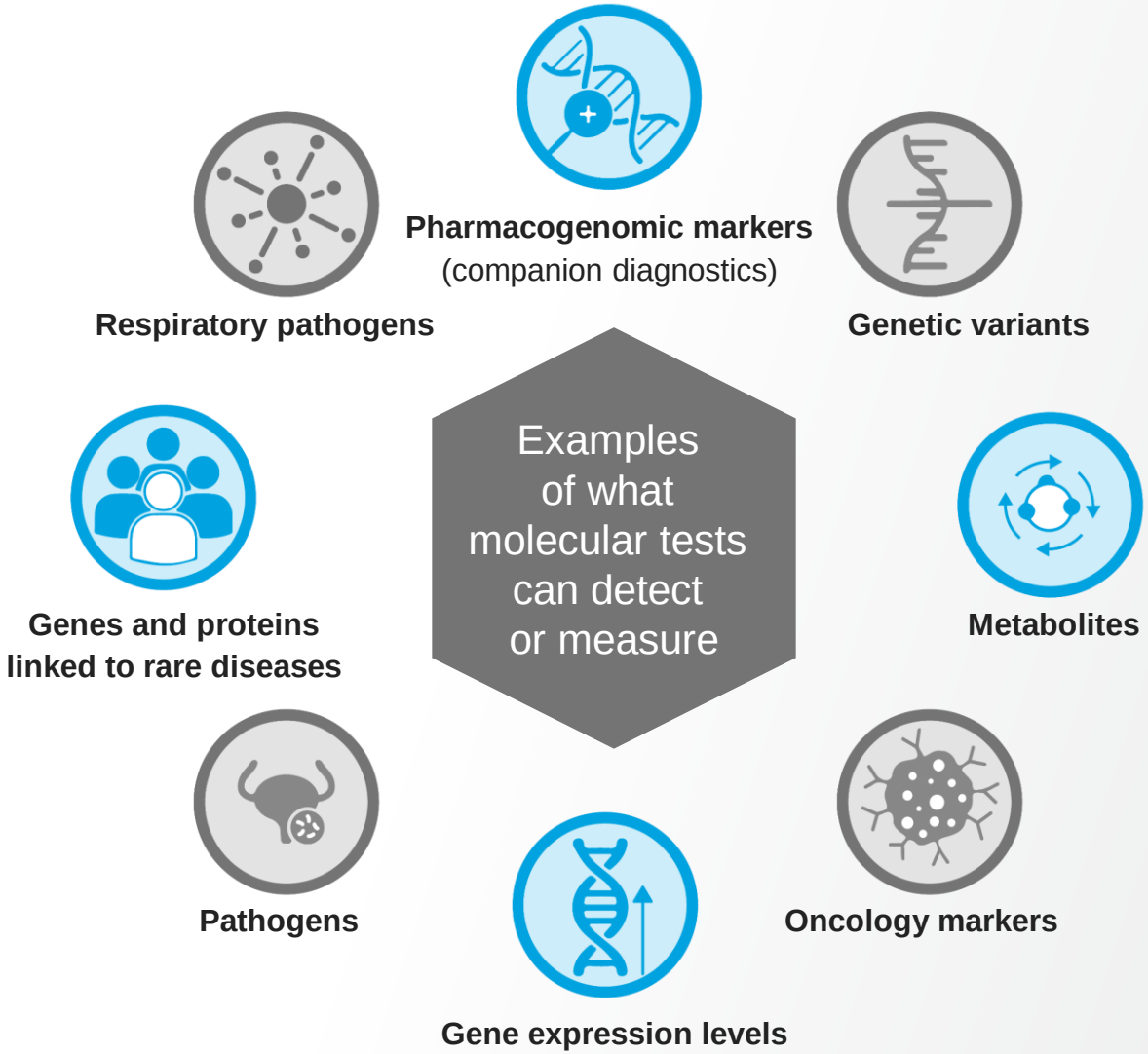
In Vitro

IVDs	LDTs
• Tests performed on body fluids (blood, urine) or cells / tissues (pap smear, biopsy)	• Tests performed on body fluids (blood, urine) or cells / tissues (pap smear, biopsy)
• Detect and/or quantify levels of desired bio markers (e.g., enzymes, protein) to diagnose cancer, body function disorders, cellular malfunctions	• Detect and/or quantify levels of desired bio markers (e.g., enzymes, protein) to diagnose cancer, body function disorders, cellular malfunctions
• Technology **DRIVEN BY PRODUCTS** from simple (clinical chemistry / ELISA) to complex (flow cytometry)	• Technology **DRIVEN BY PROCESS**

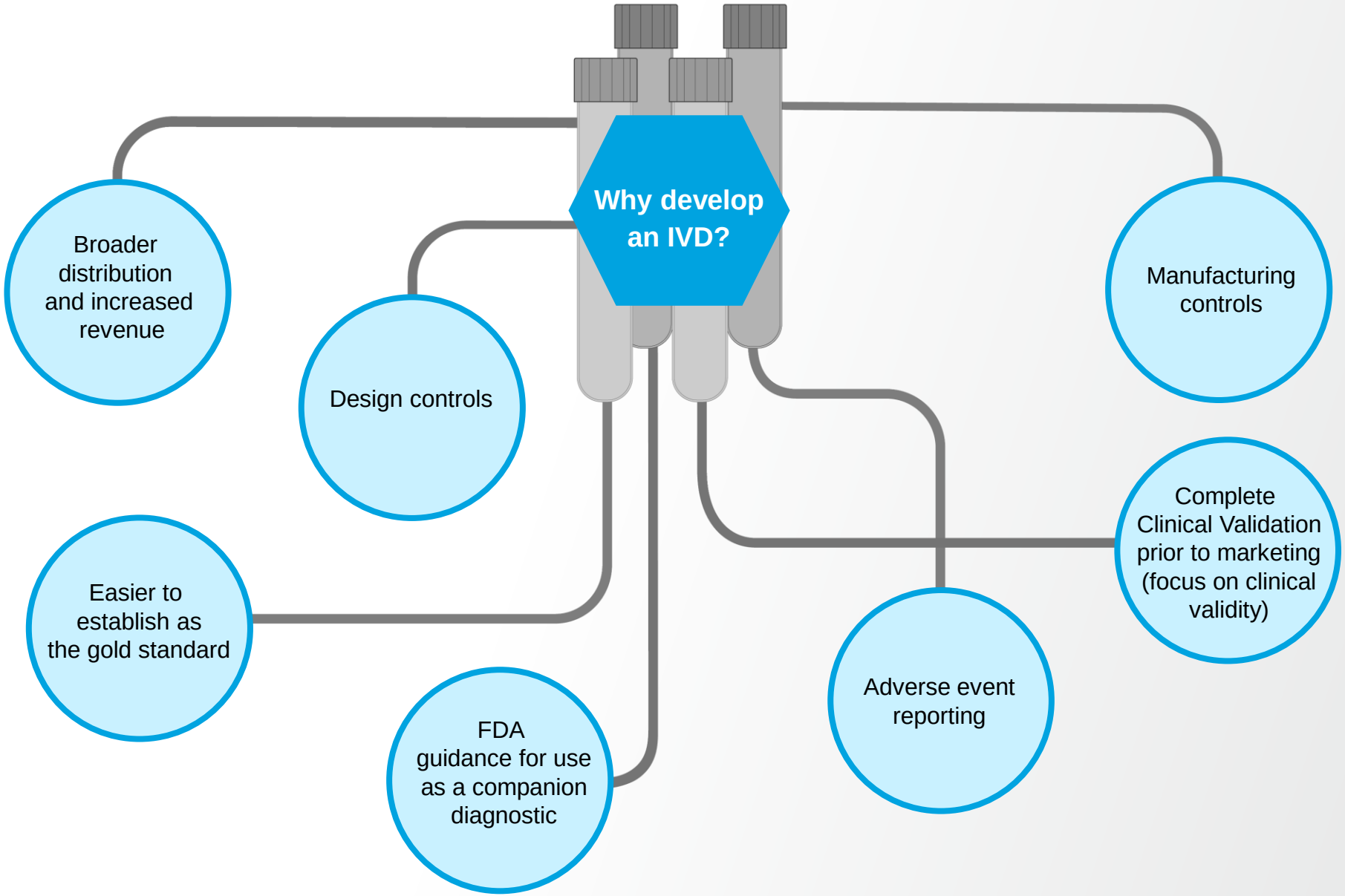
Laboratory Developed Test v. In Vitro Diagnostic Test

IVD test registered with FDA	LDT
Developed for sale to diagnostic laboratories, health clinics, or consumers	Developed by individual laboratories; not transferred, licensed, or sold
Standardized instrument qualification procedures and training required	Instrument qualification and training requirements established by individual laboratories
Must be pre-validated with a data analysis and bioinformatics report	Often developed in-house by necessity—no standard assay available
Must be clinically validated	Must be clinically verified and can be implemented quickly for emergency use (must be CLIA compliant)

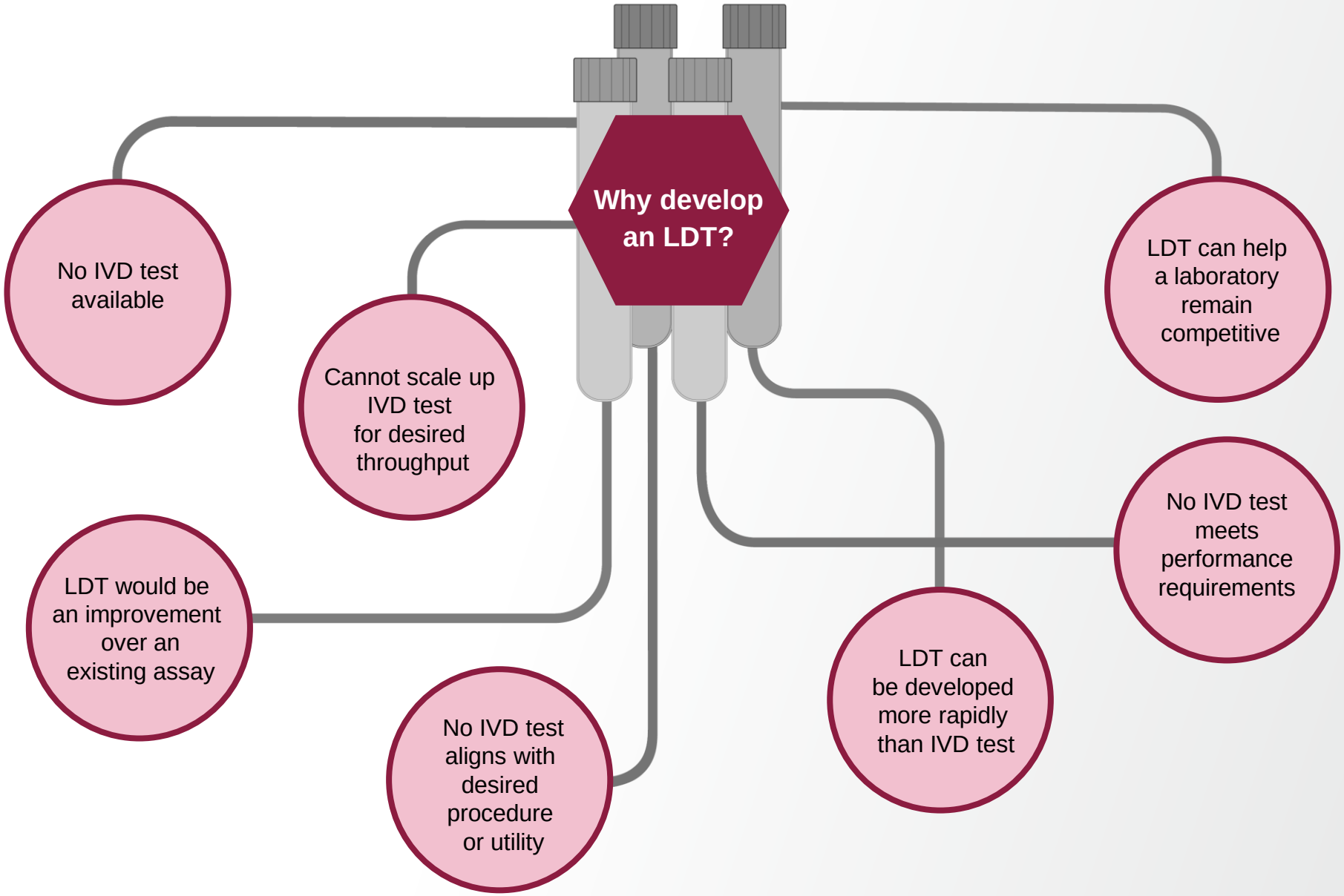
Molecular Tests Can Include A Wide Variety Of Assays For Different Applications



Reasons for Developing an IVD



Reasons for Developing an LDT



Advantages of IVDs

Advantage	Example
Quality System	Test is subject to a number of requirements including design controls, manufacturing controls and handling complaints.
Simplified inventory control	Only need to order the manufactured tests for anticipated use rather than LDTs which require inventory of the actual test, but also all of the components necessary to produce the test – reduced documentation.
Technical support	Customer can go to supplier's technical support to troubleshoot and also replace faulty products.
Clinical validity	Clinical validation of test to ensure that it identifies, measures or predicts if a clinical condition or predisposition is present or absent prior to marketing.
Broad distribution	Many laboratories can utilize the test providing greater amounts of use data which can increase (or possibly decrease) confidence in the test.

Advantages of LDTs

Advantage	Example
Control over content	Laboratories can select specific and relevant target(s).
Rapid adaptation	LDTs can be developed and modified relatively quickly to respond to market needs, such as outbreaks and rare diseases.
Lower cost per test	Technological advances have made complex analyses faster and more affordable.
Consolidation into a single test	Testing for multiple analytes provides more data per sample and may enable faster diagnosis.
Laboratory qualification	Laboratories and their quality systems are qualified rather than individual tests.

Typical LDT Process From Planning To Launch

Key questions:	Why run an LDT?	What is the test / panel?	Is test set-up properly and performing as expected?	Are test results clinically accurate?	What are best practices for LDT introduction?
Lab setup and accreditation	**Planning**	**Test configuration**	**Analytical validation**	**Clinical verification**	**Test launch***
Considerations: - CLIA certification - CAP (2-year cycle) - JCAHO - State-specific regulations	Examine available tests, technology options, and resources **Potential drivers:** - Lack of alternative test - Technological requirements (automation vs. manual) - Clinical, economic, or operational improvements - Reimbursement	- Assess available test menu - Select targets - Customization - Interpretation - Reporting	Validate analytical performance based on published clinical literature or CLSI* criteria - Sensitivity - Specificity - Reproducibility - Accuracy - Interference tests - Split samples for clinical validation	Run clinical samples to assess accuracy - Samples previously characterized by another laboratory (blind) - Compare results to check concordance	Announce test to providers Describe test utility in educational content/ forums Publish peer reviewed papers (if consistent with IP strategy)

Regulation

IVD	LDT*
Diagnostics include “reagents, instruments, and systems intended for use in the diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease.”	FDA claims that it *could* regulate LDTs, but is exercising *enforcement discretion*
Regulated by FDA as a Device when marketed to any third party – may require PMA or sponsor can use 510(k) pathway if suitable predicate device exists	Under the 1988 CLIA Amendments, all laboratories that test patient specimens must obtain a certificate of compliance or accreditation *in order to bill CMS for their services* *CLIA focuses on the laboratory and its personnel – not the test*
The sponsor can use non-approved/non-cleared FDA IVD for RUO or IUO, but MUST: (i) use proper labeling and (ii) refrain from use in clinical diagnostic procedures	LDTs are categorized into different levels of complexity (next slide)
FDA has authority to use Emergency Use Authorization (EUA) to provisionally approve IVDs during an emergency (e.g., pandemic)	For a limited time, FDA was requiring EUAs for SARS-CoV-2 LDTs – power revoked by HHS in August 2020

* https://www.fda.gov/medical-devices/in-vitro-diagnostics/laboratory-developed-tests

Test Complexity and Examples

CLIA categorization	Waived tests	Moderately complex tests	Highly complex tests
Description	• Simple to perform • Low risk of interpretation error • Little clinical significance • Many sold over the counter (OTC) for consumer use	• Usually performed with automated clinical laboratory equipment	• Require clinical laboratory expertise beyond automation • May require additional data analysis expertise
Examples	• Pregnancy tests • Strep tests • Dipsticks – Urine tests • Tests to detect drugs of abuse • Glucometers and other simple devices	• Electrolyte profiles • Chemistry profiles • Complete blood count • Urinalysis • Urine drug screen • Automated immunoassays	• Cytology • Immunohistochemistry • Peripheral smears • Flow cytometry • Gel electrophoresis • Most molecular diagnostic tests like RT-PCR, gene chip arrays, multiplexed analyses, dot blots, viral loads, expression arrays and CGH arrays

https://hub.ucsf.edu/clinical-laboratory-testing

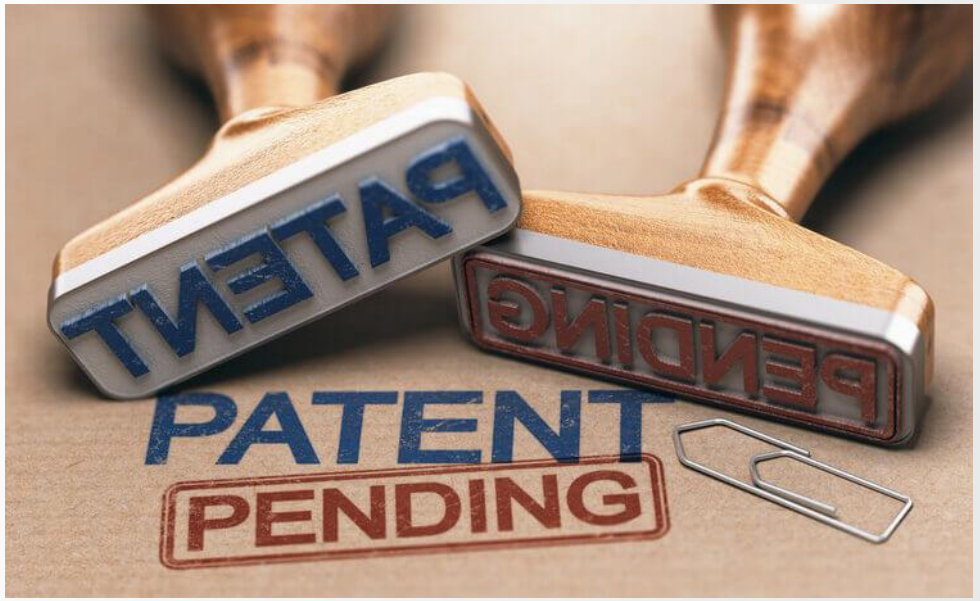
Intellectual Property Issues

Obtaining IP Protection

- Challenging if not impossible to protect:
  - Use of conventional platform technologies
  - Use of known/previously published biomarkers

- May be able to obtain patent protection for:
  - Novel platform technologies
  - Improvements to conventional platform technologies
  - Novel biomarkers
    - Mayo v. Prometheus Supreme Court case makes protection of novel biomarkers quite challenging
    - Without detail more “well-understood, routine, conventional” elements, the claims are not protectable because they recite only a high-level relationship

- One option – consider trade secret protection



Intellectual Property Issues

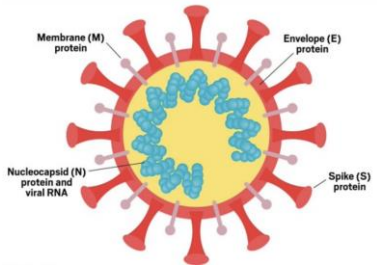
Pre-Commercialization Concerns

- Regardless of issues from Mayo case, patent infringement is still a concern
- As early as possible in the development process, engage counsel to conduct a freedom-to-operate (FTO) search and opinion
- Results of FTO search may necessitate preparation of invalidity/non-infringement opinions
  - FTO search may also guide patent-prosecution strategy
  - FTO search may also reveal the need to in-license one or more patents

LDTs in the COVID-19 Pandemic

- U.S. Centers for Disease Control and Prevention (CDC) created an LDT for SARS-CoV-2 within 10 days of genome sequencing
- University of Washington and Broad Institute each developed their own LDTs by end of January 2020
- U.S. labs also developed antibody-based assays
  - Limited deployment before FDA required EUA

What can we look for?



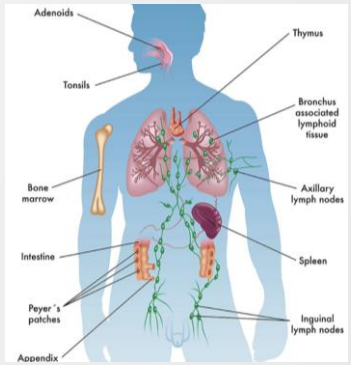
The Virus

Viral Elements

And / Or

The Infected

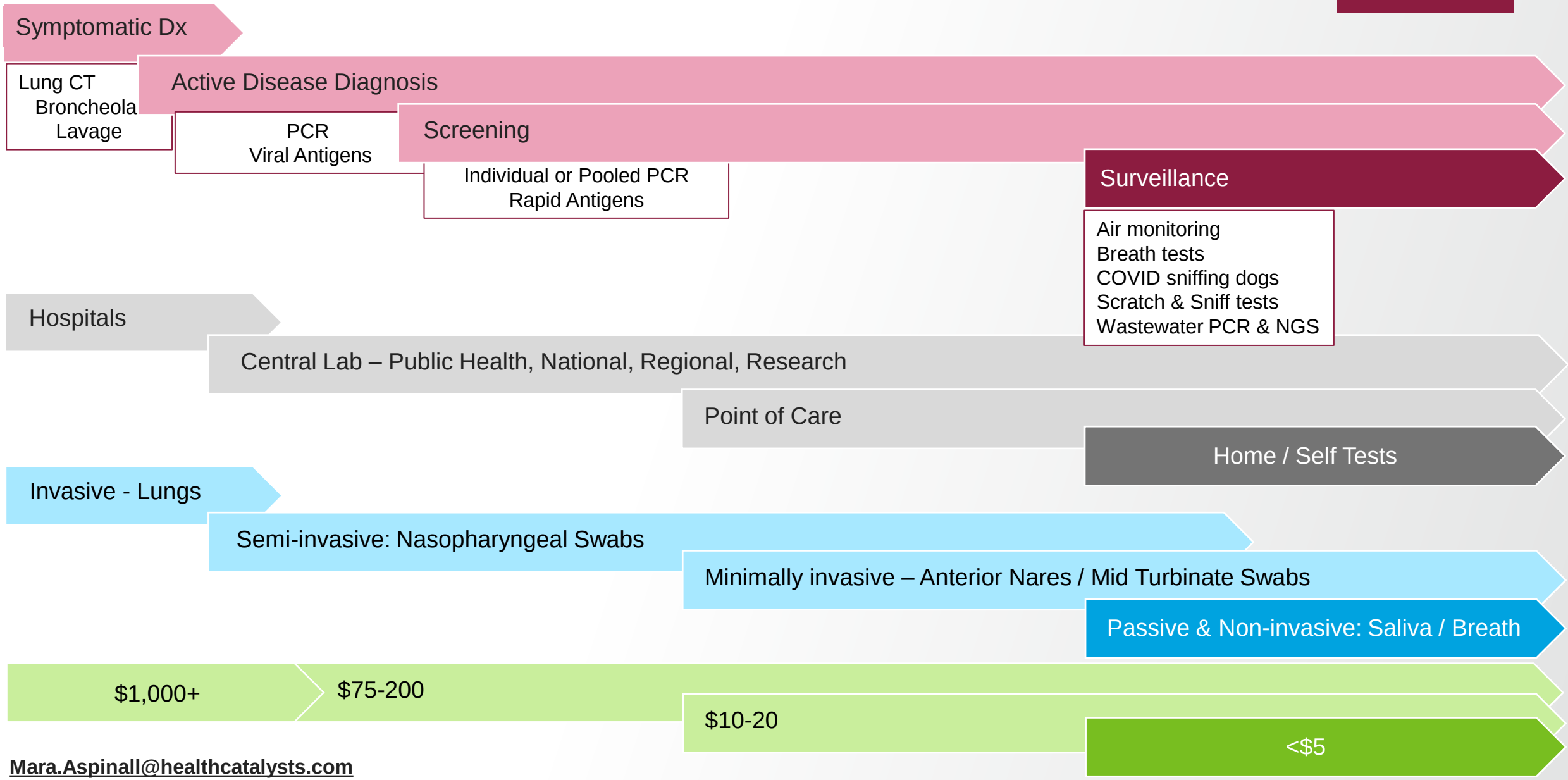
Immune Reaction



- **mRNA genome (29,811 base pairs)**
  - 80% identical to SARS 1, 50% identical to common cold
  - Unique primers designed to detect specific regions (PCR, Crispr etc.)
  - Requires two >10bp primers (ideally 18-22bp) to detect and amplify effectively
- **Viral proteins (29 different types)**
  - 4 external structural proteins
    - Most abundant is Nucleocapsid
    - Largest, most active & most unique is Spike (S1 ACE2 receptor binding; S2 TMPRSS cleavage site)
  - 25 other proteins

- **Circulating Antibodies (B Cell)**
  - Simple to analyze (e.g. present antigen; measure bound antibody from serum)
  - IgG: late but long lasting presence
  - IgM & IgA: early but brief presence
- **Circulating Activated T Cells**
  - Complex to analyze (e.g. Elispot: stimulate sample with antigen; quantify cytokine response (IFNγ,TNFα, or IL2 +)
- **Systemic biometric dynamics**
  - Oxygenation **/** Circulation (Pulse & Blood pressure)
  - Coagulation
  - Fever
  - Sense of Smell (Anosmia)
  - (VOC) Volatile Organic Compounds

The History and Progression of COVID-19 Diagnostics



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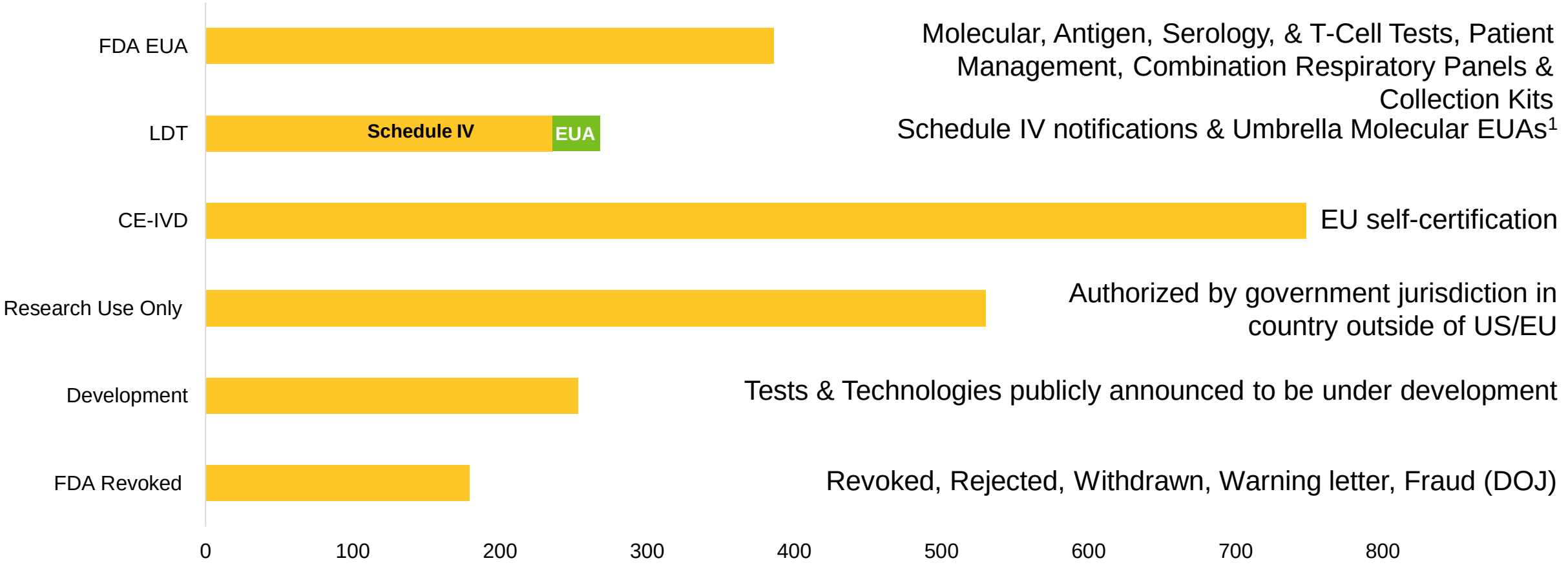
TestingCommons.com Review of COVID Tests

COVID-19 TESTING COMMONS | Arizona State University | WITH SUPPORT FROM The ROCKEFELLER FOUNDATION



1. n/a after 10/7/20 when HHS/FDA announced policy to not require authorization for any LDT
2. 19% of tests with approval internationally have been granted EUA by the US FDA

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Pandemic Total through 6/30/21

US FDA Emergency Use Authorizations

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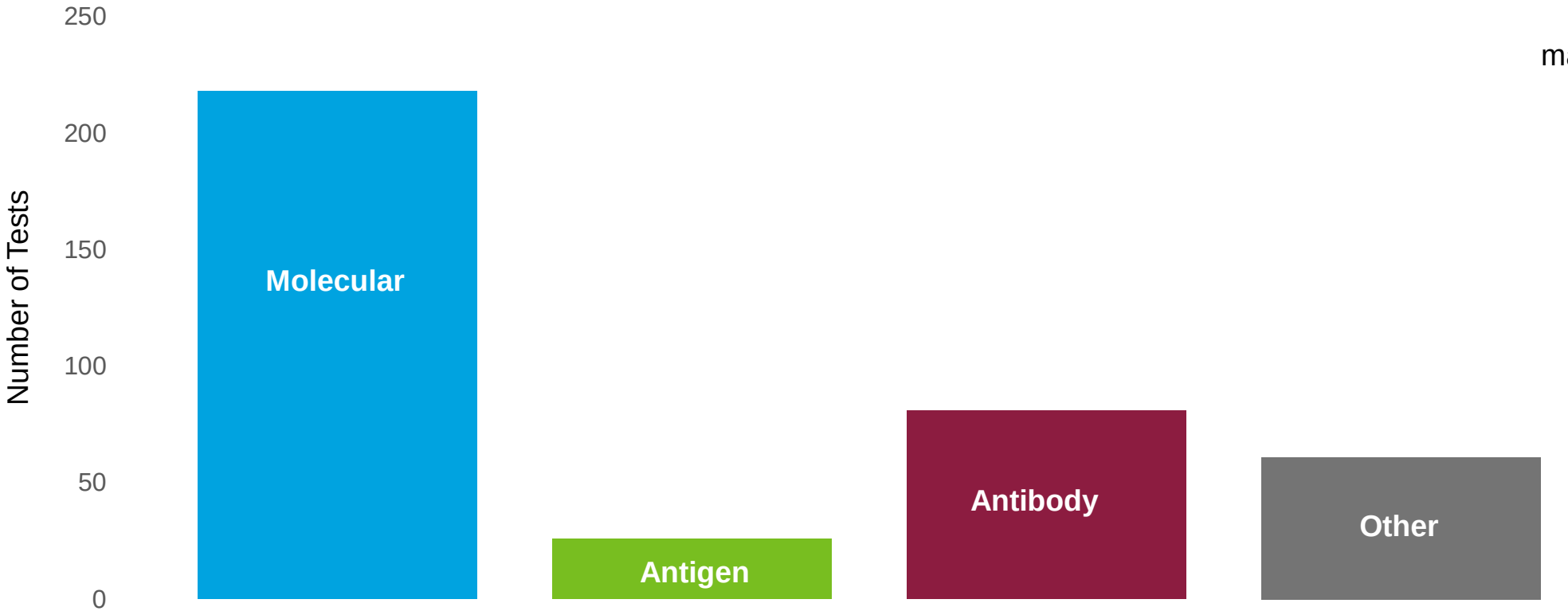

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Pandemic Total through 6/30/21

Molecular
- 88% RTqPCR
- 9% Isothermal
- 3% Sequencing
- 1% CRISPR

Antigen
- 88% Lateral Flow
- 8% Chemiluminescence
- 4% other

Antibody
- 60% ELISA
- 31% Lateral Flow
- 6% Chemiluminescence

Other
- Collection Kits
- Flu/RSV Panels
- Patient Management

Tests in Development Worldwide

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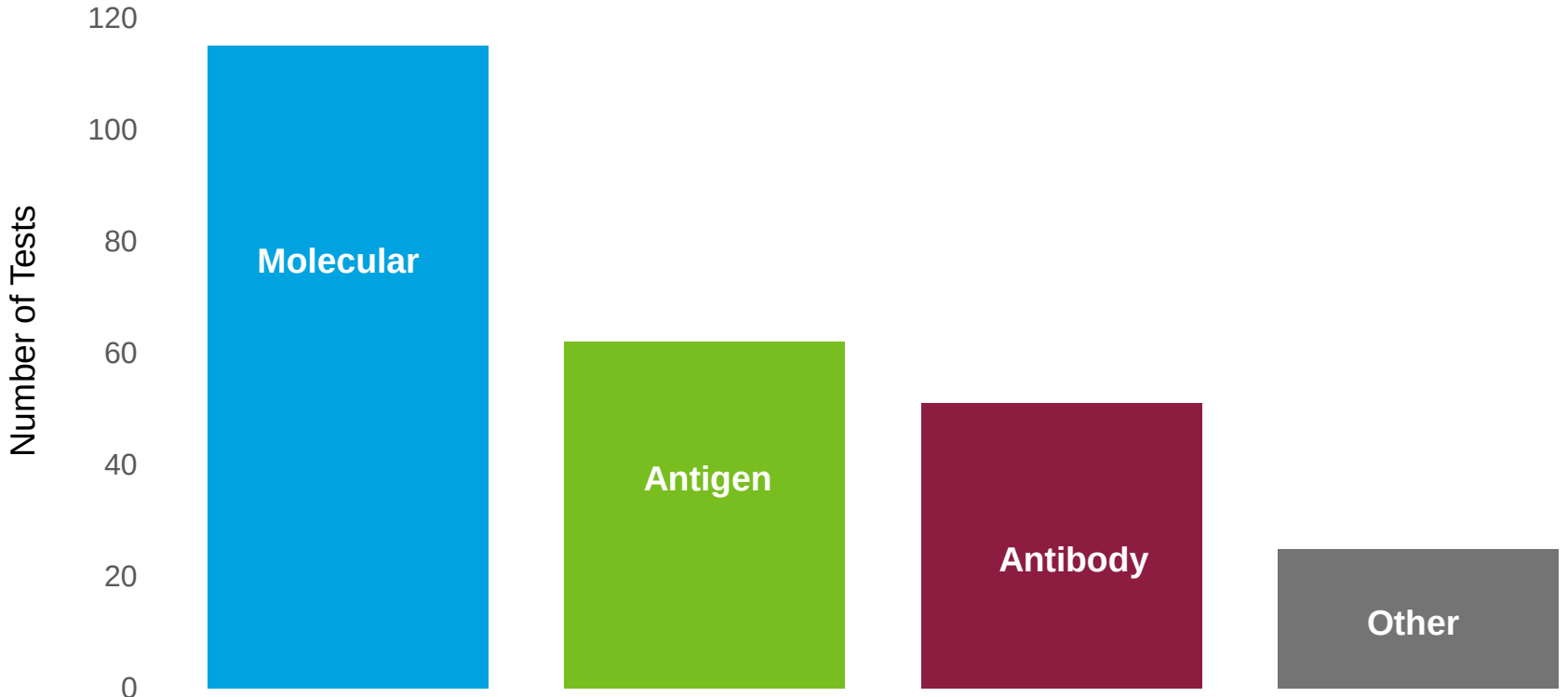

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Pandemic
Total through
6/30/21

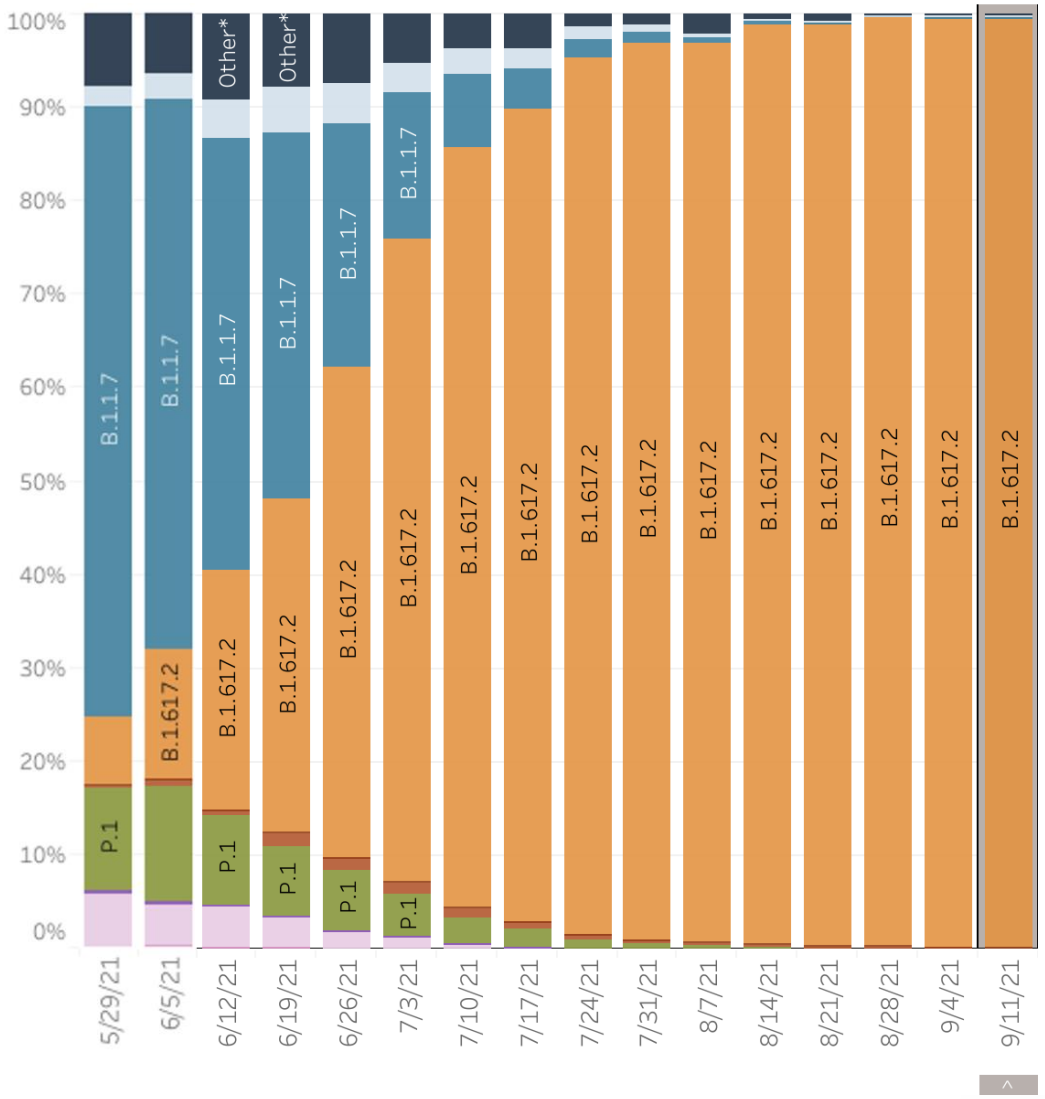
Molecular
40% RTqPCR
23% Isothermal
16% CRISPR
14% Sequencing

Antigen
56% Lateral Flow
18% ELISA
10% Breath
16% Other

Antibody
63% Lateral Flow
25% ELISA
12% Other

Other
Breath / VOC
Mass Spec
Raman Spec

…all because Delta (δ) is now 99.7% US cases



USA

WHO label	Lineage #	Type	%Total	95%PI
Alpha	B.1.1.7	VOC	0.0%	0.0-0.2%
Beta	B.1.351	VOC	0.0%	0.0-0.2%
Gamma	P.1	VOC	0.0%	0.0-0.2%
Delta	B.1.617.2	VOC	99.4%	98.6-100.0
	AY.1	VOC	0.2%	0.0-0.7%
	AY.2	VOC	0.1%	0.0-0.5%
Eta	B.1.525	VOI	0.0%	0.0-0.2%
Iota	B.1.526	VOI	0.0%	0.0-0.2%
Kappa	B.1.617.1	VOI	0.0%	0.0-0.2%
Mu	B.1.621		0.1%	0.0-0.5%
N/A	B.1.617.3	VOI	0.0%	0.0-0.2%
Other	Other*		0.2%	0.0-0.7%

* Enumerated lineages are VOI/VOC or are circulating >1% in at least one HHS region during at least one two week period; remaining lineages are aggregated as "Other".
** These data include Nowcast estimates, which are modeled projections that may differ from weighted estimates generated at later dates
# Sublineages of P.1, B.1.351 and B.1.621 are aggregated with the parent lineage and included in parent lineage's proportion. Q.1-Q.8 are aggregated with B.1.1.7. AY.3-AY.25 are aggregated with B.1.617.2.

δ=99.7%

Time to Dominance

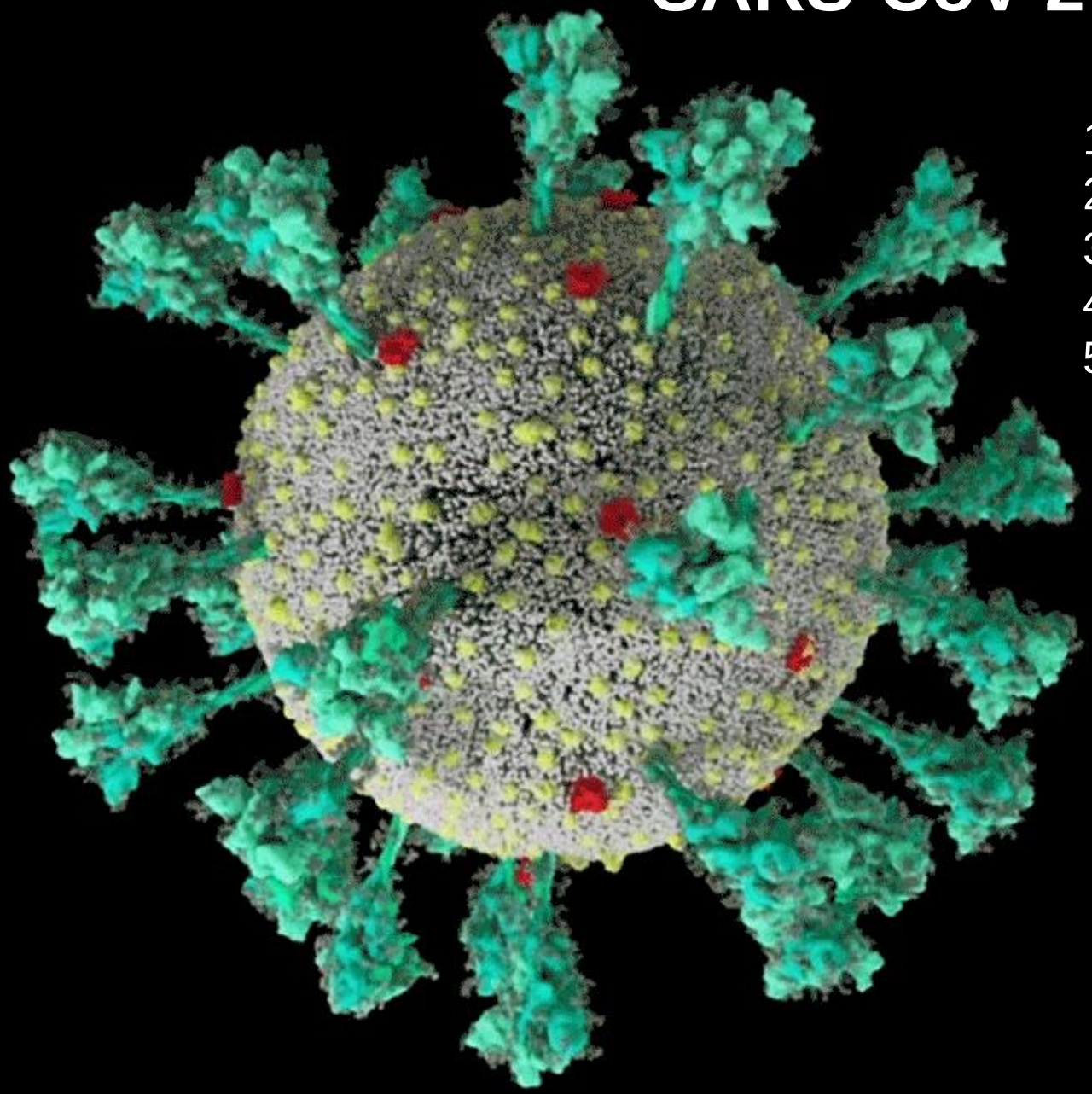
α (B.1.1.7) **3** months

δ (B.1.617) **1** month

powering the surge among unvaccinated

Source: https://covid.cdc.gov/covid-data-tracker/#variant-proportions accessed 9/20/21

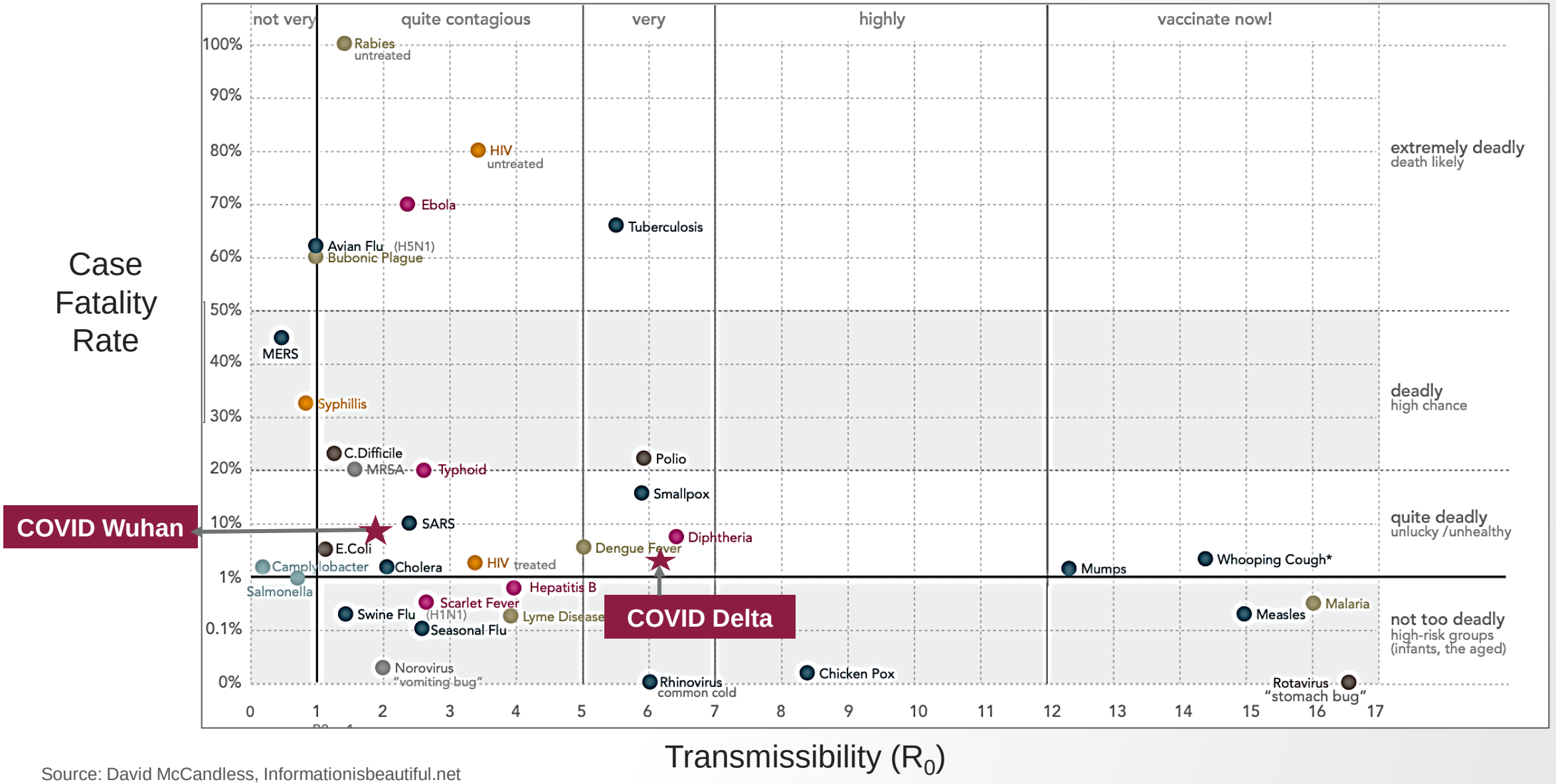
SARS-CoV-2 Variants: Five Questions



1.	Make tests less accurate?	No
2.	Increase cases and deaths?	Yes
3.	Make treatments ineffective?	Some
4.	Vaccine effectiveness?	Reduced
5.	Raise hurdle for herd immunity?	Yes

Source: Janet Iwasa, University of Utah: Nature Vol 595 7/2/21

SARS-COV-2 is not the worst epidemic threat possible



Source: David McCandless, Informationisbeautiful.net

Conclusion

- LDTs and IVDs are central to clinical patient care as well as medical research and development
  - Both types of tests will help make personalized medicine a reality for patients
- LDTs are an important locus of diagnostic innovation
- LDTs leverage a regulatory system that provides labs the ability to quickly adapt to changing needs
- LDTs are tied to laboratory processes while IVDs are tied to laboratory tests
  - Labs need to be cognizant of the processes and procedures that must be in place to correctly prepare, develop, perform and document the assay

Appendix

Specific Validation Comparisons

Validation requirements for LDTs and IVD tests

	IVD FDA test validation*	LDT validation*
Utility	Per product labeling	Determined by lab, as demonstrated in validation studies
Reproducibility (CV)	High and low controls: • Intra-run precision (10 or more samples) • Inter-run precision (10 days)	High and low controls: • Intra-run precision (10 or more samples) • Inter-run precision (10 days)
Analytical sensitivity	Determine LOD with serial low-end dilutions	Determine LOD with serial low-end dilutions
Analytical specificity	Identify interferents (mucus, normal flora, etc.)	Varies with sample type
Analytical range	Validate established package insert cutoff with 10 or more samples	Establish normal range using samples from a mixed male and female cohort
Clinical sensitivity	Verify performance per package insert with samples from patients with and without disease	Verify performance with samples from patients with and without disease
Clinical specificity	Verify performance per package insert with samples from patients with and without disease	Verify performance with samples from patients with and without disease
Method correlation study (R^2, slope)	Not usually applicable (refer to IVD label)	Comparison with a different platform
Interpretation	IVD label	Criteria established by laboratory
Documentation for inspector	• QC • Calibration • PT • Reviewed, updated, and approved procedures • training records • personnel qualifications	• Procedures • Utility (defined by laboratory)

* IVDs are regulated by FDA and need to be registered with FDA. Validation procedures and lab requirements are determined by the laboratory based on accrediting body, state and/or local policies and regulations.

Accreditation and validation parameters for LDT and IVD tests

	IVD FDA test validation*	LDT validation*
Accreditation	CLIA + CAP* or JCAHO*	CLIA + CAP or JCAHO
Assay reagents	IVD Kit	LDT Kit
Controls	Provided	Provided
Calibrators	Provided	Provided
Calibration verification (linearity)	Third party† every 6 months	Third party† every 6 months
Proficiency Testing	Third party, 2-3 tests per year	Third party, 2-3 tests per year

* CAP: College of American Pathologists

** JCAHO: Joint Commission on Accreditation of Healthcare Organizations

† Third party calibration (CAP, American Petroleum Institute, Maine Standards, American Association of Bioanalysts)