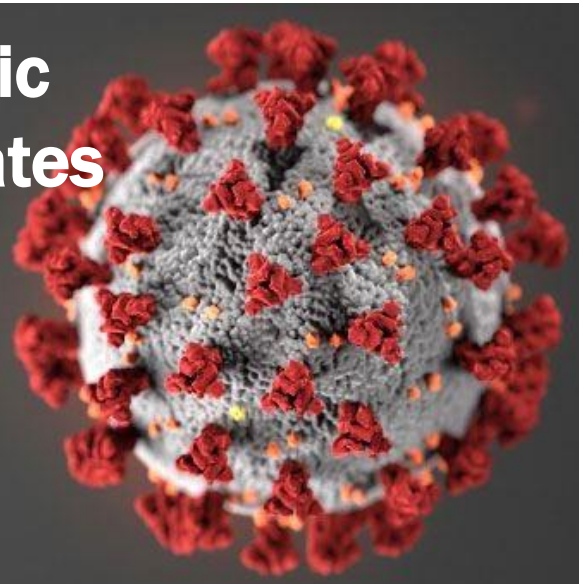
FIND

Because diagnosis matters

Diagnostics in the COVID-19 Pandemic Response: Knowledge gaps and updates on the performance of serology tests



ASLM Webinar: 08 May 2020

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What is the goal of testing for COVID-19?

***Either*:**
Stop transmission and prevent spread

- Countries with **no cases**
- Countries with 1 or more cases, imported or locally detected (**sporadic cases**)
- Countries experiencing **clusters of cases** related in time, geographic location, or common exposure

***Or*:**
Slow transmission, reduce case numbers, end community outbreaks; reduce health, social, economic impact; minimize healthcare disruptions for non-COVID-19 illness

- Countries experiencing larger outbreaks or sustained and pervasive local transmission (**community transmission**)

https://apps.who.int/iris/bitstream/handle/10665/331509/WHO-COVID-19-lab_testing-2020.1-eng.pdf

To accomplish these goals we can consider testing individuals for INFECTION and/or for EXPOSURE and it's important to select the right test.

Who should be tested?

- Use clinical (**symptoms**) and epidemiological factors (**exposure risk**) to ascertain likelihood of infection
  - **PCR testing of asymptomatic or mildly symptomatic** contacts can be considered in the assessment of individuals who have had contact with a COVID-19 case
  - Rapid collection and testing for patients meeting suspected case definition for COVID-19 is a priority for
    1. Clinical management
    2. Outbreak control
  - What might be the utility of POC testing (i.e. RDTs) for antigen or antibody-detection?

https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117

Unique features of SARS-CoV-2 that should be considered when using RDTs

- SARS-CoV-2 is a respiratory pathogen, unlike HIV, dengue, Zika, chikungunya
- Immune response may be atypical
  - HIV, flaviviruses, other viruses: IgM is detectable in the blood during active infection and then wanes after a few weeks; IgG levels rise after the acute phase
  - SARS-CoV-2: preliminary studies suggest that *both* IgM and IgG rise after the first few days of infection and may remain high for weeks (more data needed)

- There may be high levels of virus days before the onset of symptoms – between 6-44% of transmission may occur before symptom onset

- In a pandemic situation, where there are no specific treatments and the **goal is to minimize spread** of the infection, strive to select tests with the ***highest possible sensitivity*** to minimize the possibility of missing active cases…
  - To reduce the burden on confirmatory testing, a positive result from a screening test (even with low specificity and thus a higher probably of false positivity) may not require confirmation
  - In this scenario, all individuals who screen positive should be directed to home-isolate or be admitted to a healthcare facility, if symptoms are indicative of hospitalization

…but given that prevalence in most populations will be low, specificity is critical to ensure high PPV

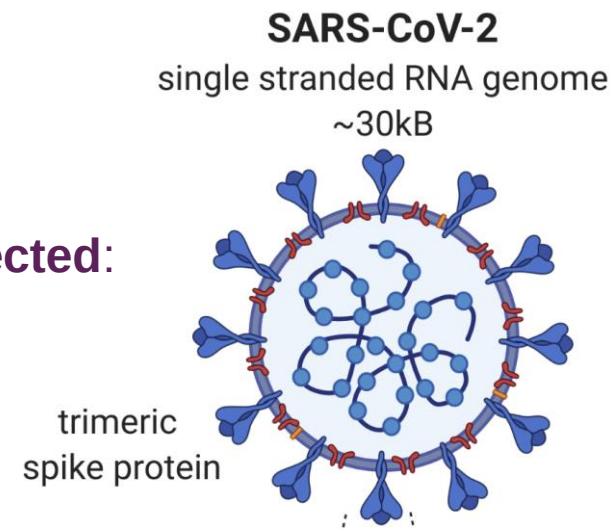
What do we know about SARS-CoV-2-specific antibodies and what can antibody tests tell us?

- Ab tests detect **the host response**; take several days to become positive; **likely most accurate 10+ days post infection**
  - Can target the **Nucleocapsid (N) protein** which is very abundant, and highly immunogenic, but is internal to the virus so likely not for neutralizing antibodies
    - Very conserved across coronaviruses so may have specificity issues
  - Can target the **Spike (S) protein**, which is responsible for viral entry into the host cell, and is likely the best target for for neutralizing antibodies
    - Very divergent across coronaviruses so likely more specific

- Ab tests **cannot distinguish between active and previous** infection on their own

- Ab tests **cannot currently confirm immunity to reinfection**

- Antibodies are the **best biomarker to estimate the number of people previously infected**:
  - Enables more accurate estimates of case fatality rates
  - Serial sampling could enable estimates of incidence
  - Prevalence estimates can help inform testing strategies, populations at higher risk



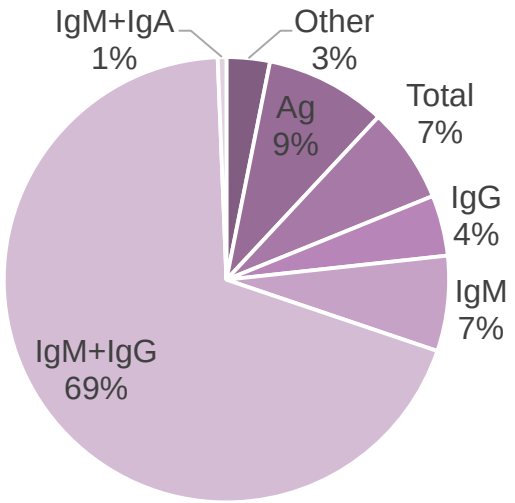
doi: https://doi.org/10.1101/2020.04.15.20066407

There is an overwhelming number of Immunoassays available...and more are being developed

FIND Pipeline 07 May 2020

159 RDTs

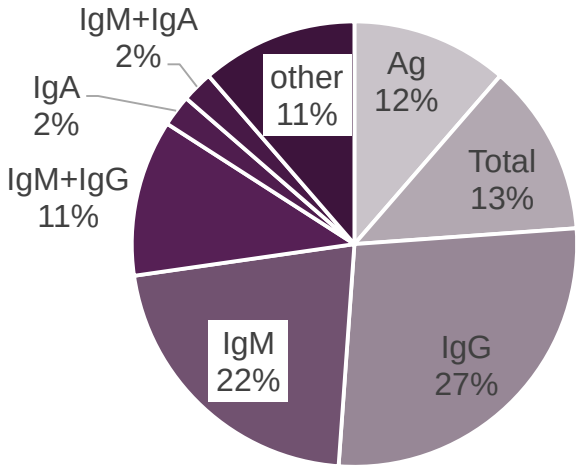
- 128 companies



- The majority of RDTs detect IgM+IgG

90 ELISA or Automated IAs

- 50 Companies



- The ELISAs/IAs mostly detect IgM, IgG or both
- If target antigen is described, the majority are specific for N protein

https://www.finddx.org/covid-19/pipeline/

What do we know about performance? (1) US FDA EUA

- US FDA has granted EUA to the below Serology tests:
  - *"In the early days of an infection when the body's immune response is still building, antibodies may not be detected. This limits the test's effectiveness for diagnosing COVID-19, and this is one reason serology tests should not be used as the sole basis to diagnose COVID-19."*

Date EUA Issued	Manufacturer	Diagnostic (Letter of Authorization)	Technology	Perfromance
05/04/2020	EUROIMMUN US Inc.	Anti-SARS-CoV-2 ELISA (IgG)	Serology IgG	HCP, Recipients, IFU
05/02/2020	Roche Diagnostics	Elecsys Anti-SARS-CoV-2	Serology Antibody	HCP, Recipients, IFU
04/30/2020	Wadsworth Center, New York State Department of Health	New York SARS-CoV Microsphere Immunoassay for Antibody Detection	Serology Total Antibody	HCP, Recipients, EUA Summary
04/29/2020	Bio-Rad Laboratories, Inc	Platelia SARS-CoV-2 Total Ab assay	Serology Total Antibody	HCP, Recipients, IFU
04/26/2020	Abbott Laboratories Inc.	SARS-CoV-2 IgG assay	Serology IgG only	HCP, Patients, IFU
04/24/2020	Autobio Diagnostics Co. Ltd.	Anti-SARS-CoV-2 Rapid Test	Serology IgM and IgG	HCP, Recipients, IFU
04/24/2020	DiaSorin Inc.	LIAISON SARS-CoV-2 S1/S2 IgG	Serology IgG only	HCP, Recipients, IFU
04/24/2020	Ortho-Clinical Diagnostics, Inc.	VITROS Immunodiagnostic Products Anti-SARS-CoV-2 IgG Reagent Pack	Serology IgG only	HCP, Recipients, IFU
04/15/2020	Mount Sinai Laboratory	COVID-19 ELISA IgG Antibody Test	Serology IgG	HCP, Patients, EUA Summary
04/14/2020	Ortho Clinical Diagnostics, Inc.	VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total Reagent Pack	Serology Total Antibody	HCP, Patients, IFU
04/14/2020	Chembio Diagnostic System, Inc	DPP COVID-19 IgM/IgG System	Serology IgM and IgG	HCP, Patients, IFU
04/01/2020	Cellex Inc.	qSARS-CoV-2 IgG/IgM Rapid Test	Serology IgM and IgG	HCP, Patients, IFU

https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd

What do we know about performance? (1) US FDA EUA – cont.

- Performance data submitted by Suppliers; US FDA in partnership with NCI and BARDA have started to conduct independent performance validation studies – results available for EuroImmun
  - PCR positives and historic unexposed controls (negatives)

Company	Target	Format	Sensitivity	95% CI	n	Specificity	95% CI	n
Abbott	IgG	High Throughput ELISA	100.0%	(95.8-100)	88	99.6%	(99-99.9)	1070
Autobio	IgM+IgG	Lateral Flow	88.1%	(84.6-90.9)	405	99.0%	(97.2-99.7)	312
Bio-Rad	Pan-Ig	High Throughput ELISA	92.2%	(81.5-96.9)	51	100%	(98.7-99.9)	687
Cellex	IgM+IgG	Lateral Flow	93.8%	(88.2-96.8)	128	96.0%	(92.8-97.8)	250
Chembio	IgM+IgG	Lateral Flow	93.5%	(79.3-98.2)	31	94.4%	(88.9-97.3)	125
Diasorin	IgG	High Throughput ELISA	97.6%	(87.4-99.6)	41	99.3%	(98.6-99.6)	1090
EuroImmun*	**IgG**	**ELISA**	**90.0%**	**(74.4-96.5)**	**30**	**100.0%**	**(95.4-100)**	**80**
Ortho-Clinical	IgG	High Throughput ELISA	87.5%	(75.3-94.1)	48	100%	(99.1-100)	407
Ortho-Clinical	Pan-Ig	High Throughput ELISA	83%	(68.1-93.1)	36	100%	(99-100)	400
Roche	Pan-Ig	High Throughput ELISA	100%	(88.3-100)	29	100%	(99.7-99.9)	5272

**independently verified by NCI*

https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance

What do we know about performance? (2) COVID Dx Project: Independent Evaluation Results

- Collaborators from UCSF, UC-Berkeley, Innovative Genomics Institute and Chan Zuckerberg Biohub performing head-to-head evaluations of LFAs and ELISAs
  - Sample panel: 130 plasma or serum samples from 80 symptomatic SARS-CoV-2 RT-PCR-positive individuals; 108 pre-COVID-19 negative controls; and 52 recent samples from individuals who underwent respiratory viral testing (Biofire Panel) but were not diagnosed with Coronavirus Disease2019 (COVID-19).
- The percent seropositive increased with time, peaking at **81.8-100.0% in samples taken>20 days after symptom onset**

		Supplier											
Assay	Days Since Onset	BioMedomics	Bioperfectus	DecomBio	DeepBlue	Innovita	Premier	Sure-Bio	UCP Biosciences	VivaDiag	Wondfo	Epitope ELISA	In-House ELISA
IgM	1-5d	26.9%	40.7%	32.0%	44.4%	14.8%	37.0%	11.1%	25.9%	29.2%		18.5%	
	6-10d	61.1%	74.3%	66.7%	77.8%	33.3%	71.4%	42.9%	58.3%	62.9%		52.8%	
	11-15d	73.5%	80.0%	85.3%	80.0%	37.5%	80.0%	62.9%	74.3%	83.9%		77.1%	
	16-20d	76.2%	76.2%	70.0%	76.2%	28.6%	76.2%	66.7%	71.4%	71.4%		66.7%	
	>20d	81.8%	100.0%	90.9%	90.9%	16.7%	90.9%	72.7%	90.9%	90.0%		81.8%	
IgG	1-5d	23.1%	25.9%	28.0%	22.2%	25.9%	22.2%	18.5%	25.9%	29.2%		40.7%	
	6-10d	52.8%	65.7%	66.7%	50.0%	47.2%	51.4%	54.3%	50.0%	62.9%		77.8%	
	11-15d	67.7%	77.1%	85.3%	60.0%	75.8%	62.9%	71.4%	71.4%	80.7%		88.6%	
	16-20d	66.7%	66.7%	70.0%	71.4%	64.3%	66.7%	66.7%	66.7%	66.7%		76.2%	
	>20d	81.8%	90.0%	90.9%	81.8%	66.7%	81.8%	90.9%	81.8%	90.0%		90.9%	
IgG +/or IgM	1-5d	30.8%	40.7%	32.0%	44.4%	25.9%	37.0%	18.5%	25.9%	29.2%	40.0%	40.7%	37.0%
	6-10d	63.9%	77.1%	66.7%	77.8%	55.6%	71.4%	54.3%	58.3%	62.9%	66.7%	80.6%	72.2%
	11-15d	76.5%	85.7%	85.3%	80.0%	75.8%	82.9%	71.4%	77.1%	83.9%	81.8%	88.6%	91.4%
	16-20d	81.0%	81.0%	70.0%	81.0%	64.3%	81.0%	71.4%	71.4%	71.4%	81.0%	81.0%	81.0%
	>20d	81.8%	100.0%	90.9%	90.9%	83.3%	90.9%	90.9%	90.9%	90.0%	81.8%	90.9%	81.8%

https://covidtestingproject.org/ https://doi.org/10.1101/2020.04.25.20074856

What do we know about performance? (2) COVID Dx Project: Independent Evaluation Results – cont.

- Test specificity ranged from **84.3-100.0% in pre-COVID-19 specimens**

Specificity

Assay	BioMedomics	Bioperfectus	DecomBio	DeepBlue	Innovita	Premier	Sure-Bio	UCP Biosciences	VivaDiag	Wondfo	Epitope ELISA	In-House ELISA
	Supplier											
IgM	87.9%	97.1%	90.7%	84.3%	96.3%	98.2%	100.0%	98.1%	95.0%		97.2%	
IgG	96.3%	98.1%	91.6%	99.1%	100.0%	99.1%	100.0%	98.1%	96.0%		90.7%	
IgG +/or IgM	86.9%	95.2%	89.7%	84.3%	96.3%	97.2%	100.0%	98.1%	95.0%	99.1%	89.8%	99.1%

https://covidtestingproject.org/ https://doi.org/10.1101/2020.04.25.20074856

What do we know about performance? (3) FIND Data Aggregation

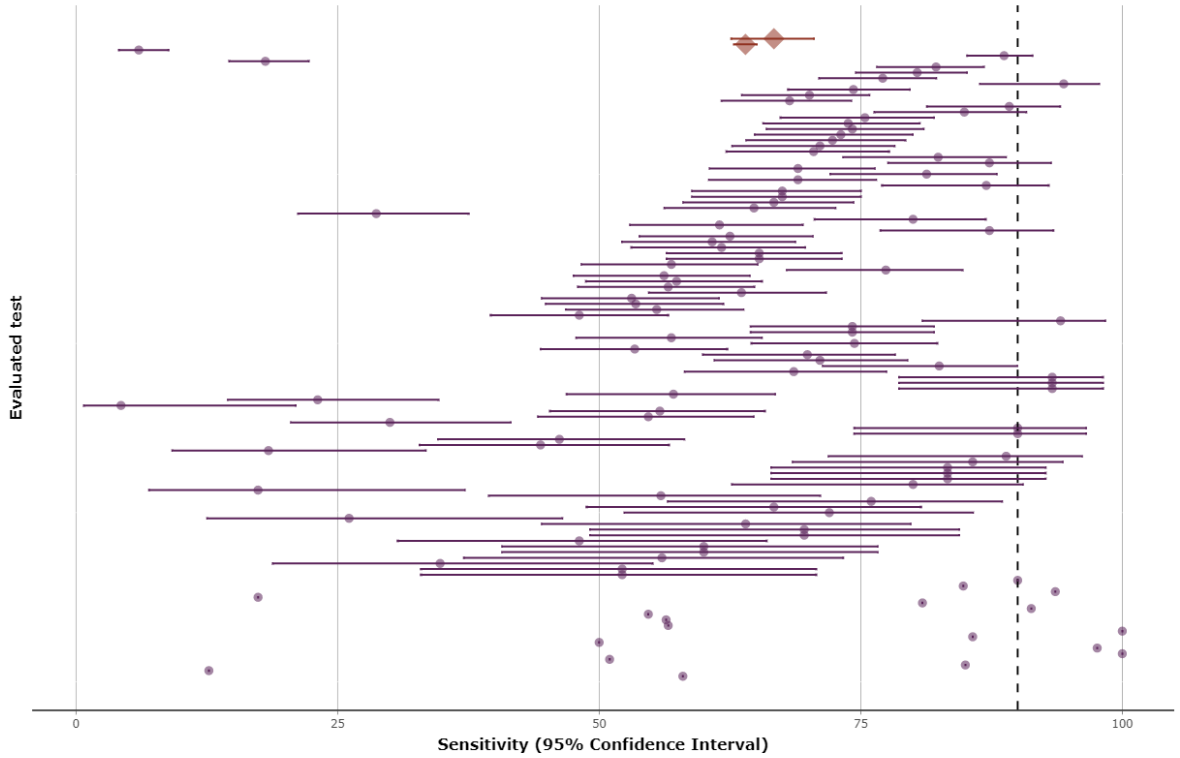
- FIND is reviewing publicly available data (published or preprints) and has an open call for partners and laboratories to directly submit performance data on commercially available IVDs for SARS-CoV-2 NAT, Ag or Ab tests
  - 44 studies from 15 countries (05 May 2020): 19 from publicly available resources; 25 submitted via web-form
  - Data on 77 different tests (29 Molecular, 2 Ag, **46 Ab**) from 70 companies
  - Majority of tests evaluated in one or two studies
  - Limited data on performance of molecular & antigen-based tests

https://www.finddx.org/covid-19/dx-data/

What do we know about performance? (3) FIND Data Aggregation:

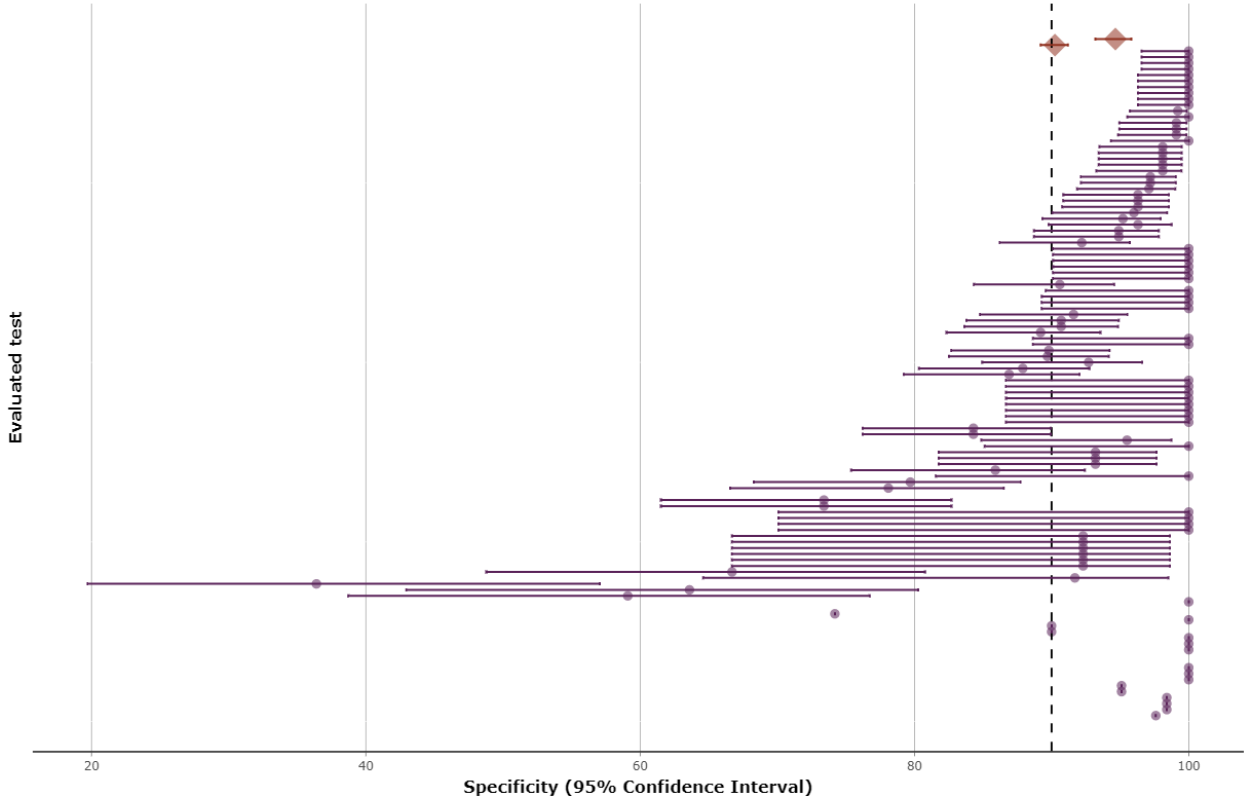
Overall sensitivity is poor

Sensitivity



Meta-analysis Fixed Effect: **66.7 [62.6-70.5]**
Meta-analysis Random Effect: **64.0 [62.9-65.1]**

Specificity

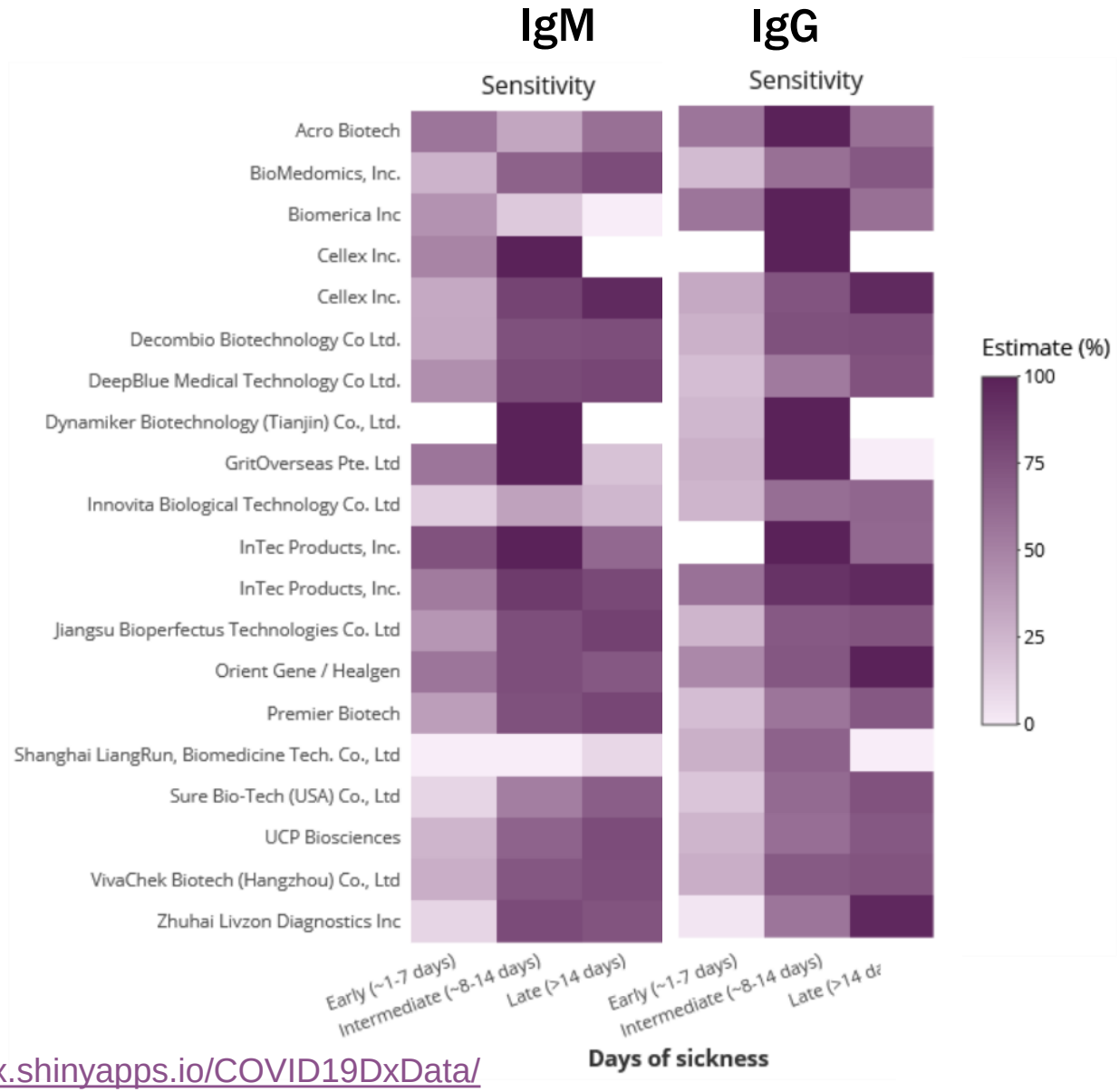


Meta-analysis Fixed Effect: **90.34 [89.2-91.2]**
Meta-analysis Random Effect: **94.65 [93.2-95.8]**

https://finddx.shinyapps.io/COVID19DxData/

What do we know about performance? (3) FIND Data Aggregation:

Sensitivity according to days from symptom onset is most informative



- Overall:
  - sample sizes are small (n < 50)
  - Poor performance within ~7 days post symptom onset
  - Gradual increase in performance after ~7 days from symptom onset
  - Specificity evaluation mostly on samples from healthy controls – limited geographic diversity, i.e. samples with antibodies to various endemic infections, e.g. malaria, HIV, dengue, etc.

https://finddx.shinyapps.io/COVID19DxData/

FIND is conducting limited performance evaluations of molecular tests and immunoassays for SARS-CoV-2 to support accurate, affordable, accessible testing in LMIC

Background:

- Although many tests are rapidly entering the market and achieving Emergency Use Authorization by National Regulatory Agencies, there is a need to generate **independent data on assay performance** to inform product selection:
  - To ensure global access to a diversity of accurate and high-quality testing modalities
  - To design testing strategies to inform clinical management, prevention, and containment

FIND's approach:

- We launched two Expressions of Interest (EOI) for molecular and immunoassay test suppliers to participate in independent evaluations (end Feb for NAT; end March for IA – both Ag and Ab)
  - Received: > 150 NAT, 19 Ag IA, 95 Ab IA applications
  - Products were selected based on reported performance, regulatory status, QMS, and LMIC distribution capacity
    - 21 manual NAT, 5 Ag RDT, 26 RDT, 7 ELISA initially selected
  - We are continuing to review applications on a rolling basis for ongoing evaluations

- We are actively monitoring the product pipeline and using our knowledge of IVD companies to **spur test innovations** to address performance or use-case gaps

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

Antibody Test Evaluation Study Overview

Study design	Retrospective, multicenter
Study Sites*	USA, Europe, South Africa, South America (n = 9) - Each RDT will be evaluated at two sites - Each ELISA will be evaluated at minimum one site (Europe) and some will be evaluated at a second site
Use Case	Detection of serostatus to determine exposure to COVID-19, intended for 1) triage of COVID-suspected[1] patients, 2) aid in diagnosis of COVID-suspected[1] patients, and 3) assessment of recovery in COVID-19-convalescent patients. [1] as defined by country or WHO case definitions
Study Samples	- De-identified, remnant plasma or serum from a minimum of 100 COVID-19 RT-PCR positive from acute and convalescent individuals across sub-categories of days post symptom onset ▪ N = 10 for Day 0-3, N = 20 for Day 4-7, N = 30 for Day 8-14, N = 20 for Day 15-28, N = 20 for Day 29+) - Minimally 100 (ideally 300) COVID-19 negative samples ▪ historic controls, including some confirmed for other respiratory infections ▪ Some sites: PCR negative suspect cases - Addition of 10 Malaria Pos and Dengue Pos samples
Reference	RT-PCR EuroImmun IgG (S1) Assay

March:
- EOI launch, application review

April:
- study design/site selection, procurement

May:
- study start on a rolling basis
- First results

June:
- studies continue
- Results available

** More sites to be added overtime*

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

Test Utility (post-test performance) is dependent on accuracy and pre-test probability (prevalence)

- No test is perfect -- every test returns some false positive and false negative results -- therefore broad use of the tests, when not appropriately informed by other relevant information, could identify too many false-positive individuals.

Target population	Example Prevalence Range
Symptomatic healthcare workers	High to Very high (10 - ≥ 30%)
Healthcare workers with significant exposure	Medium to High (5 - 10%)
Contacts of index patient	Low to High (2 - 10%)
Community testing/contract tracing of hotspots	Medium to High (5 - ≥ 10%)
Symptomatic general population	Low (2%)
Asymptomatic general population	Very low to Low (≤ 2%)

- Given timing of antibody expression and expected prevalence in populations being screened for active infection, the **use of serology tests to screen for active infection is unlikely to be beneficial** as PPV will remain low

- In order to more appropriately plan public health measures and understand chains of transmission, it is critical to define prevalence therefore **use of serology tests to screen for exposure (ie prior infection in individuals exposed ≥ 10 days) will be beneficial**. Should select tests with high PPV.

- Data are rapidly becoming available that define the accuracy of specific serological test products to detect antibodies but the **correlation with effectiveness and duration of protective immunity remains to be elucidated**

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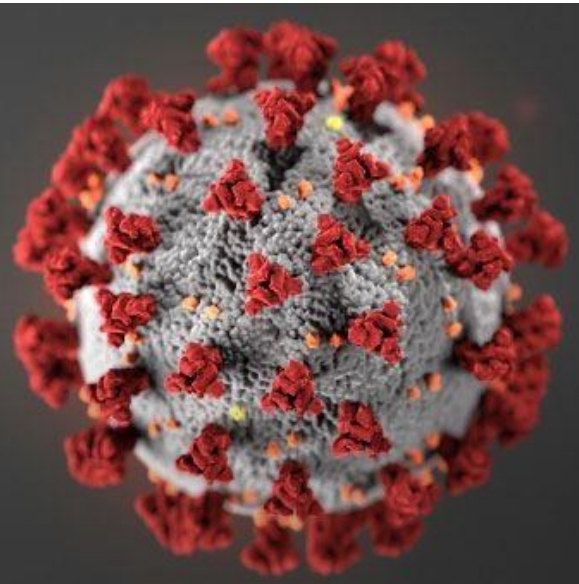
THANK YOU



For more information please contact the FIND Pandemic Preparedness team:
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www.finddx.org/covid-19