


University of California
San Francisco

Covid Testing for Dermatologists

An update on PCR, antibodies, what they mean, and how to interpret results

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19 May 2020



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1st UCSF Covid toes case

44-year old female w 3 weeks of severe fatigue and dry cough presented with a CC of “I woke up with lollipop toes.”

Covid PCR negative







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Negative Covid PCR test was unsurprising

Mild/Subclinical infection
(virus present)

10-14 days

Covid Toes as “convalescent” manifestation?

>60 Covid toes cases at UCSF
All our tested patients have been SARS-CoV-2 PCR negative

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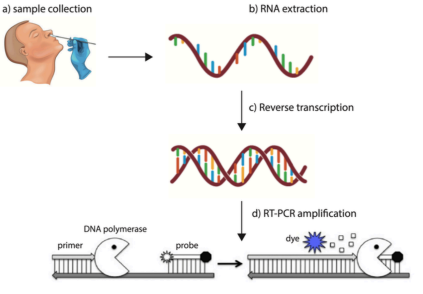
SARS-CoV-2 PCR: DIRECT test for virus detection

a) sample collection

b) RNA extraction

c) Reverse transcription

d) RT-PCR amplification



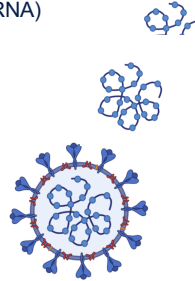
PCR primers are designed to match SARS-CoV-2 RNA sequences

<https://www.globalbiotechinsights.com/articles/20247/the-worldwide-test-for-covid-19>

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PCR testing details

- Detects viral genome (replicating virus and residual RNA)
- Viral nucleic acids present in body fluids:
 - YES respiratory epithelia (swab)
 - YES sputum/ET tube secretions (prolonged+)
 - YES stool (prolonged+, not infectious)
 - NO blood
 - NO urine

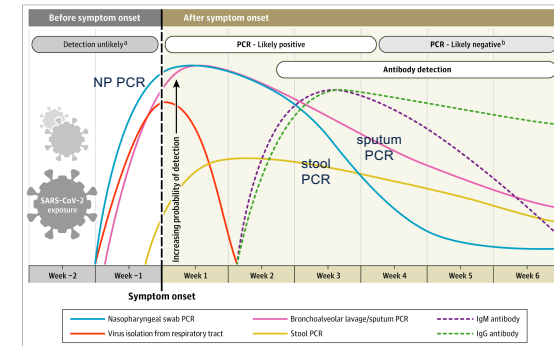


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Wolfel et al. *Nature*. 2020 Apr 1. doi: 10.1038/s41586-020-2196-x.



WHEN SHOULD THE PATIENT GET PCR TESTED? ASAP



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Sethuraman N, Jeremiah SS, Ryo A. Interpreting Diagnostic Tests for SARS-CoV-2. *JAMA*. Published online May 06, 2020. doi:10.1001/jama.2020.8259



LIMITATION OF PCR testing



the virus must be there!
finite window of active shedding EARLY
~day -8 to +8, 10^5 - 10^7 copies/ml then falls

in mild/subclinical infection, can easily miss the window
interpreting negative PCR tests depends on sample timing!

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To et al. *Lancet Infect Dis*. 2020;20(5):565-574. doi:10.1016/S1473-3099(20)30196-1



Exanthems are most likely to present in the window where PCR for viral DNA detection is useful

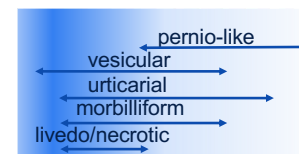


Table 2. Temporal relationship with other manifestations of COVID-19.

Timing of cutaneous signs with respect to other symptoms	Pseudo-chilblain	Vesicular	Urticaria	Maculopapules	Livedo/necrosis	Total
Before, n (%)	5 (7)	5 (15)	3 (4)	8 (5)	1 (5)	22
Same time, n (%)	24 (34)	19 (56)	43 (61)	108 (61)	18 (86)	212
After, n (%)	42 (59)	10 (29)	25 (35)	60 (34)	2 (10)	139
Total	71	34	71	176	21	373

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Galván Casas et al. *Br J Dermatol*. 2020;10.1111/bjd.19163. doi:10.1111/bjd.19163



Most but not all Covid toes patients test PCR neg

Pediatric Dermatology May 2020 x3 papers
Cordoro et al. all 6 negative (San Francisco)
Colonna et al. all negative (Italy)
Andina et al. 1 of 19 PCR+ (Spain)

JAAD May 2020
Masson et al., 277 patients ~15% tested,
 23% of PCR+ patients (<10) had acral lesions



Additional case reports of PCR+ adults
 during URI/systemic symptoms
 with prolonged pernio (>1 month)
 & several additional in the AAD registry

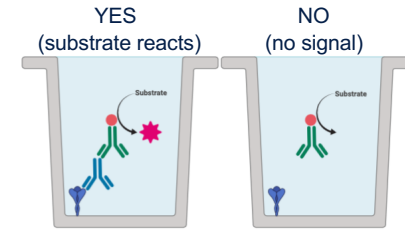
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What about antibody testing? INDIRECT assay

Measures prior pathogen exposure and + humoral (Ab) immune response
 Q: Is there detectable SARS-CoV2 protein-reactive antibody present in serum?

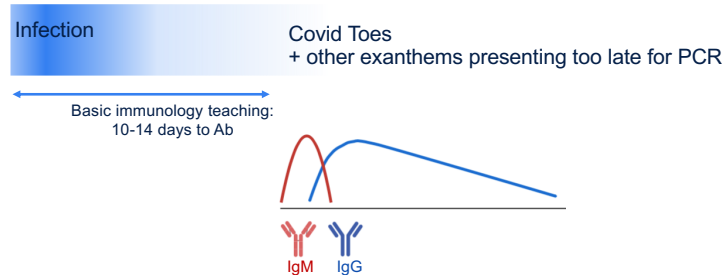


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How does antibody testing complement PCR tests? TIMING



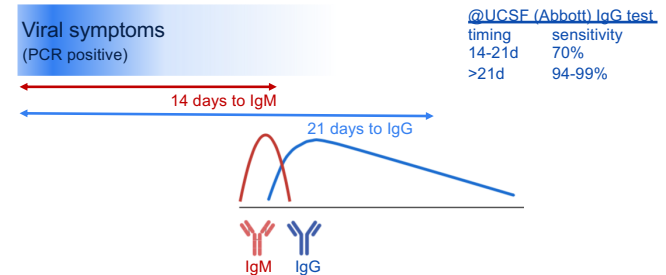
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ADVANTAGE OF Ab testing: potentially longer window

Covid Ab detectable 14-21d+ after symptom onset



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BIOLOGICAL CHALLENGES OF COVID-19 ANTIBODY TESTING:

- MULTIPLE ANTIGENS
- UNUSUAL KINETICS OF IMMUNOGLOBULIN PRODUCTION
- POTENTIAL FOR CROSS-REACTIVITY
may detect pre-existing antibodies to other coronaviruses
- ANTIBODY TITERS DEPEND ON INFECTION SEVERITY

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MULTIPLE COVID-19 protein antigens

spike protein (full-length & RBD)

Peiris et al. Severe acute respiratory syndrome. *Nature Medicine Supplement*, 2004, 10 (12)
Wrapp et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* (2020); 367 (6483): 1260-1263

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during immune response, multiple viral antigen-antibody reactions occur

in the lab, enzyme-linked or colorimetric assays test *specific* antibody-antigen interactions

anti-spike antibody present?
anti-envelope antibody?
anti-nucleocapsid antibody?

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KINETICS OF ANTIBODY RESPONSE

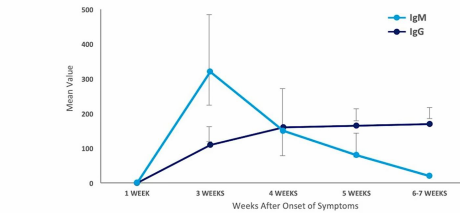
Papers from China suggest variable antibody production, sometimes very early and/or concurrent with PCR+

Long et al. *Nat Med* (2020) <https://doi.org/10.1038/s41591-020-0897-1>

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KINETICS OF ANTIBODY RESPONSE – PROLONGED IgM+ PHASE?

Quantitative Results for SARS-CoV2 IgM and IgG



A.T. Xiao et al. *J Infect* (2020) doi: <https://doi.org/10.1016/j.jinf.2020.03.012>

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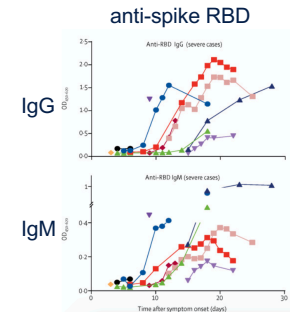
Xiao et al. *J Infect* (2020) doi: <https://doi.org/10.1016/j.jinf.2020.03.012>

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KINETICS OF ANTIBODY RESPONSE

IgM and IgG rise together rather than sequentially (also true for IgA, not shown)

Rapid antibody responses detectable day 4-7 → plateau day 14



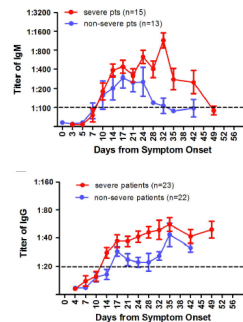
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To et al. *Lancet Inf Dis* (2020): [https://doi.org/10.1016/S1473-3099\(20\)30181-1](https://doi.org/10.1016/S1473-3099(20)30181-1)

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KINETICS OF ANTIBODY RESPONSE

Another example of rapid seroconversion and near-simultaneous rise in IgM/IgG



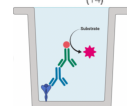
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CROSS-REACTIVITY with OTHER CORONAVIRUSES - IT'S OUT THERE!

Antigen	Antibody	Sample type	Specificity	References
Spike (S)	Entire S	IgM, IgG	Patient serum	Not reported (10, 11)
	IgG	Patient serum	Cross-react with SARS-CoV and MERS-CoV	(14)
	Not indicated	Patient plasma	Cross-react with SARS-CoV	(19)
	IgM, IgG, IgA	Patient serum or plasma	Not reported	(13)
S1 subunit	IgG, IgA	Patient serum	Cross-react with SARS-CoV only	(14)
	Not indicated	Patient plasma	Not reported	(19)
	IgG	Patient serum	Cross-react with SARS-CoV only	(14)
	Not indicated	Patient plasma	Cross-react with SARS-CoV	(19)
Receptor-binding domain (RBD)	IgG	Mouse serum	SARS-CoV RBD-induced antibodies cross-react to SARS-CoV-2 RBD	(20)
	IgM, IgG, IgA	Patient serum or plasma	Not reported	(13)
	IgG	Patient serum	Cross-react with SARS-CoV only	(14)
	Not indicated	Patient plasma	Cross-react with SARS-CoV	(19)

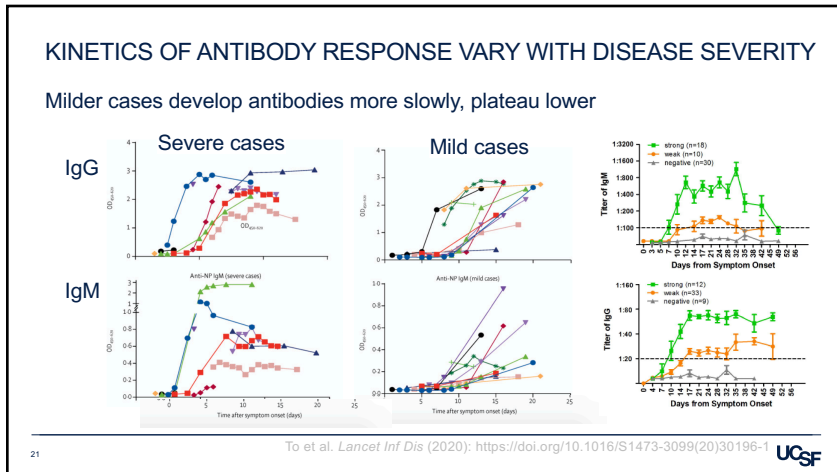
Imagine a patient w previous SARS infection, their anti-SARS Ab may react in an anti-COVID19 serology test (false positive)



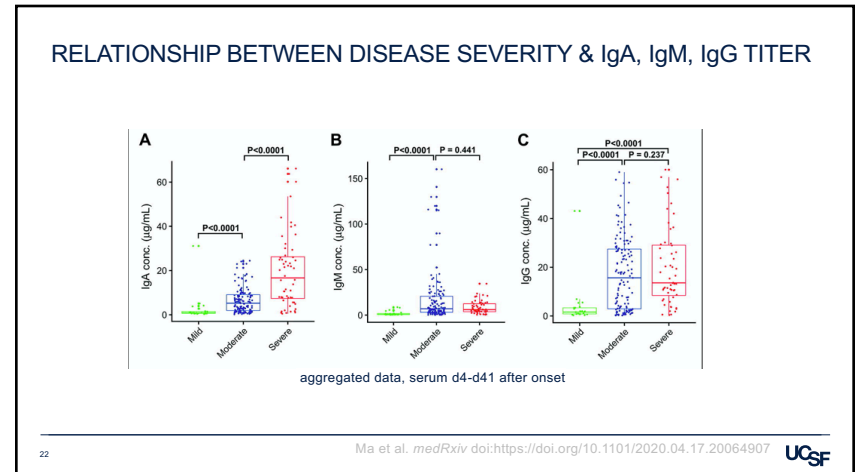
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Lee et al. *Front Immunol* (2020) <https://doi.org/10.3389/fimmu.2020.00879> Review

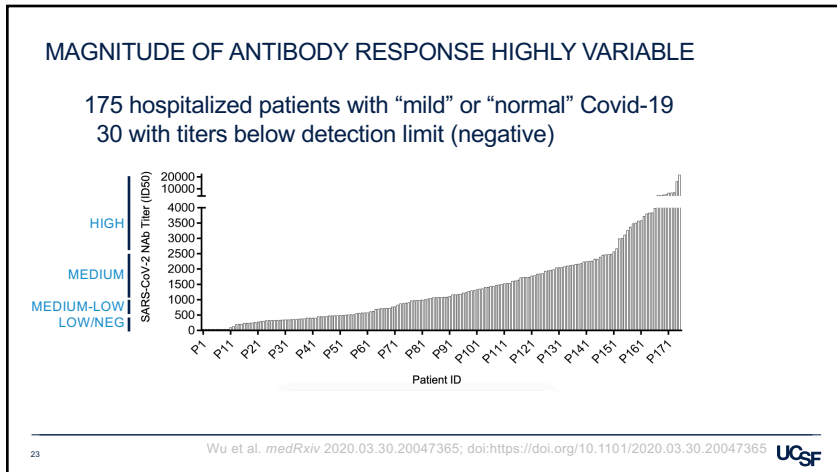
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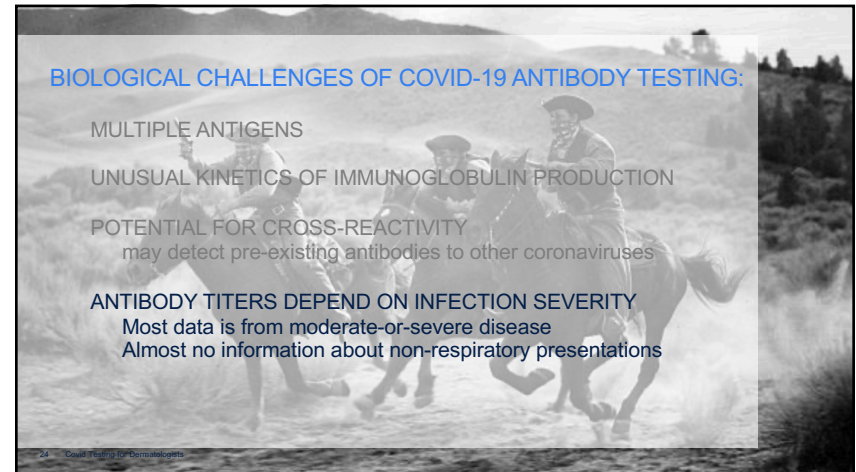
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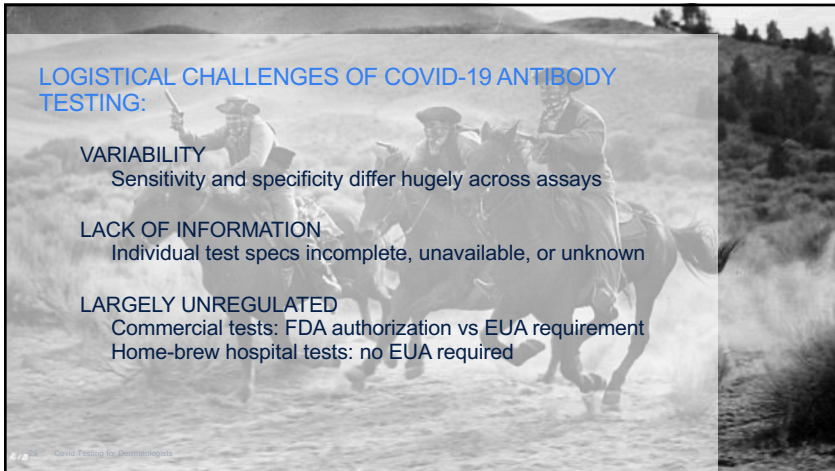
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LOGISTICAL CHALLENGES OF COVID-19 ANTIBODY TESTING:

- VARIABILITY**
Sensitivity and specificity differ hugely across assays
- LACK OF INFORMATION**
Individual test specs incomplete, unavailable, or unknown
- LARGELY UNREGULATED**
Commercial tests: FDA authorization vs EUA requirement
Home-brew hospital tests: no EUA required

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EXPLOSION OF TESTING OPTIONS COMPLICATES INFORMATION

>150 COVID19 IgG, IgM, and total immunoglobulin tests available in U.S.

Only 6 FDA authorized (5/17/2020):

- Abbott (ARCHITECT system) (IgG)
- Roche (total)
- Ortho-Clinical VITRIS (IgM/IgG)
- EUROIMMUN AG (IgG)
- Chembio Diagnostic Systems (IgM/IgG)
- Autobio (IgM/IgG)

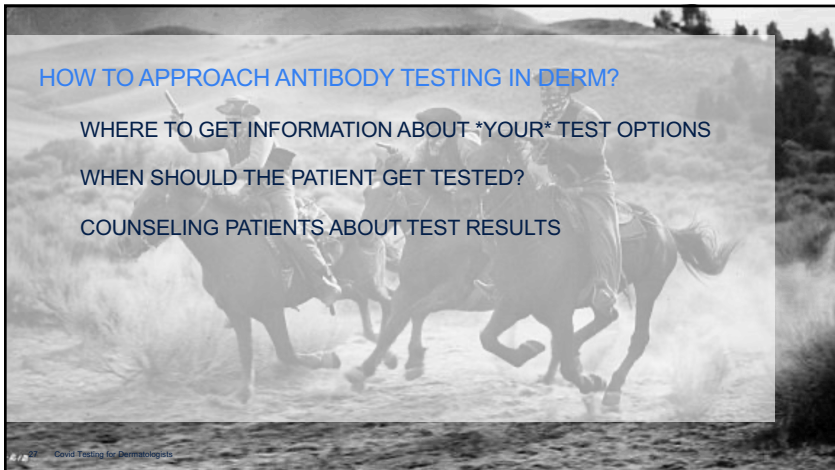
Many don't disclose whether they detect IgG, IgM, or total immunoglobulin
Many don't disclose protein antigen information

<https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-testing-sars-cov-2#serology>

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HOW TO APPROACH ANTIBODY TESTING IN DERM?

- WHERE TO GET INFORMATION ABOUT *YOUR* TEST OPTIONS
- WHEN SHOULD THE PATIENT GET TESTED?
- COUNSELING PATIENTS ABOUT TEST RESULTS

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DIFFERENT TESTS ARE AVAILABLE EVERYWHERE

TALK TO YOUR CLINICAL PATHOLOGIST

FDA WEBSITE – CAN LOOK UP SPECS ON INDIVIDUAL TESTS

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

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U.S. FOOD & DRUG ADMINISTRATION

Home / Medical Devices / Medical Device Safety / Emergency Situations (Medical Devices) / EUA Authorized Serology Test Performance

EUA Authorized Serology Test Performance

Abbott Architect SARS-CoV-2 IgG

Developer: Abbott
Test: Architect SARS-CoV-2 IgG
Technology: High Throughput ELISA

Antibody	Performance Measure	Estimate of Performance	95% Confidence Interval
IgG	Sensitivity (PPA)	100% (88/88)	(95.8%, 100%)
IgG	Specificity (NPA)	99.6% (1064/1070)	(99.0%, 99.9%)
IgG	PPV at prevalence = 1%	92.9%	(83.4%, 98.1%)
IgG	NPV at prevalence = 5%	100%	(99.8%, 100%)

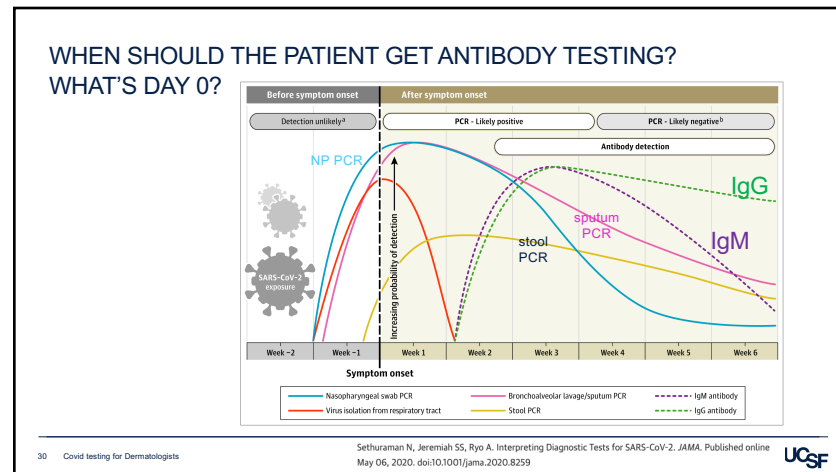
Test Facts:

- Information for Healthcare Providers
- Information for Recipients
- Instructions for Use

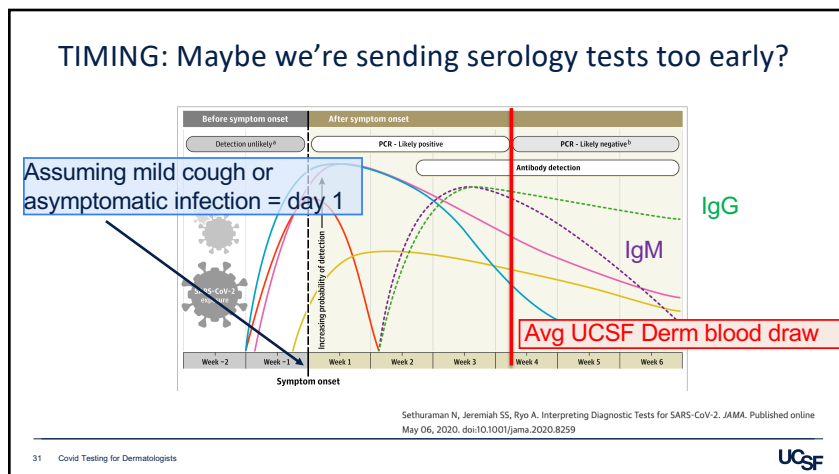
<https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance>

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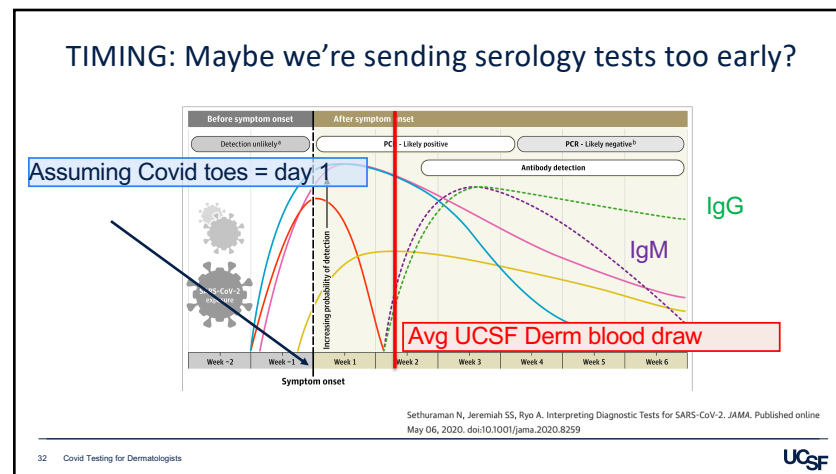
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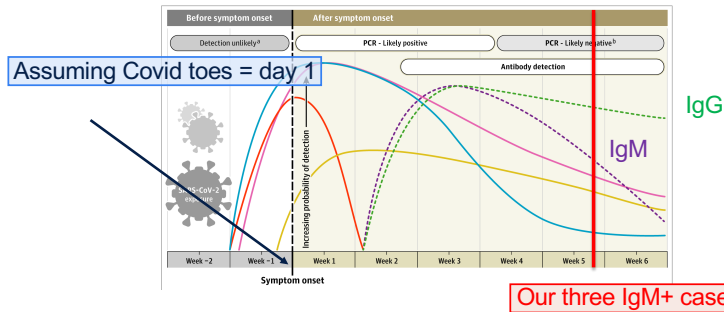


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These models reflect PCR+ patients w systemic disease.
Do pts w mild non-respiratory disease (ambulatory derm) behave differently?



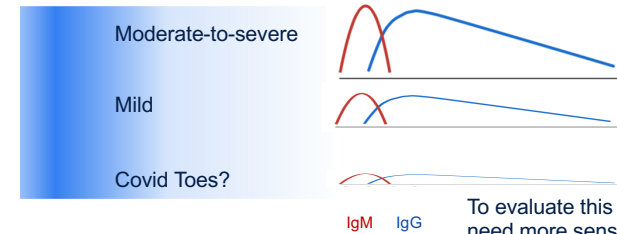
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MILD DISEASE/LOW MAGNITUDE PROBLEM

Very low antibody levels may preclude diagnosis in ambulatory patients w mild/transient infection, such as Covid toes



To evaluate this hypotheses, need more sensitive serologic tests.

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HOW TO INTERPRET AND COUNSEL DERMATOLOGY PATIENTS ABOUT TEST RESULTS

NEGATIVE ANTIBODY TEST DOES NOT RULE OUT COVID
TIMING
SENSITIVITY
MAGNITUDE OF HUMORAL IMMUNE RESPONSE*

POSITIVE TESTS ARE RARE, SHOULD BE CORROBORATED
FOLLOW-UP IgM WITH IgG
SERIAL MONITORING

REMAIN OPEN-MINDED ABOUT TIME-TO-SEROCONVERSION

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FINAL SLIDE: GOOD NEWS

NYC Covid patients who recovered at home (and close contacts!) had moderate-titer Abs that effectively neutralize viral entry *in vitro*

Table 1. Cohort characteristics

				Average duration		Average Sx Severity (0-10)	ELISA binding (AUC)				Neutralization (NT50)
				Sx total	Sx onset to visit		RBD		S		
Gender	n	Average age	Case/Contact	Sx total	Sx onset to visit		IgG	IgM	IgG	IgM	
Male	37	42 (21-68)	27/10	10 (0-21)	31 (21-42)	5 (0-9)	2.31	2.18	4.72	1.52	880
Female	31	41 (19-75)	21/10	9 (1-28)	30 (17-48)	4.4 (2-9)	1.95	2.10	4.62	1.43	814

Avg 30d post-symptom onset
Prevalence of antibody:

anti-spike: 40% IgG 21% IgM
anti-RBD: 88% 66%

Convergent Antibody Responses to SARS-CoV-2 Infection in Convalescent Individuals
Davide F. Robbiani, Christian Gaebler, Frauke Muecksch, Julio Cetrulo Lorenzi, Zijun Wang, Alice Cho, Mariana Agudelo, Christopher Barnes, Shlomo Fink, Thomas Haggel, Thiago Oliveira, Charlotte Vian, Ariane Hurley, Katrina Millard, Rhonda Kost, Melissa Cipolla, Anna Gazumyan, Kristie Gordon, Filippo Bianchini, Spencer Chen, Victor Ramos, Rosalvi Patel, Juan Dixon, Inna Shmeliovich, Pilar Mendez, Harald Harweg, Lili Nogueira, Raza Padi, Jill Horowitz, Fabian Schmidt, Yuka Weisblum, Hans-Heinrich Hoffmann, Eleftherios Michailidis, Alison Ashbrook, Eric F. Walter, John P. Kelly, Kathryn Harty-Tuoman, Nicholas Koranda, Pauline Hoffman, Anthony Weiss, Charles Rice, Theodoros Hatzigeorgiou, Pamela Bjorkman, Paul Benoit, Marina Caskey, Michel Nussenzweig
bioRxiv 2020.05.13.092619; doi: <https://doi.org/10.1101/2020.05.13.092619>

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UCSF Immunology

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Jason Cyster

AAD Covid Registry

Esther Freeman

