Covid Testing for Dermatologists
An update on PCR, antibodies, what they mean, and how to interpret results

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Assistant Professor of Dermatology
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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

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

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

https://www.globalbiotechnologystarts.com/articles/20247/the-worldwide-test-for-covid-19

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

LIMITATION OF PCR testing

the virus must be there!
finite window of active shedding EARLY
~day -8 to +8, 10^4-10^7 copies/ml then falls
in mild/subclinical infection, can easily miss the window
interpreting negative PCR tests depends on sample timing!

WHEN SHOULD THE PATIENT GET PCR TESTED? ASAP

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

<table>
<thead>
<tr>
<th>Exanthem</th>
<th>Pemio-like</th>
<th>Vesicular</th>
<th>Urticarial</th>
<th>Morbilliform</th>
<th>Livedo/necrotic</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbness, X (%)</td>
<td>3 (17) 3 (17)</td>
<td>3 (17)</td>
<td>3 (17)</td>
<td>3 (17)</td>
<td>3 (17)</td>
<td>71</td>
</tr>
<tr>
<td>Sore throat, X (%)</td>
<td>24 (24) 19 (24)</td>
<td>19 (24)</td>
<td>24 (24)</td>
<td>24 (24)</td>
<td>19 (24)</td>
<td>212</td>
</tr>
<tr>
<td>After, X (%)</td>
<td>42 (56) 10 (29)</td>
<td>10 (29)</td>
<td>25 (29)</td>
<td>25 (29)</td>
<td>25 (29)</td>
<td>25 (29)</td>
</tr>
<tr>
<td>Total</td>
<td>71 34 71</td>
<td>71</td>
<td>174</td>
<td>21</td>
<td>577</td>
<td></td>
</tr>
</tbody>
</table>
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 perniosis (>1 month)
& several additional in the AAD registry

What about antibody testing? INDIRECT assay

Q: Is there detectable SARS-CoV2 protein-reactive antibody present in serum?

Yes (substrate reacts) NO (no signal)

How does antibody testing complement PCR tests?
TIMING

Infection Covid Toes + other exanthems presenting too late for PCR

Basic immunology teaching:
10-14 days to Ab

IgM IgG

ADVANTAGE OF Ab testing: potentially longer window

Covid Ab detectable 14-21d+ after symptom onset

Viral symptoms (PCR positive)

@UCSF (Abbott) IgG test
timing sensitivity
14-21d 70%
>21d 94-99%

IgM IgG
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

MULTIPLE COVID-19 protein antigens

KINETICS OF ANTIBODY RESPONSE

Papers from China suggest variable antibody production, sometimes very early and/or concurrent with PCR+
KINETICS OF ANTIBODY RESPONSE – PROLONGED IgM+ PHASE?

Quantitative Results for SARS-CoV2
IgM and IgG

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

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

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

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antibody</th>
<th>Sample type</th>
<th>Specificity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spike (S)</td>
<td>Entire S IgM, IgG</td>
<td>Patient serum</td>
<td>Not reported</td>
<td>(10, 11)</td>
</tr>
<tr>
<td></td>
<td>IgM</td>
<td>Patient serum</td>
<td>Cross-react with SARS-CoV and MERS-CoV</td>
<td>(16)</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>Patient serum</td>
<td>Not indicated</td>
<td>(16)</td>
</tr>
<tr>
<td></td>
<td>IgM, IgG, IgA</td>
<td>Patient plasma</td>
<td>Cross-react with SARS-CoV</td>
<td>(16)</td>
</tr>
<tr>
<td></td>
<td>IgM, IgG</td>
<td>Patient serum or plasma</td>
<td>Not reported</td>
<td>(16)</td>
</tr>
<tr>
<td></td>
<td>IgM, IgG</td>
<td>Patient serum</td>
<td>Cross-react with SARS-CoV only</td>
<td>(16)</td>
</tr>
<tr>
<td>S1 subunit</td>
<td>IgM, IgA</td>
<td>Patient serum</td>
<td>Not indicated</td>
<td>(16)</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>Patient serum</td>
<td>Not indicated</td>
<td>(16)</td>
</tr>
<tr>
<td></td>
<td>IgM, IgA</td>
<td>Patient plasma</td>
<td>Cross-react with SARS-CoV only</td>
<td>(16)</td>
</tr>
<tr>
<td>S2 subunit</td>
<td>IgG</td>
<td>Patient serum</td>
<td>Not indicated</td>
<td>(16)</td>
</tr>
<tr>
<td></td>
<td>IgA</td>
<td>Patient serum</td>
<td>Not indicated</td>
<td>(16)</td>
</tr>
<tr>
<td></td>
<td>IgM, IgG</td>
<td>Mouse serum</td>
<td>SARS-CoV-2 RBD-escape antibodies</td>
<td>(57)</td>
</tr>
</tbody>
</table>

Imagine a patient with previous SARS infection, their anti-SARS Ab may react in an anti-COVID19 serology test (false positive)
KINETICS OF ANTIBODY RESPONSE VARY WITH DISEASE SEVERITY

Milder cases develop antibodies more slowly, plateau lower

To et al., Lancet Inf Dis (2020). https://doi.org/10.1016/S1473-3099(20)30196-1

RELATIONSHIP BETWEEN DISEASE SEVERITY & IgA, IgM, IgG TITER

Ma et al., medRxiv doi:https://doi.org/10.1101/2020.04.17.20064907

MAGNITUDE OF ANTIBODY RESPONSE HIGHLY VARIABLE

175 hospitalized patients with “mild” or “normal” Covid-19
30 with titers below detection limit (negative)

Wu et al., medRxiv 2020.03.30.20047365; doi:https://doi.org/10.1101/2020.03.30.20047365

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

Most data is from moderate- or-severe disease
Almost no information about non-respiratory presentations
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

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

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

DIFFERENT TESTS ARE AVAILABLE EVERYWHERE

TALK TO YOUR CLINICAL PATHOLOGIST

FDA WEBSITE – CAN LOOK UP SPECS ON INDIVIDUAL TESTS

**EUA Authorized Serology Test Performance**

**Abbott Architect SARS-CoV-2 IgG**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Sensitivity (95% CI)</th>
<th>Specifity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>100% (98%-100%)</td>
<td>99% (98%-100%)</td>
</tr>
<tr>
<td>IgA</td>
<td>99.5% (99%-100%)</td>
<td>99.4% (99%-100%)</td>
</tr>
<tr>
<td>IgM</td>
<td>100% (98%-100%)</td>
<td>99% (98%-100%)</td>
</tr>
</tbody>
</table>

**Timing:**

- **IgG:**
  - 14-21d: 70%
  - >21d: 94-99%

**Test Factors:**
- Information for Healthcare Providers
- Information for Recipients
- Instructions for Use

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**When Should the Patient Get Antibody Testing?**

**What’s Day 0?**

- NP PCR
- Sputum PCR
- Stool PCR

**IgG**

**IgM**

**TIMING:**

- **Maybe we're sending serology tests too early?**

**Assuming mild cough or asymptomatic infection = day 1**

**Avg UCSF Derm blood draw**

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**Sensitivity of SARS-CoV-2 Antibody Tests for COVID-19**

- **IgG**
- **IgM**

**Assuming Covid toes = day 1**

**Avg UCSF Derm blood draw**
These models reflect PCR+ patients with systemic disease. Do pts with mild non-respiratory disease (ambulatory derm) behave differently?

Assuming Covid toes = day 1

Our three IgM+ cases

Covid Testing for Dermatologists

MILD DISEASE/LOW MAGNITUDE PROBLEM

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

To evaluate this hypotheses, need more sensitive serologic tests.

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

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

Avg 30d post-symptom onset

Prevalence of antibody:

anti-spike: 40% IgG 21% IgM

anti-RBD: 88% 66%
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Antonia Gallman
Jason Cyster

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Esther Freeman