Testing

Herd Immunity & Vaccines

Disease & Treatments
# How Do Molecular Tests Work?

## I - Types of SARS-CoV-2 Diagnostic Tests

<table>
<thead>
<tr>
<th>Molecular (a.k.a. PCR)</th>
<th>Antigen</th>
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Table modified from Service, RF. (2020, August 3). Radical shift in COVID-19 testing needed to reopen schools and business, researchers say. *Science* doi:10.1126/science.abe1546. Table created with BioRender.com
How Do Molecular Tests Work?
II - COVID-19 Diagnostics Testing through RT-qPCR

1. Nasopharyngeal swab <15 min
   Cotton swab is inserted into nostril to absorb secretions.

2. Collected specimen 0-72 h
   Specimen is stored at 2-8°C for up to 72 hours or proceed to RNA extraction.

3. RNA extraction ~45 min
   Purified RNA is extracted from deactivated virus.

4. RT-qPCR ~1 h per primer set
   Purified RNA is reverse transcribed to cDNA and amplified by qPCR.

5. Test results real time
   Positive SARS-CoV-2 patients cross the threshold line within 40.00 cycles (< 40.00 Ct).

Primers and probes for screening
- E_Formal: ACAGTACGGTTAATGTTAATACGCT
- E_Probe1: FAM-ACACTGACCTCATCTGCTCTGCCTC-BBQ
- E_Reverse: ATATTGCACGCTACGAGCATCA
- RdRp_Formal: GTGARATGTGACGTGTGACG
- RdRp_Probe1: FAM-CCAGTGAAACCTTCACTTGATGAC-BBQ
- RdRp_Probe2: FAM-CAGTGAACTCCTACAGGAGATGAC-BBQ
- RdRp_Reverses: CARATGTAAASACACTATTACGATA

* N gene testing is not further used because it is slightly less sensitive.
How Do Molecular Tests Work?

III - Fluorescent Probe-Based Quantitative PCR (qPCR)

1. Initiation
2. Denaturation (95°C)
3. Primer annealing (60°C)
4. Extension (72°C)

Key concept:
- Fluorescent Reporter
- Quencher
- Ground state fluorophore
- Excited state fluorophore

Results:
- Amplification (Positive)
- No amplification (Negative)

Fluorescence vs. Cycles
How Do Molecular Tests Work?
III - Nicking Enzyme Amplification Reaction (NEAR, IDNow)

- Key Differences from PCR
  - Cutting site (restriction site) in primer
  - Presence of enzyme to make cuts (restriction enzyme - Nt.BstNBI)
  - Strand-displacing polymerase (Bst)

- Advantages
  - Faster - reaction can be run at a single temperature (isothermal)
  - More sensitive - >2 fold amplification per cycle (PCR)

- Disadvantages
  - Specificity - common to see non-specific products; high background

Why Are Antigen Tests Less Accurate?

I - How Lateral Flow Antigen Testing Works

1. Sample is added to the sample pad and begins to flow up the membrane.
2. Antigen binds to complexes of antibody and dye or other detection particle on the conjugate pad. This forms an antibody:antigen complex.
3. Antibody:antigen complexes bind to test antibodies. Dye is released upon binding.
5. Interpret results.

Positive

Negative

Inconclusive

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Why Are Antigen Tests Less Accurate?
II - Differences in Detection Between Molecular & Antigen Tests

**Molecular Tests**
Amplify Target (Viral Genome)

**Antigen Tests**
No Target (Viral Protein)
Amplification
Why Are Antigen Tests Less Accurate?

II - All Tests Have Limits …

- **PCR-based tests** can detect small amounts of viral genetic material, so a test can be positive long after a person stops being infectious.

- **Rapid antigen tests** detect the presence of viral proteins and can return positive results when a person is most infectious.

- **Antibody tests** detect the body's immune response to the virus and are not effective at the earliest phase of infection.

![Graph showing the probability of detection over time from symptom onset.](Gugliemi_Nature_2020)
Why Are Antigen Tests Less Accurate?

IV - ... But Each Test Has A Purpose

**Types of SARS-CoV-2 Tests**

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Testing
Miscellaneous Quick Answers

Why Were There Shortages in Testing?
• Production (materials, facilities, staff, etc)
• Testing (materials, instrumentation, CLIA certified labs & staff, etc)
• Allocation of Tests & Materials

Why Does It Take Over A Day To Get Results?
• Point-of-Care / Rapid Tests
  • Abbott IDNow (molecular: ~15 min/test, 1 test/run)
  • Cepheid Xpress (molecular: ~35 min/test, 2-80 tests/run)
  • Quidel Sofia2 (antigen: ~15 min/test, 1 test/run)
  • Ellume COVID-19 home test (antigen: ~15 min/test, 1 test/run)
• RT-qPCR (molecular)
  • Straight from collection,: ~ 120 min/test, 10-90 tests/run*
    • *depends on size of plate, pooling, # of primer sets
  • Bottlenecks: transport to lab, certified staff, time from sample collection, sample back-log, etc
Testing

Herd Immunity & Vaccines

Disease & Treatments
What is Herd Immunity & How is it Achieved?

**Principles of HERD IMMUNITY & SOCIAL DISTANCING**

- **Onset of infection**
  - Immunization: Red flag
  - Social distancing: Red flag

- **Spread**
  - Infection passes freely among individuals

- **End outcome**
  - Healthy
  - Social distancing
  - Infected
  - Immunity acquired
  - Deceased

---

- **Onset of infection**
  - Immunization: Red flag
  - Social distancing: Green flag

- **Spread**
  - Isolated individuals slow spread of infection

- **End outcome**
  - Healthy
  - Social distancing
  - Infected
  - Immunity acquired
  - Deceased

---

- **Onset of infection**
  - Immunization: Green flag
  - Social distancing

- **Spread**
  - Infection cannot pass freely among individuals

- **End outcome**
  - Healthy
  - Social distancing
  - Infected
  - Immunity acquired
  - Deceased

(created with biorender.com)
Herd immunity is achieved when, on average, one infected person in a generates less than one secondary case ($R < 1$)

\[ R = (1-p_C)(1-p_I)R_0 \]

- $R$ = effective reproduction number
- $p_C$ = reduction of transmission due to non-pharmaceutical interventions (NPI)
- $p_I$ = proportion of immune individuals
- $R_0$ = basic reproduction number; varies

When is Herd Immunity Achieved?
The Relationship Between $R_0$ & $p_I$

- Herd immunity is achieved when, on average, one infected person in a generates less than one secondary case ($R<1$)

$$R = (1-p_C)(1-p_I)R_0$$

$R = \text{effective reproduction number}$
$p_C = \text{reduction of transmission due to non-pharmaceutical interventions (NPI)}$
$p_I = \text{proportion of immune individuals}$
$R_0 = \text{basic reproduction number; varies}$

- Without NPI ($p_C = 0$), herd immunity ($R<1$) is achieved when:

$$p_I = 1-1/R_0$$

<table>
<thead>
<tr>
<th>Disease</th>
<th>$R_0$</th>
<th>$p_I$ (as a%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seasonal Influenza</td>
<td>1.28 (median)</td>
<td>22% (median)</td>
</tr>
<tr>
<td>Measles</td>
<td>12 - 18</td>
<td>92 - 94%</td>
</tr>
<tr>
<td>SARS-CoV-2</td>
<td>2.5 - 4</td>
<td>60 - 75%</td>
</tr>
</tbody>
</table>

### What Types of Vaccines Are In Development?

#### I - Types of Vaccines

<table>
<thead>
<tr>
<th>Type</th>
<th>How It Works</th>
<th>Similar To</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>viral antigen(s) encoded by DNA produced in host cells by sequential</td>
<td>West Nile (horses)</td>
</tr>
<tr>
<td></td>
<td>transcription-to-translation <em>in vivo</em></td>
<td></td>
</tr>
<tr>
<td>mRNA</td>
<td>viral antigen(s) encoded by mRNA are synthesized <em>in vitro</em> and produced</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>in host cells by translation <em>in vivo</em></td>
<td></td>
</tr>
<tr>
<td>viral-vectored</td>
<td>genes encoding viral antigen(s) epackaged into (non)replicating viral</td>
<td>Ebola</td>
</tr>
<tr>
<td></td>
<td>vectors; viran antigens synthesized in host cells by sequential</td>
<td></td>
</tr>
<tr>
<td></td>
<td>transcription-to-translation <em>in vivo</em></td>
<td></td>
</tr>
<tr>
<td>live attenuated</td>
<td>virus is pathogenicity is weakened or eliminated prior to infection;</td>
<td>MMR</td>
</tr>
<tr>
<td></td>
<td>mimics live virus infection</td>
<td>Chickenpox</td>
</tr>
<tr>
<td>inactivated</td>
<td>virus physically or chemically inactivated with virus particle (and</td>
<td>Influenza</td>
</tr>
<tr>
<td></td>
<td>antigens) intact</td>
<td>Polio</td>
</tr>
<tr>
<td>virus-like particles</td>
<td>viral antigens co-expressed to form non-infectious particles;</td>
<td>HPV</td>
</tr>
<tr>
<td></td>
<td>resemble real virions but lack virus genome</td>
<td></td>
</tr>
<tr>
<td>protein subunit</td>
<td>viral antigen(s) manufactured <em>in vitro</em> in bacteria, yeast, insect, or</td>
<td>whooping cough</td>
</tr>
<tr>
<td></td>
<td>mammalian cells</td>
<td>HBV</td>
</tr>
</tbody>
</table>

(created in biorender.com
modified from Dai & Cai Nat. Rev. Immuno. (2020))
What Types of Vaccines Are In Development?

II - The Mechanism of SARS-CoV-2 Entry as a Vaccine Target

1. Cleavage of SARS-CoV-2 S protein
2. Activation of S2 domain
3. Fusion of viral and host membranes

Target cell
What Types of Vaccines Are In Development?

III - Major Targets Used in COVID-19 Vaccine Candidates

- Pfizer/BioNTech/Fosun Pharma (mRNA)
- Moderna/NIAID (mRNA)
- Oxford/AstraZeneca (viral vector)
Why Does COVID-19 Have Varying Severity?

I - A Very Brief & Simplified Overview of Immunity

**INNATE IMMUNITY**

- **sensitivity:** virus vs. bacteria vs. fungi vs. parasite
- **sensors:** pattern-recognition receptors
- **functions:** basic pathogen removal, activates proper adaptive response for type of pathogen
- **strengths:** fast-acting first line of defense
- **weaknesses:** non-specific, amnesia

**ADAPTIVE IMMUNITY**

- **sensitivity:** SARS-CoV-1 vs. MERS vs. SARS-CoV-2
- **sensors:** antigen receptors
- **functions:** neutralize threat (antibodies from B cells), kill infected cells (T cells), learn from and remember best methods of attack
- **strengths:** memory, specificity
- **weaknesses:** takes a while to get into gear
Why Does COVID-19 Have Varying Severity?

II - COVID-19 Susceptibility & the Type I Interferon Pathway

red = neutralizing autoantibodies or loss-of-function genetic variants in subsets of patients with severe COVID-19

Viral particles are sensed by various PRRs, including cytosolic sensors. Type I IFNs are potent antiviral cytokines produced by innate immune cells. They bind a specific cell-surface receptor and signal through the JAK-STAT pathway to induce expression of ISGs that encode other antiviral proteins and various transcription factors. Subsets of patients with severe COVID-19 have loss-of-function genetic variants in several members of the type 1 IFN pathway (red) or neutralizing autoantibodies against type I IFNs, specifically IFN-α2 and IFN-ω.

modified from Beck & Aksentijevich Science (2020)
Why Are There So Many Long-Haulers?
Data Below Not Yet Peer-Reviewed; Take with a Grain of Salt

• COVID-19 as a vascular disease (Chioh et al medRxiv 2020)
  • persistent immune activation —> endothelial cell dysfunction

• Cytokine profile suggests long-haulers have the capability to activate T cells, but inability to recruit them (Patterson et al bioRxiv 2020)
  • additional support for COVID-19 as a vascular disease
References
Information Up-To-Date as of December 28, 2020

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Graphics
• Created with BioRender.com
Additional Resources
To Quench Your Thirst For Knowledge

Testing
- How Coronavirus Antibody, Genetic and Antigen Tests Work
- Coronavirus Test: Real Time RT-PCR - Animation
- Coronavirus Antibody Test - Animation

Herd Immunity & Vaccines
- Virology Lectures 2020 #19 - Vaccines
- COVID-19 Vaccine & Therapeutics Tracker
- What Does 95% Effective Mean? Teaching the Math of Vaccine Efficacy
- There Are Four Types of COVID-19 Vaccines: Here's How They Work

Disease & Treatments
- COVID-19 Vaccine & Therapeutics Tracker
- Immunology of COVID-19: Current State of the Science

Other / General
- This Week in Virology
- New Mutant Strain
  - Preliminary Genomic Characterization of an Emergent SARS-CoV-2 Lineage in the UK Defined By a Novel Set of Spike Mutations
  - Mutant Coronavirus in the United Kingdom Sets Off Alarms, But Its Importance Remains Unclear
  - Why the New COVID-19 Mutations Might Not Be As Scary As You Think