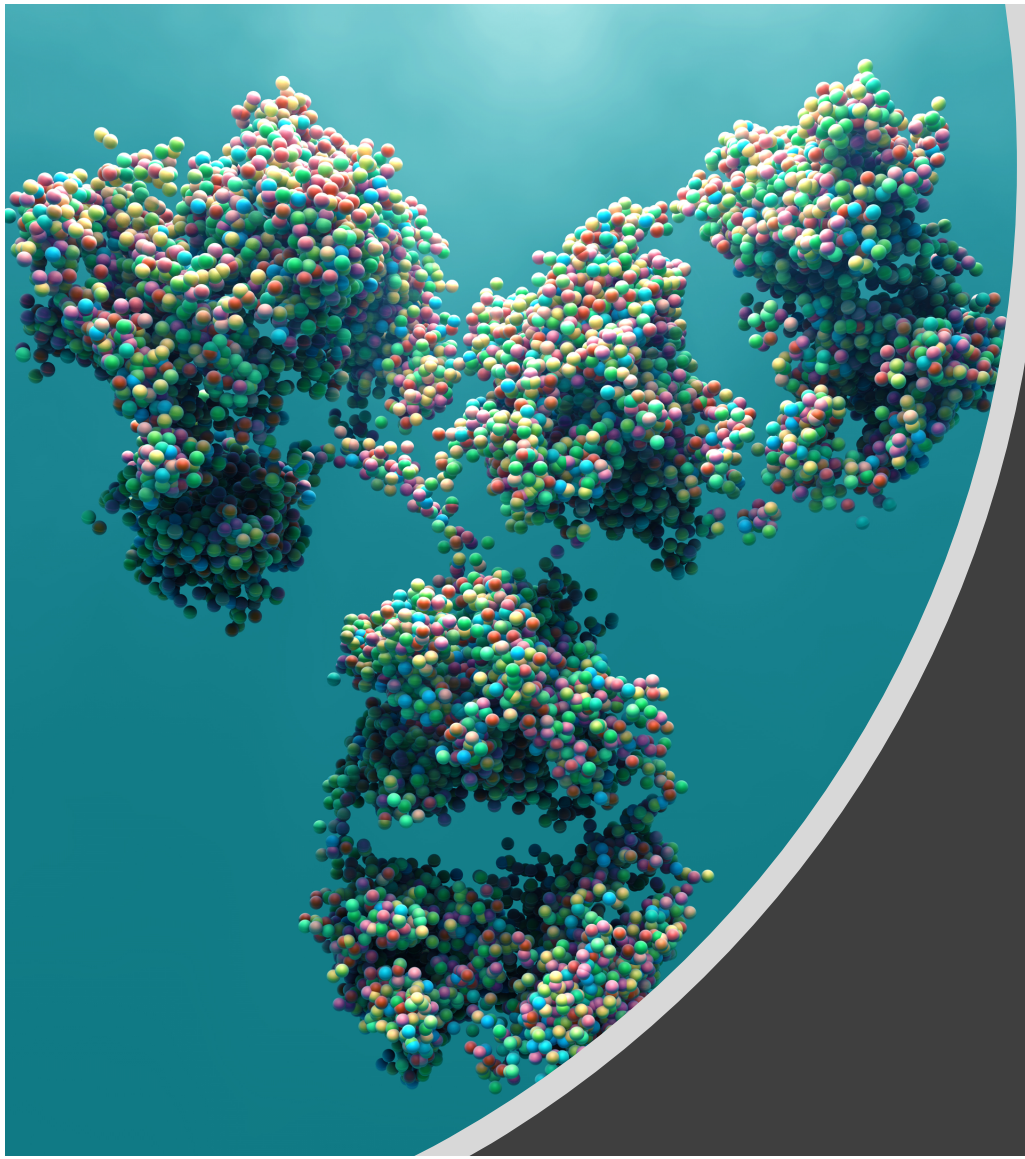
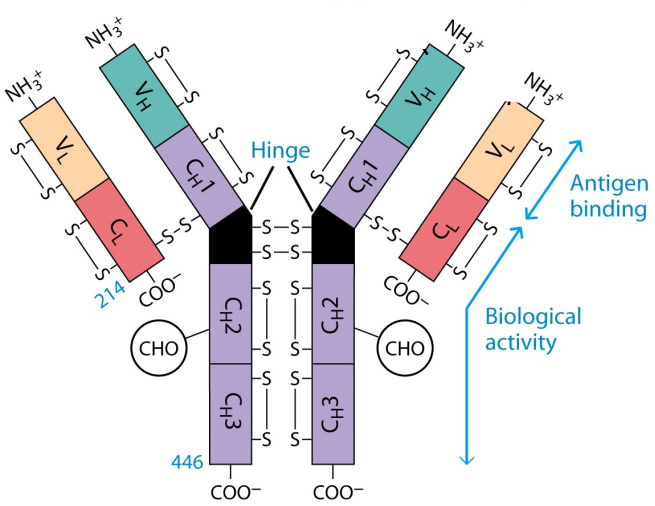


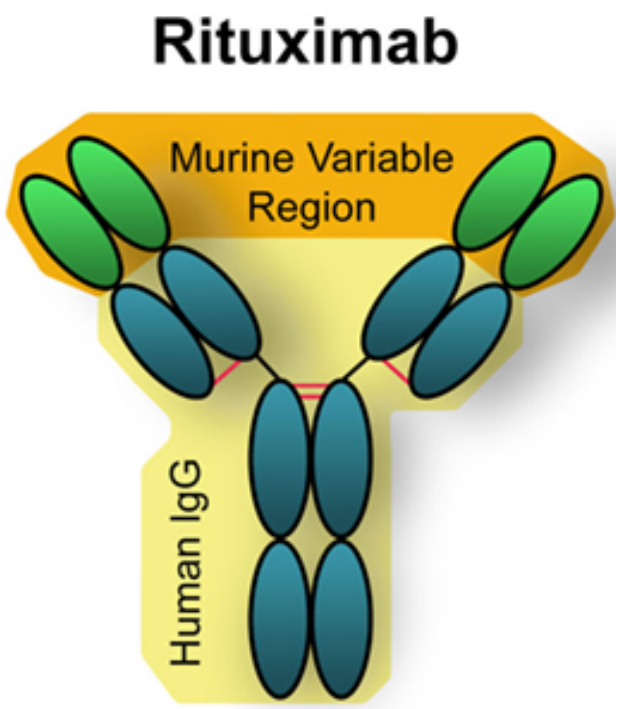
Antibodies as medicine and diagnostics

Engineering antibodies for medicine and diagnostics

- Humanized monoclonal antibodies as drugs
  - Rituximab (1997)
  - LY-CoV555 (2020)
- Antibody fragments (scFvs): bispecific antibodies
  - Blinatumomab (2009)
- Antibody drug conjugates
  - Trodelvy (2020)
- Lateral flow immunochromatographic assay
  - human chorionic gonadotropin, hormone (pregnancy) test (1970s)
  - COVID-19 lateral flow assay (2020)



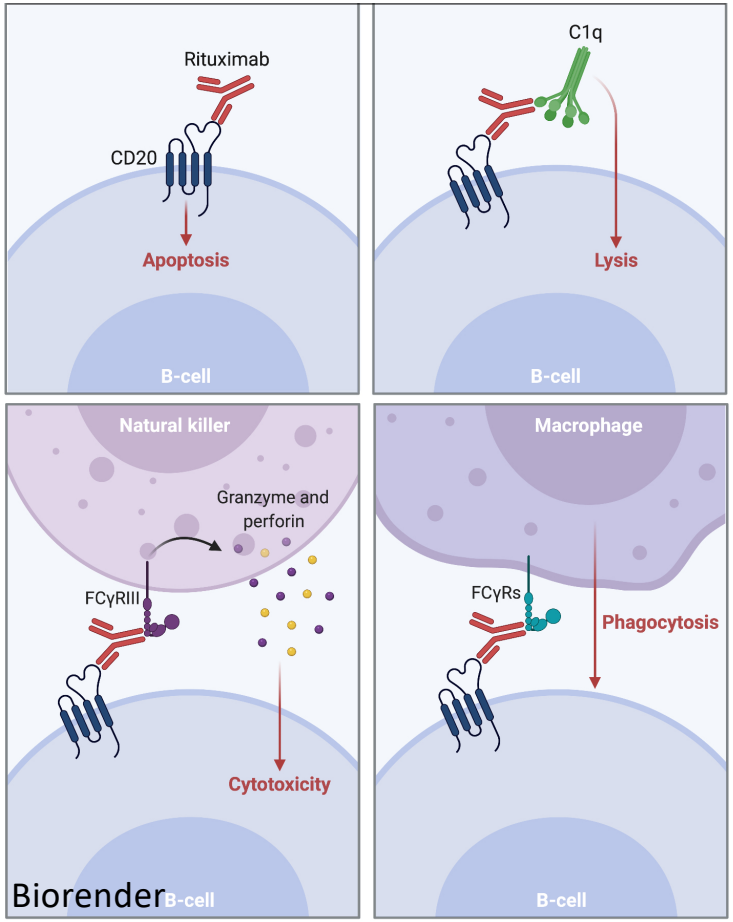
Rituximab is treatment for cancer and autoimmune diseases



- First antibody FDA approved for cancer treatment in 1997
- Antigen is CD20
- Produced in CHO cells
- Approved for treatment of:
  - non-Hodgkin's lymphoma
  - chronic lymphocytic leukemia
  - rheumatoid arthritis
  - multiple sclerosis

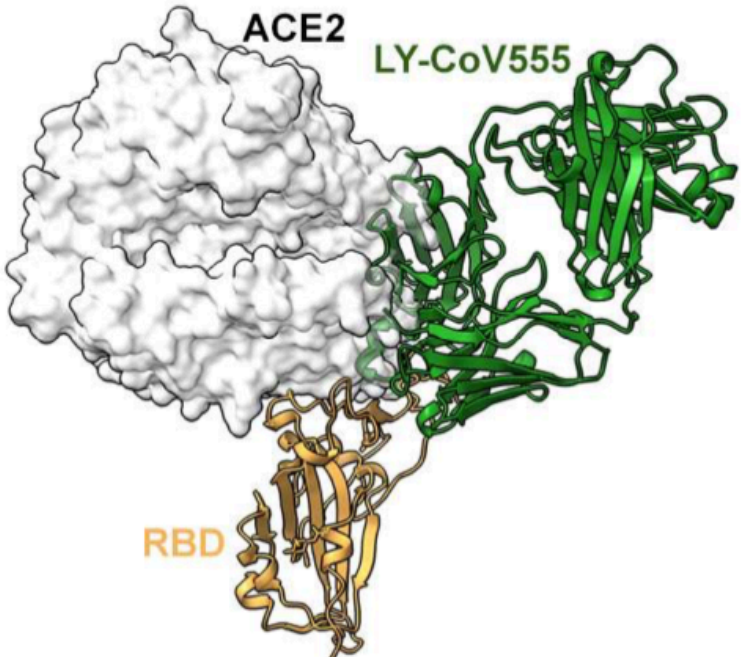
Pierpont, T. et al. Past, Present, and Future of Rituximab-The World's First Oncology Monoclonal Antibody Therapy. Front Oncol. 2018.

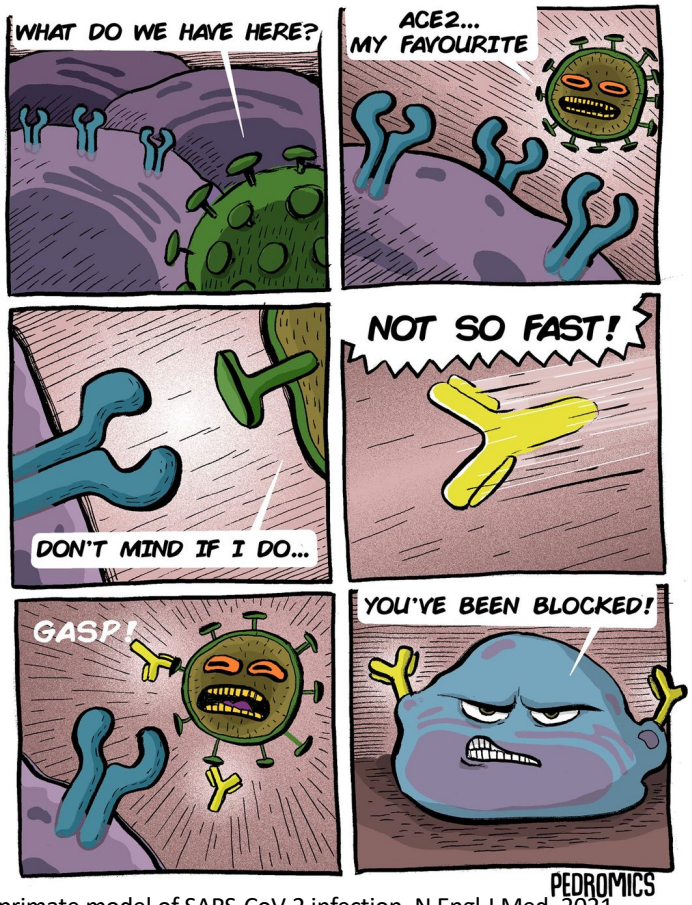
Rituximab targets and kills CD20+ B cells



- Binding of Rituximab to CD20+ cells can result in:
  - CD20 mediated apoptosis
  - Complement-dependent cytotoxicity (direct lysis)
  - antibody-dependent cell-mediated cytotoxicity (Natural Killer cell)
  - antibody-dependent phagocytosis (macrophage phagocytosis)
- Need better mouse models to study effects of immunotherapy to reduce resistance and side effects
  - Anti-human CD20 mouse models don't mimic the human immune system well enough

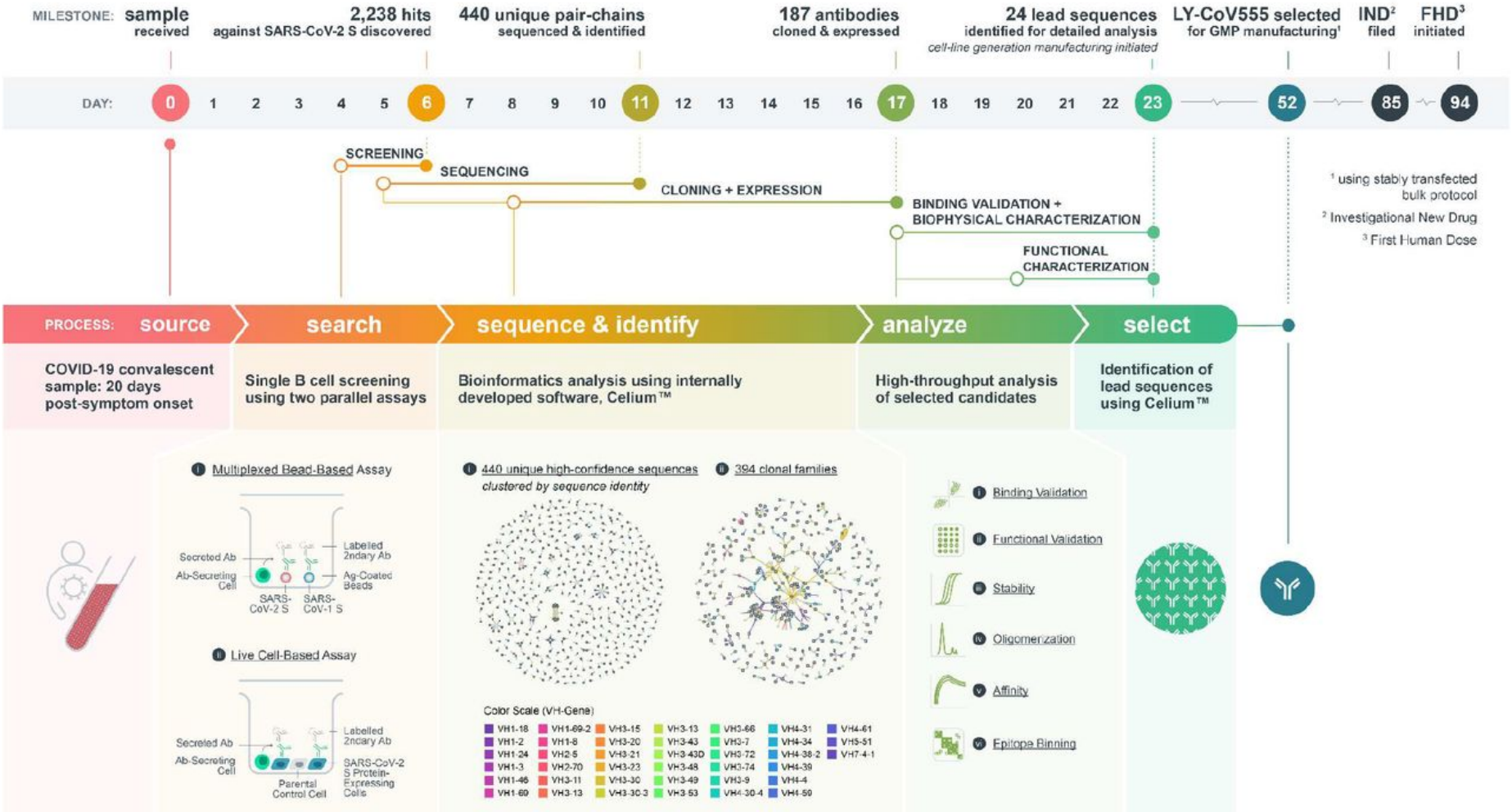
Bamlanivimab (LY-CoV555) first monoclonal antibody therapy approved for treatment of COVID-19





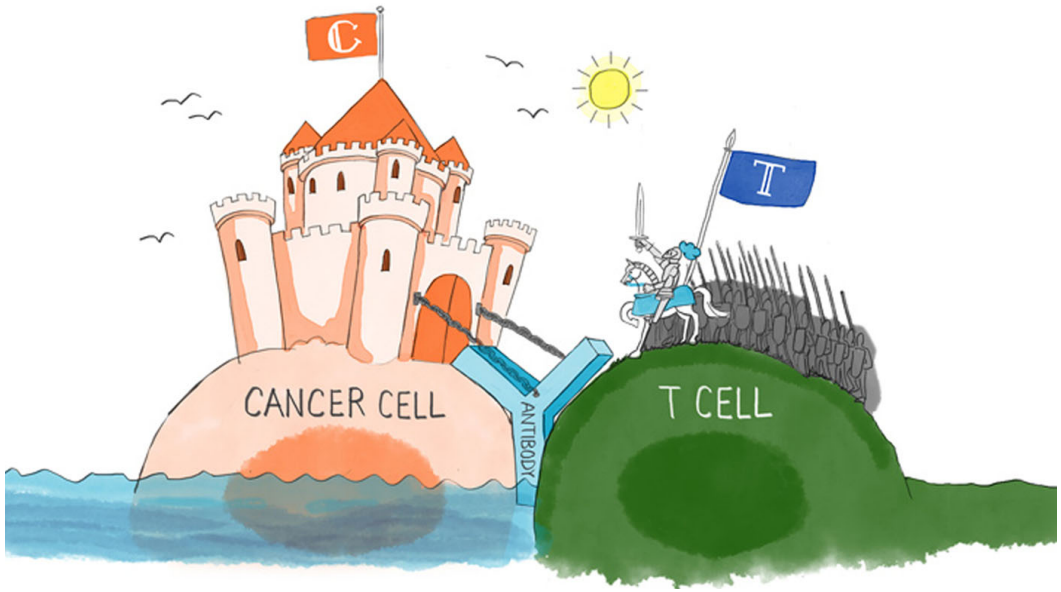
Jones, B. et al. LY-CoV555, a rapidly isolated potent neutralizing antibody, provides protection in a non-human primate model of SARS-CoV-2 infection. N Engl J Med. 2021.

Bamlanivimab development timeline



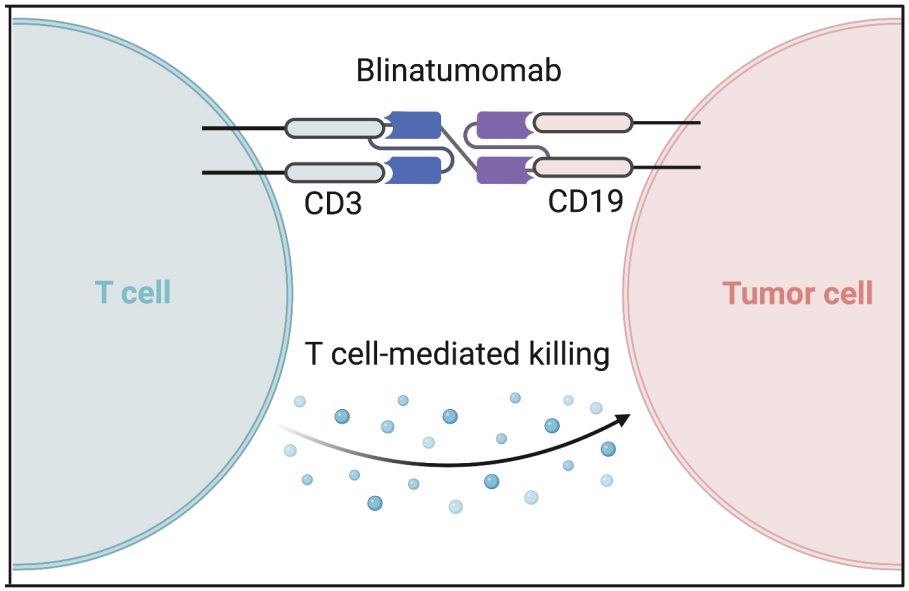
Jones, B. et al. LY-CoV555, a rapidly isolated potent neutralizing antibody, provides protection in a non-human primate model of SARS-CoV-2 infection. N Engl J Med. 2021.

Recombinant antibody production led to development of bispecific antibodies

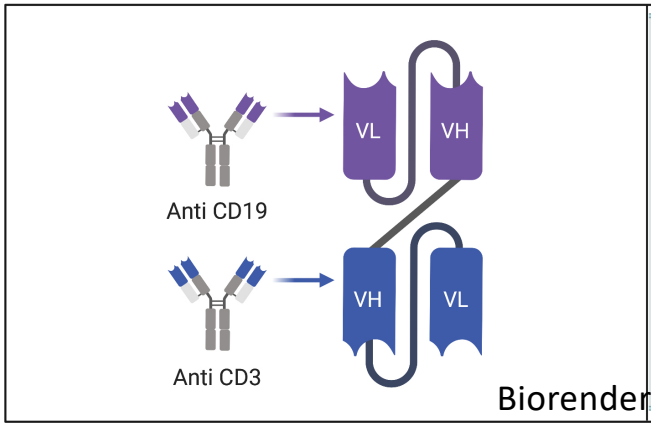


- A bispecific antibody contain two different antigen binding sites (scFvs) in one molecule
- Bispecifics generated in 1980s but not approved for use as drugs until 2009
- Blinatumomab: a bispecific T cell engager (BiTE) antibody against CD19/CD3 for refractory acute lymphoid leukemia

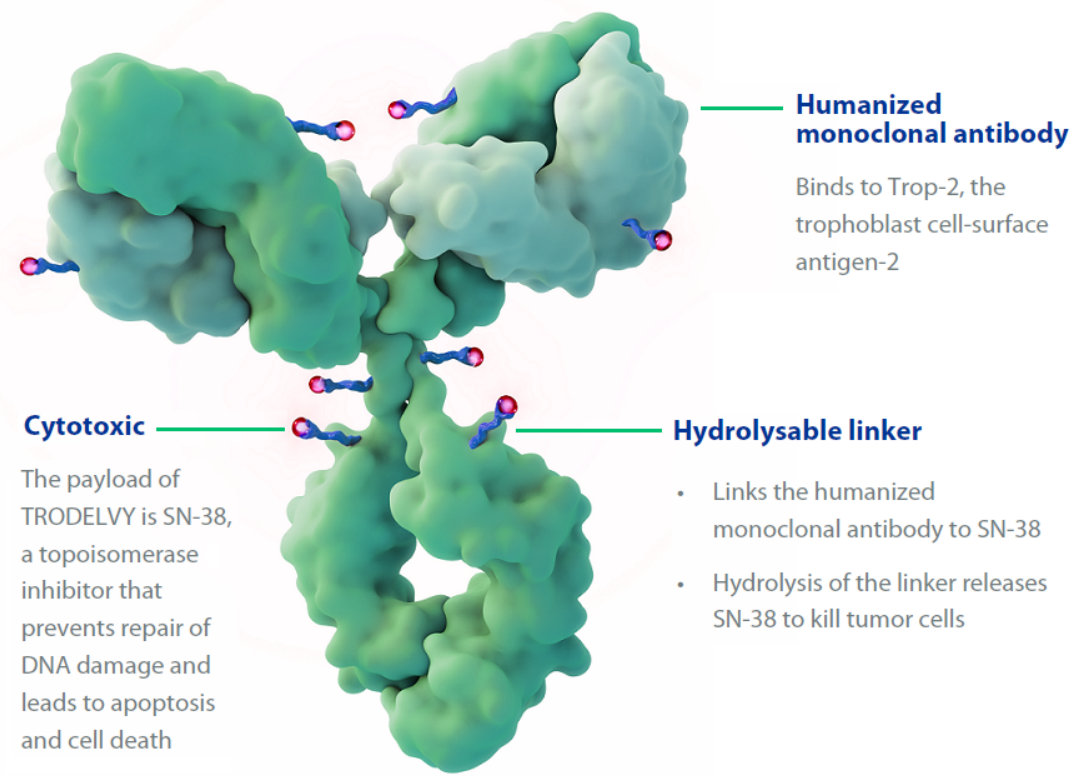
Blinatumomab complexes a T cell with a CD19+ cancer cell resulting in lysis



- Continuous intravenous administration of the drug is required
- CD19+ targets acute lymphoid leukemia
- CD3+ targets T cells and activates T cell cytotoxicity to kill the leukemia cell

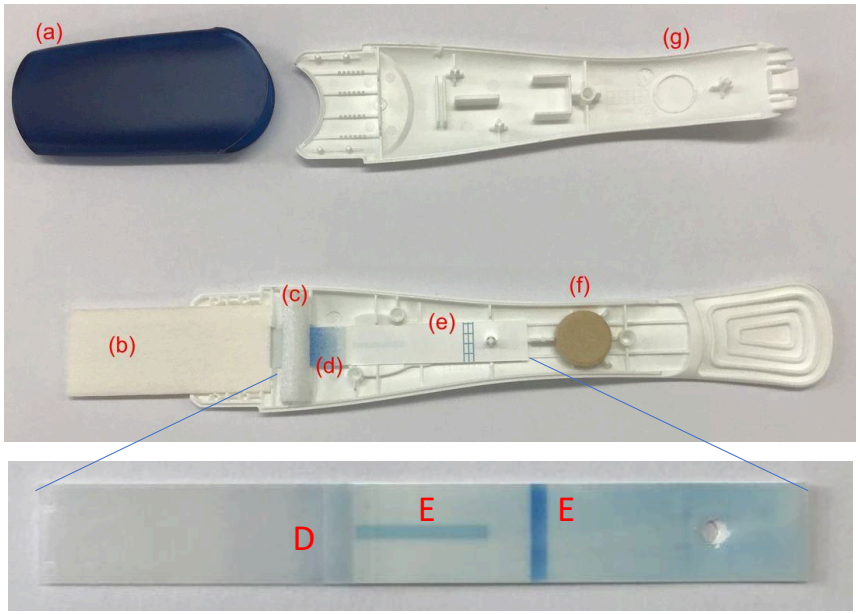


Trodelvy, drug conjugated monoclonal critical for patients with limited treatment options



- Triple-negative breast cancer (TNBC) tumor cells limited treatment options are available
- Trop-2 is a surface protein highly expressed by many cancers, including approximately 90% of TNBC tumors
- Once inside the tumor cell, the linker connecting the antibody to the cytotoxic SN-38 is cleaved, releasing the active drug inside

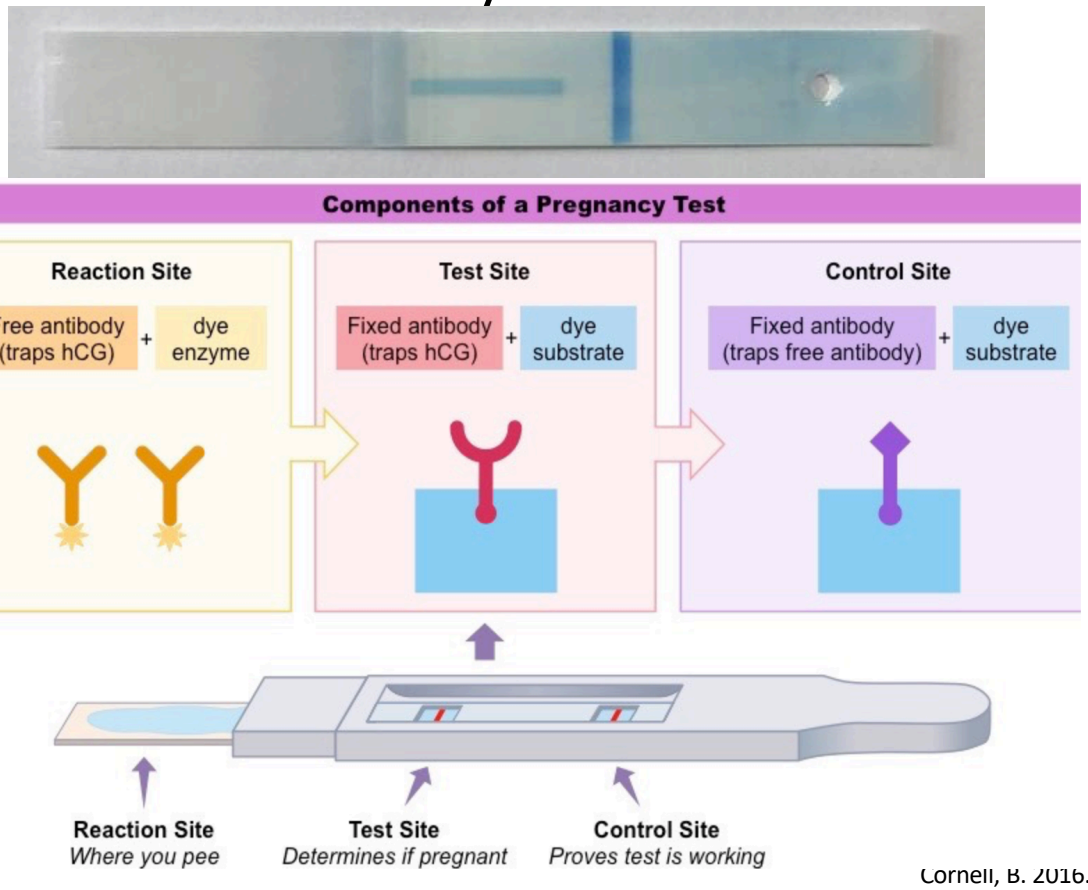
A pregnancy test is a Lateral flow immunochromatographic assay



- A pregnancy test is a widely used antibody based diagnostic device
  - anti-hCG (human chorionic gonadotropin, hormone) is a hormone that increases as a pregnancy progresses
- (B)Absorbent pad, a filter helping to remove any proteins or bacteria in the urine that may affect the assay's performance, leaving mostly water and the hCG protein.
- (D) Conjugate pad with free antibody specific to hCG that is conjugated to blue dye
- (E) Nitrocellulose membrane with two antibody test lines

https://www.futurelearn.com/info/courses/creating-the-amazing-engineering-the-future/0/steps/65276

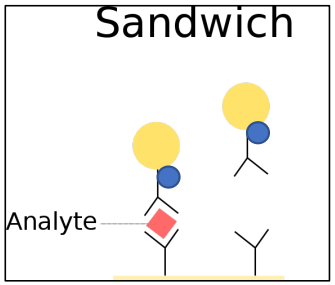
This design is used in many diagnostic devices and only necessitates a specific antibody



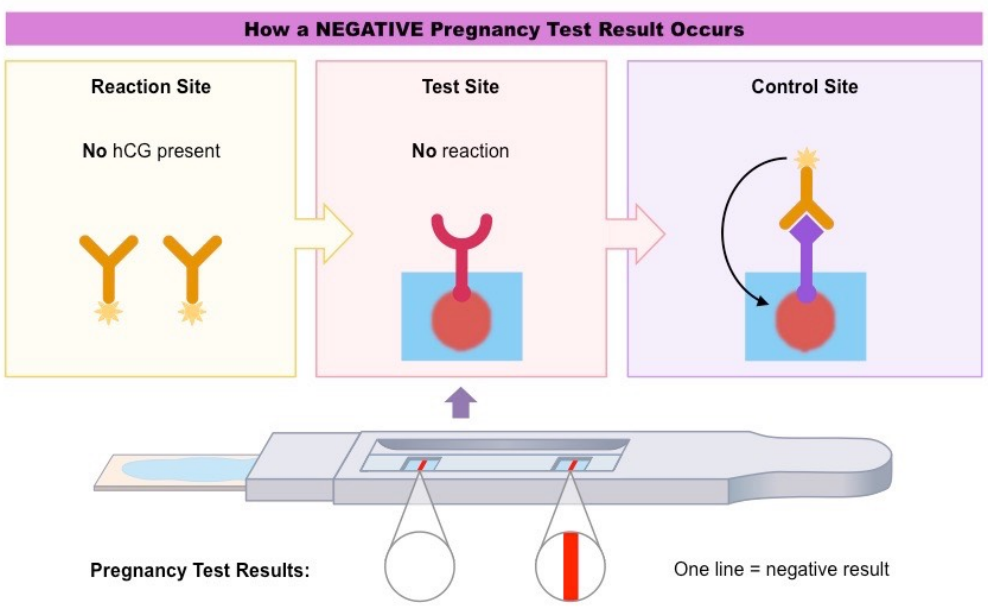
- 3 sites for antibody-antigen interactions:
  - Reaction site: free antibody first encounters hCG antigen
  - Test site: antibody-antigen complex can bind
  - Control site: all antibodies captured here to indicate test complete

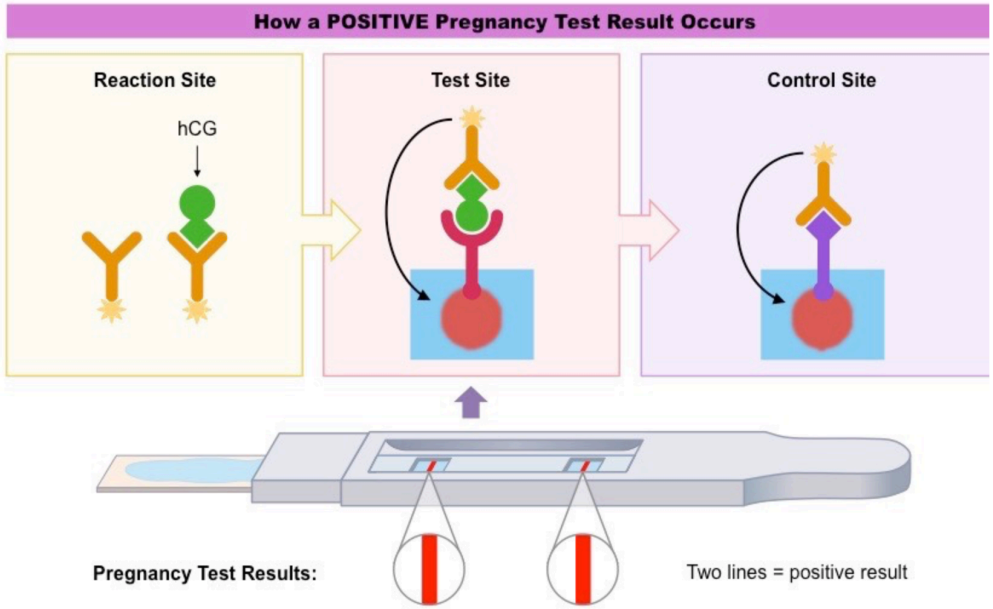
Cornell, B. 2016. *Referencing*. [ONLINE] Available at: http://ib.bioninja.com.au. [Accessed Jan 2021]

This diagnostic device is modified from a sandwich ELISA



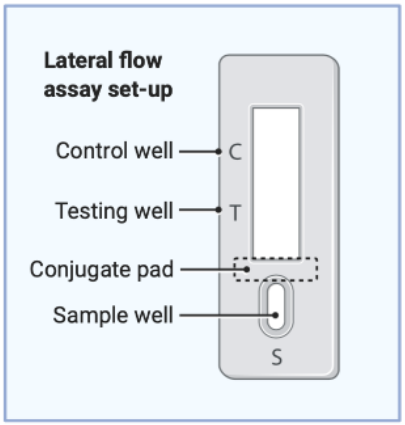
- If test **negative,** free antibodies flow through strip and end at control site (nothing at test site)
- If test is **positive**: hCG binds free antibodies also bind to the antibodies in the test strip, stopping them from flowing through the test strip (some also bind at control).

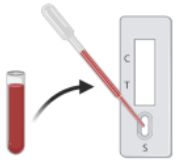




Cornell, B. 2016. *Referencing*. [ONLINE] Available at: http://ib.bioninja.com.au. [Accessed Jan 2021]

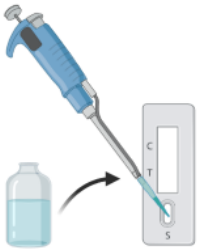
Serologic Diagnostic Test: COVID-19 Detection





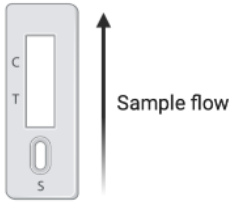
1 Sample loading

Add drop of blood or serum in sample well (S).



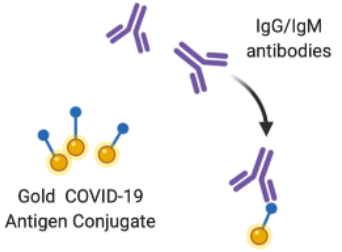
2 Buffer loading

Add dilution phosphate saline buffer to sample well.



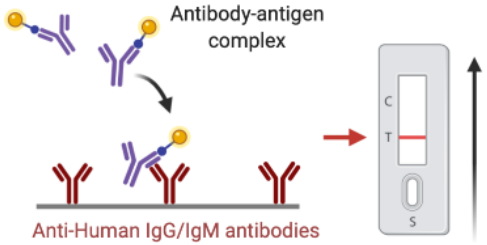
3 Sample incubation

Capillary action moves sample across lateral flow test.



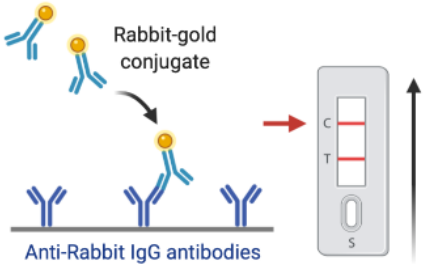
4 Antibody-antigen recognition

Antibodies with specificity for COVID-19 bind to gold COVID-19-antigen conjugates in conjugate pad.



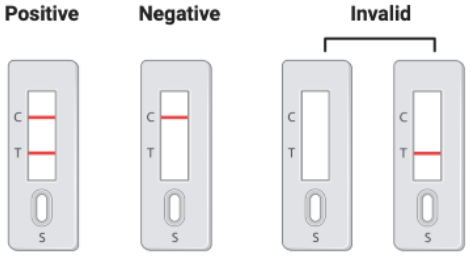
5 COVID-19 antibody detection

Sample enters testing well (T) and COVID-19 antibody-antigen complex binds to immobilized anti-human IgG/IgM antibodies.



6 Control antibody detection

Rabbit antibody-gold conjugate binds to immobilized anti-rabbit IgG antibodies.



7 Interpreting results

Positive: one strip each in C well and T well
Negative: one strip in C well

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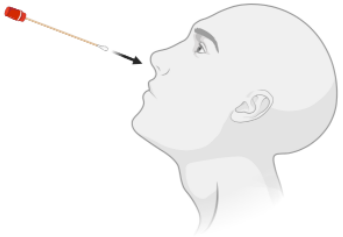
COVID-19 Diagnostic Test through RT-PCR

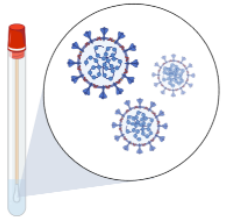
1 Nasopharyngeal swab <15 min

Cotton swab is inserted into nostril to absorb secretions.



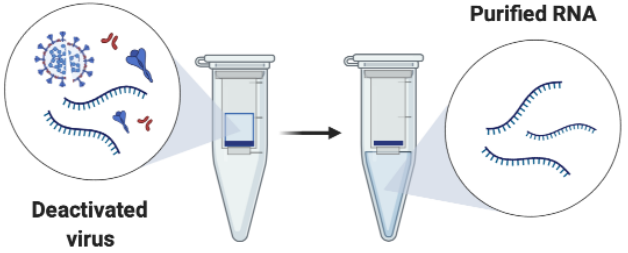
2 Collected specimen 0-72 h

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



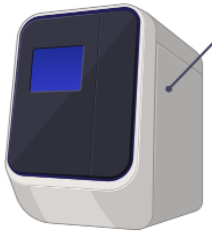
3 RNA extraction ~45 min

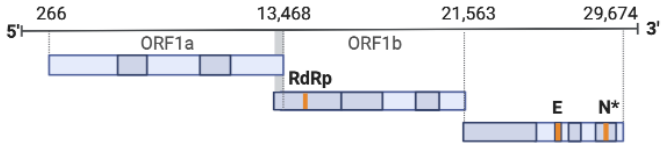
Purified RNA is extracted from deactivated virus.



4 RT-qPCR ~1 h per primer set

Purified RNA is reverse transcribed to cDNA and amplified by qPCR.





Primers and probes for screening

E gene First-line screening tool

E_Forward: ACAGGTACGTTAATAGTTAATAGCGT
E_Probe1: FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ
E_Reverse: ATATTGCAGCAGTACGCACACA

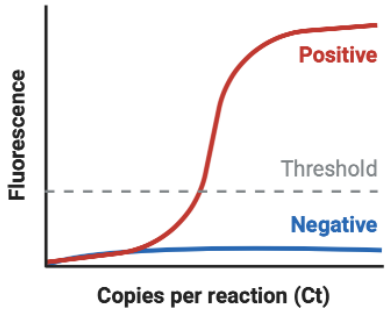
RdRp gene Confirmatory testing

RdRp_Forward: GTGARATGGTCATGTGTGGCGG
RdRp_Probe1: FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ
RdRp_Probe2: FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ
RdRp_Reverse: CARATGTTAAASACACTATTAGCATA

* N gene testing is not further used because it is slightly less sensitive.

5 Test results real-time

Positive SARS-CoV2 patients cross the threshold line within 40.00 cycles (< 40.00 Ct).



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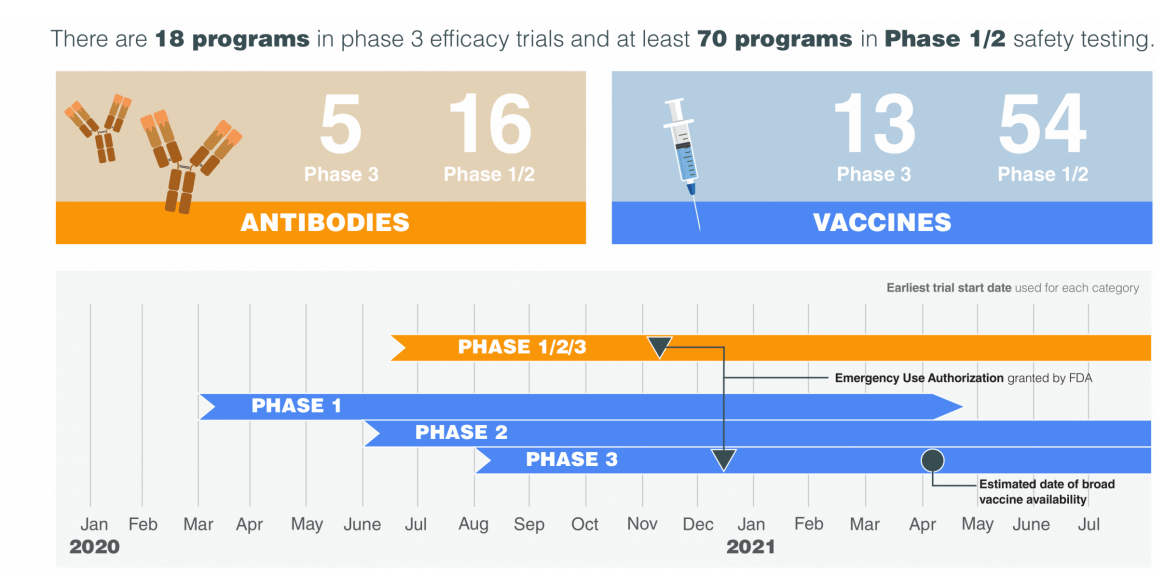
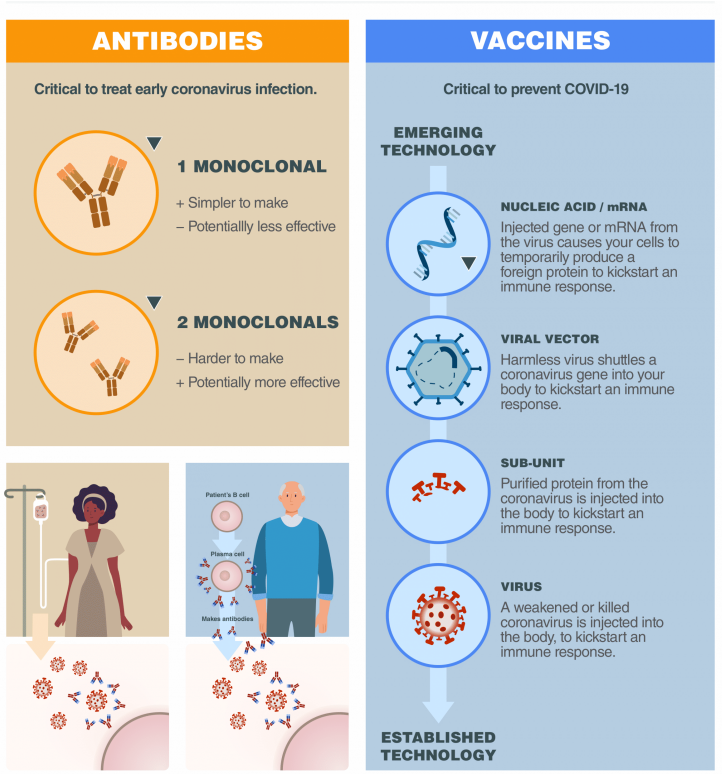
Stunning pace of science in 2020, what's to come?

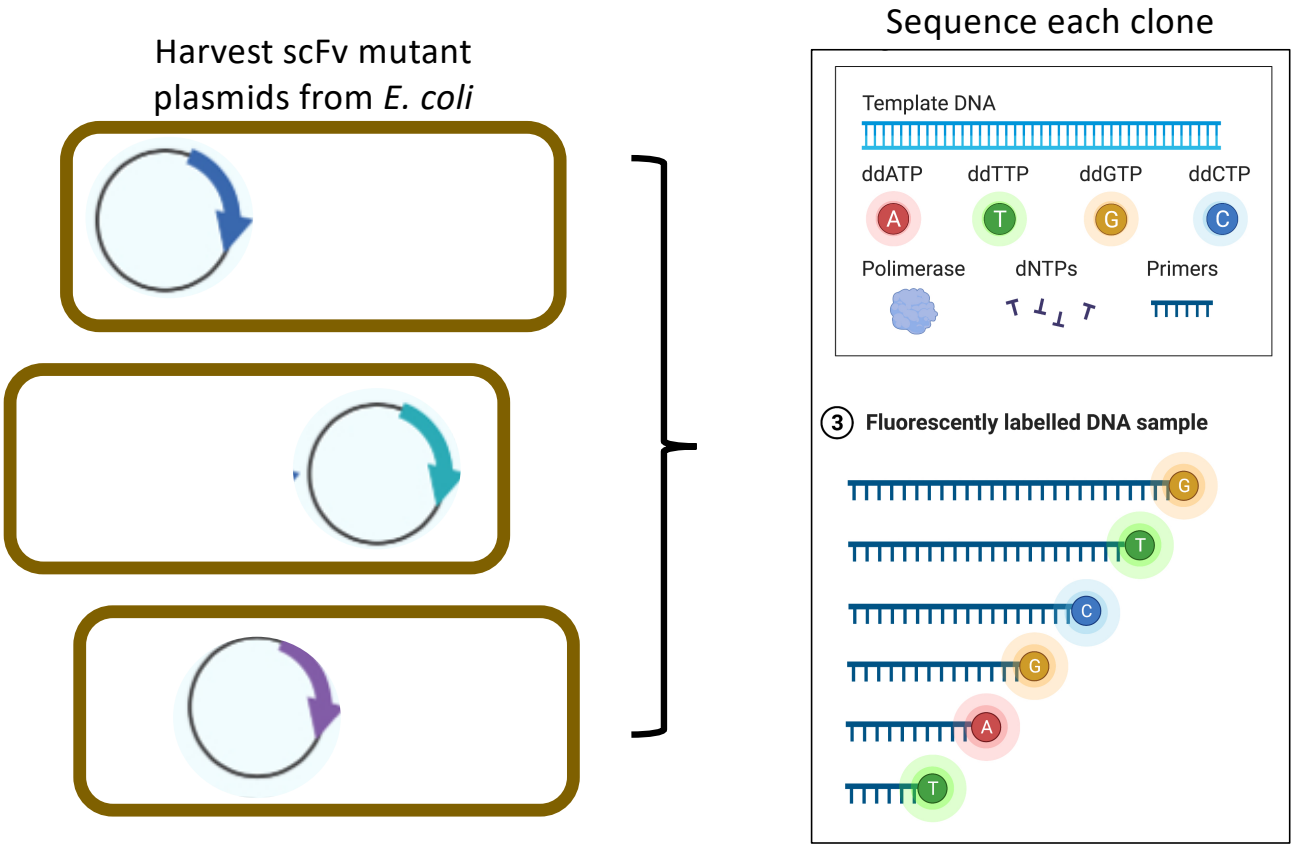


Published Dec. 8, 2020



https://www.forbes.com/sites/johncumbers/2020/12/08/the-stunning-pace-of-progress-one-year-in-covid-19-treatments-and-vaccines-are-close-at-hand-infographic

Today in lab, M1D4: Purify and sequence clone



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NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

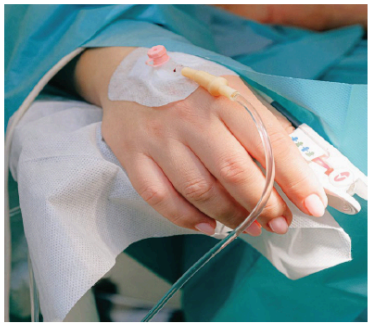
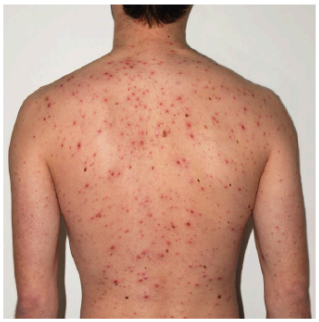
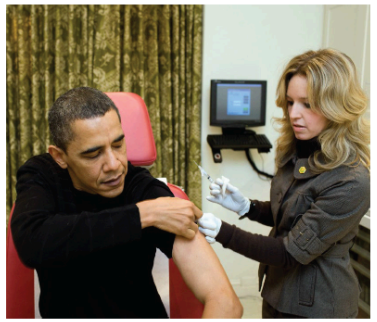
It Takes a Herd: How Can We Use Immunity to Combat an Emerging Infectious Disease?

by

Adam J. Kleinschmit
Department of Natural and Applied Sciences
University of Dubuque, Dubuque, IA

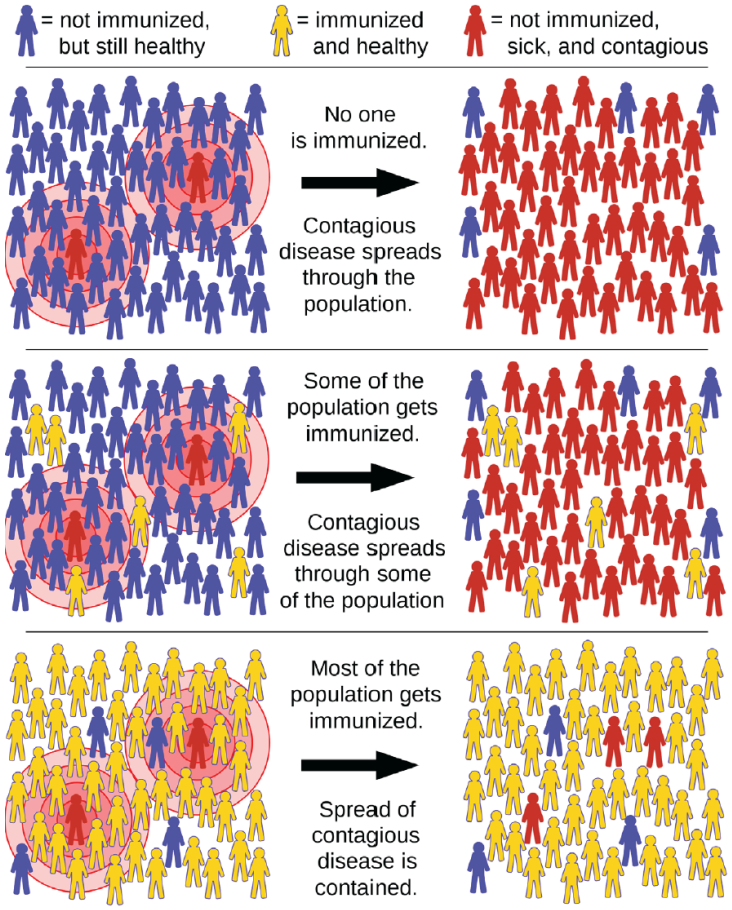
Immunity, ability to resist disease through preventing development of pathogen or by counteracting effects of the pathogen

- Active immunity: host immune's system directly produces neutralizing antibodies
- Passive immunity: antibodies produced in a donor organism and transferred to recipient

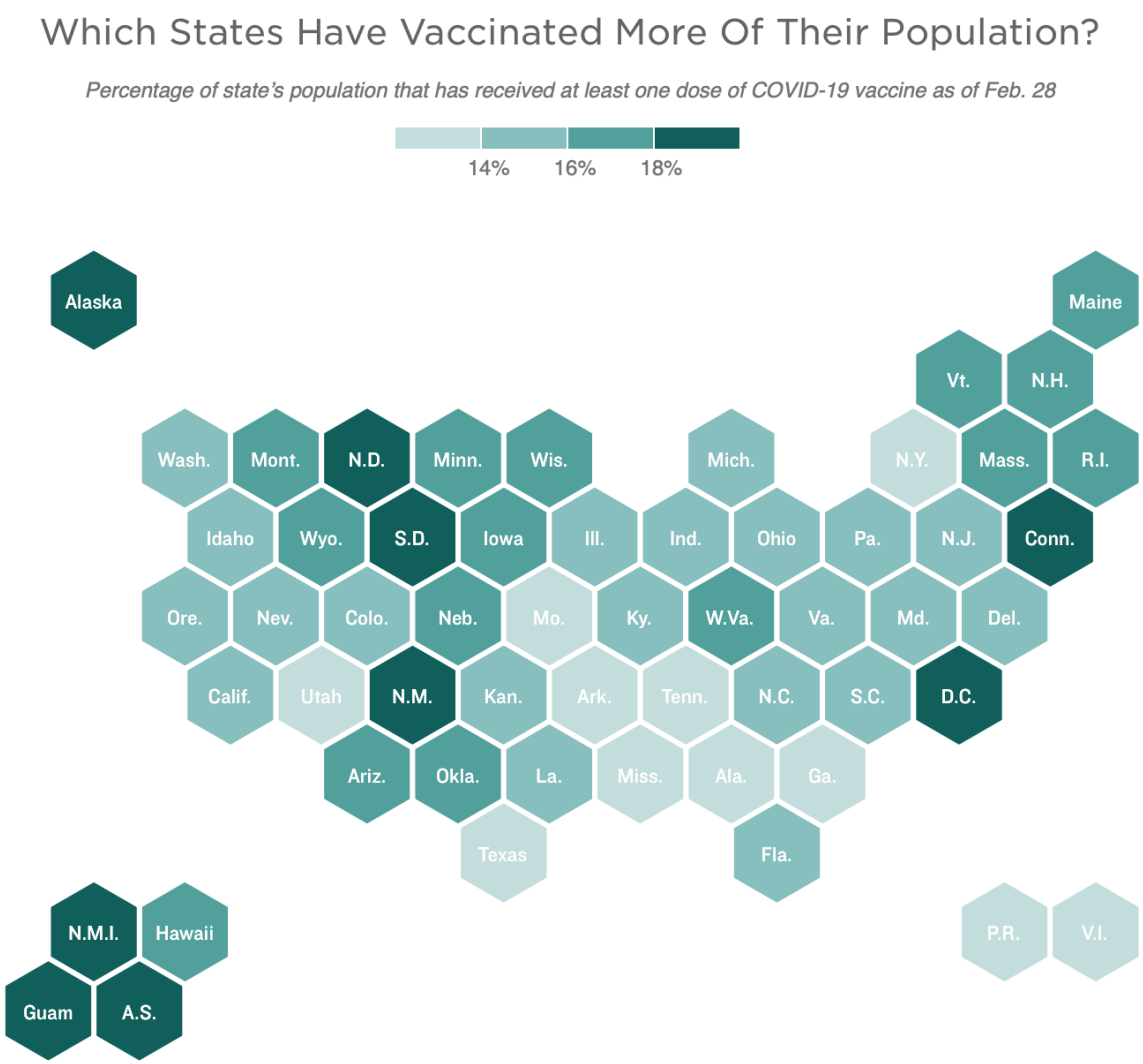
	Natural	Artificial
Passive	(A) Antibodies passed in breast milk or through placenta. 	(B) Antibodies harvested and transferred from another person, animal, or genetically engineered microbe. 
Active	(C) Illness and recovery. 	(D) Vaccination. 

Herd immunity, indirect protection from infectious disease when a sufficient percentage of a population has become immune

- In most cases, a diverse population can't all develop active immunity due to medical or physiological reasons
- The number of people that need immunity for herd protection depends on how transmittable the pathogen is
- Estimates for COVID-19 suggest ~70%* of the US population of 330million would need immunity to provide the benefit of herd immunity
- Massachusetts at 17.9% with one dose



*https://www.mayoclinic.org/diseases-conditions/coronavirus/in-depth/herd-immunity-and-coronavirus/art-20486808



https://www.npr.org/sections/health-shots/2021/01/28/960901166/how-is-the-covid-19-vaccination-campaign-going-in-your-state

Structure of our Discussion Today!

- Part 1, Individual Research (press releases/news articles):
  - (1) Reaching herd immunity,
  - (2) acquired active immunity through vaccination,
  - (3) naturally acquired immunity by infection
  - (4) acquired passive immunity through the creation of therapeutic monoclonal antibody cocktails.
- Part 2, Debrief Groups
  - Share expertise across groups
- Part 3, Whole Group Discussion
  - Discuss recent Massachusetts State policies that are designed to quickly reach herd immunity while providing vaccine rollout in an ethical way

Ethics discussion guidelines

- **We are accountable for our words and their impact.**
- Listen actively and with an ear to understanding others' views. Without interrupting.
  - Don't just think about what you are going to say while someone else is talking.
- Criticize ideas, not individuals.
  - We all can learn something from each other, even if your views don't necessarily align.
- Avoid blame, speculation, and inflammatory language.
- Avoid assumptions about any member of the class or generalizations about social groups.
  - Do not ask individuals to speak for their (perceived) social group.
- Personal information that comes up in the conversation should be kept confidential.
  - Take the message out of the classroom, not the messenger.

TLL staff, collated from Centers of Teaching and Learning at the University of Michigan, Columbia University, Cornell University, and UC Berkeley