





<u>COVID-19 Trials and Testing:</u> <u>Principles and</u> <u>Pharmacology</u> <u>for the Clinical Provider.</u>









<u>Thank You</u> <u>To WADEM</u> <u>Our Support Staff</u> <u>My Molecular Biology Mentors</u> <u>Drs. Freeman,Burris,Roux,Easton, etc</u> <u>My M.Sc. Forensic Science Professors</u> <u>GO GATORS!</u> <u>Abacus Pharma International (RDT)</u>

You, Our Audience on the Frontlines





DISCLOSURES: LIVE RAPID DIAGNOSTIC TEST DEMO (not endorsement) <u>Medical Director</u> <u>Haiti x 10 yrs</u> Contact Information / Website <u>COVID-RAPID.COM</u>





Covid-Rapid SARS-CoV-2 IGM/IGG AB (WB/S/P) One Step Rapid Test Cassette

40 TESTS

LOT: COV1252004C MFD: 20200425 EXP: 2021-04 IVD

Manufactured by Sure Bio-Tech for Abacus Pharma International USA 220 S Washington Alexandria, VA 22314 USA

RX Only ISO 📑 🕤 🔍 👘 🔲 Made in China

Sean G. Smith: Nurse-Paramedic Austere/ Emergency/ Critical-Care Contact Information / Website FaceBook, Twitter, LinkedIn, IG CriticalCareProfessionals@ <u>GMail.com</u>





What We ARE Going to Talk About.

Basic Molecular Biology / Biochemistry of various agents under consideration in the treatment of COVID-19, as well as briefly touch on the science and challenges of developing a

vaccine for the SARS-CoV2 virus. Additionally, we will explore current tests used in COVID-19, with a specific focus on Polymerase Chain Reaction (PCR) to detect viral genetic material and Immunoassay Rapid Diagnostic Tests (RDTs) designed to demonstrate exposure to viral antigens. We will clearly and concisely examine the science, pros, cons, and potential pitfalls in each of these areas, as well as how they relate to day to day clinical practice.



What we are NOT Going to Talk About:

Aside from isolated potential live questions/answers, We will not spend a lot of time dwelling on specific studies or policies. That Information is: beyond the intended purpose of this lecture, readily available on the web, provided as a resource, or may be answered by myself off-line. NB: Any ONE of these Therapeutic or Testing Options could easily comprise an entire day's worth of lecture in and of itself. This seminar is to plant seeds of inquiry in the garden of your mind, point you in the right directions by curating resources, and answer selected guestions both live and later, off-line.









Resources: Deliberately Limited

Readily Available on Internet Screen Shots In Body SCCM / WADEM / AACC Selected InfoGraphics from JAMA/BMJ/NEJM Selected InfoGraphics from "Compound Interest" CompoundChem.com MULTILINGUAL / GLOBAL LAYMAN / MOLECULAR BIOLOGIST







A recent study showed that csall recent studies are false.



RAPID DIAGNOSTIC TEST DEMONSTRATION / WHY?

https://miami.cbslocal.com/video/program/1506/4533753-firsthand-look-at-how-covid-19-antibody

-tests-are-administered/?









TREATMENT NB: US References But Global Scope



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Coronavirus Disease 2019 (COVID-19)

Symptoms

Testing	+
Prevent Getting Sick	+
If You Are Sick	+
Daily Life & Coping	+
People Who Need Extra Precautions	+
Pets & Other Animals	+
Travel	+
Frequently Asked Questions	
Cases. Data & Surveillance	+

Communities, Schools & Workplaces

Information for Clinicians on Investigational Therapeutics for Patients with COVID-19

Print Page

Updated April 25, 2020

There are no drugs or other therapeutics presently approved by the U.S. Food and Drug Administration (FDA) to prevent or treat COVID-19. Current clinical management includes infection prevention and control measures and supportive care, including supplemental oxygen and mechanical ventilatory support when indicated.

The National Institutes of Health have published interim guidelines for the medical management of COVID-19 🗹 prepared by the COVID-19 Treatment Guidelines Panel.

These guidelines contain information about investigational therapeutics and will be updated as new information emerges and drugs and other therapeutic interventions are approved for use by FDA.

Persons seeking information about registered clinical trials for COVID-19 in the United States can search for such information here: ClinicalTrials.gov \square .

Page last reviewed: April 25, 2020 Content source: National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases

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Site Updated: June 16, 2020

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Home /What's New

What's New	
Introduction	
Overview	+
Critical Care	+
Antiviral Therapy	+
Immune-Based Therapy	+
Antithrombotic Therapy	
Concomitant Medication	IS
Panel Roster	
Panel Financial Disclosu	re
Guideline PDFs Section Only (PDF) Full Guideline (PDF)	<u>222 KB)</u> . <u> 1 MB)</u>

What's New in the Guidelines

Last Updated: June 16, 2020

June 16, 2020 Update: On June 15, The Food and Drug Administration revoked the emergency use authorization (EUA) that permitted the use of chloroquine and hydroxychloroquine donated to the Strategic National Stockpile to treat certain patients with COVID-19. In light of this announcement, the following sections of the COVID-19 Treatment Guidelines have been updated to remove the information regarding the EUA:

- Antiviral Drugs Under Investigation
- Chloroquine or Hydroxychloroquine
- Table 2b

Key Updates to the Guidelines

Special Considerations in Children

This section now includes a preliminary description of multisystem inflammatory syndrome in children (MIS-C), a condition that has been associated with COVID-19 in children and young adults. This section will be updated as more data become available.

Potential Antiviral Drugs Under Evaluation for the Treatment of COVID-19

The recommendations for using remdesivir, chloroquine, and hydroxychloroquine to treat COVID-19 have been revised based on data from recently published clinical trials and observational cohort studies. This section and Table 2a now include detailed summaries of the study results. The revised recommendations are listed below,





We'll Be Exploring / Referencing The Molecular Biology of Selected Examples of Each of These:

Anti-Viral Therapy Immune Modulators Antithrombotic Concomitant Rx / "Others" (HCQ, Steroids, etc)







<u>Testing</u> Again, US Based References, But Global Scope



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General FAQs

	that mean it is CLIA waived? (Updated 5/9)	
Daaddraaa	Q: When tests are offered prior to or without an EUA under the FDA's Policy for Coronavirus Disease-2019 Tests, what is their CLIA categorization?	
Disclosure /	Q: Are EUA-authorized SARS-CoV-2 diagnostic tests limited to use in individuals who are symptomatic for COVID-19? (Updated 6/16)	
COI with API RDT	Q: Is any EUA-authorized SARS-CoV-2 diagnostic test authorized for the broad screening of asymptomatic individuals for COVID-19? (New 6/16) \checkmark	
	Q: Can an EUA-authorized SARS-CoV-2 diagnostic test be used for surveillance for COVID-19? (New 6/16) \checkmark	
	Q: Can I offer my test for home use, including self-collection of a specimen, testing, and interpreting results, under the Policy for Coronavirus Disease-2019 Tests? (Updated 5/6) 🗸	
	Q: Can I offer my test for self-collection of a specimen at home and shipping to a laboratory for testing? (Updated 5/29) \checkmark	
	Q: Do all COVID-19 tests require a prescription? Do they need to be ordered by a physician? (Updated 5/9)	
	Q: I operate a pharmacy and would like to offer COVID-19 testing. What tests are available for use? Where do I get test kits? (Updated 5/9)	
	Q: I am developing a new COVID-19 diagnostic test and need assistance with funding to complete test development. Are there any resources available to me? (New 5/26)	

eBooks











Timing Contamination Sensitivity Specificity Seroconversion (or lack thereof) Persistence (both for testing and for presumed immunity) ~ 12 months, Cross Reaction (e.g. previous exposure to SARS-COV-1) Two Antibodies, so depending on Immunoassay, "double trouble". Sean G. Smith: Nurse-Paramedic Austere/ Emergency/ Critical-Care



- **Timing** (see chart..."sweet spot" for all 3 is ~ 14 days, sooner or later may give "false" results.)
- **Contamination** (PCR... QA/QC Forensics vs Healthcare: Who is watching the watcher and how closely?)
- Seroconversion (or lack thereof)
- **Persistence** (both for testing and for presumed immunity) IgG ~ 12 months...best current guess.)
- **Cross Reaction** (e.g. previous exposure to SARS-COV-1 lgG)





Two Antibodies: IgM and IgG, so depending on Immunoassay, "double trouble". IgM testing has been shown in some cases to have lower sensitivity and specificity than IgM.

Sensitivity (greater true positive, less false negative)

Specificity (greater true negative, less false positive)

Another Clinical Take Home: "Reinfection" unlikely. Occam's Razor: Faulty sampling/processing.







RAPID DIAGNOSTIC TEST DEMONSTRATION RESULT



How does PCR testing for COVID-19 work?

Polymerase chain reaction (PCR) testing can detect even very small amounts of viral genetic material in a sample by duplicating it many times over through a complex laboratory process called amplification.

Amplification creating

millions of copies

28 21 38 PCR Ordes

(1) A test sample is swabbed from the back of the nose and processed to isolate genetic material.

2 Small pieces of specifically engineered genetic material, called primers, are introduced and bind to the isolated viral genetic material, initiating amplification.

IIImm

TTTTTTTT

IIIuuu





3 Fluorescent markers bound to the copies during PCR are released and can be detected when amplification occurs.

Positive result When there is viral genetic material in the sample, amplification occurs, releasing enough fluorescent markers to be detected.

Negative result If there is no viral genetic material in the sample, amplification will not occur and no fluorescent markers will be detected.

HOW DO THE TESTS FOR CORONAVIRUS WORK?

HOW CURRENT TESTS WORK

A swab is taken of the inside of a patient's nose or the back of their throat. This sample is then sent to a lab to test.



The RNA of the virus is extracted and purified. An enzyme, reverse transcriptase, converts the RNA to DNA.

2

3

4

The DNA is mixed with primers, sections of DNA designed to bind to characteristic parts of the virus DNA. Repeatedly heating then cooling DNA with these primers and a DNAbuilding enzyme makes millions of copies of virus DNA.



Fluorescent dye molecules bind to the virus DNA as it is copied. Binding makes them give off more light, which is used to confirm the presence of the virus in the sample.



POSITIVE AND NEGATIVE TESTS

The fluorescence increases as more copies of the virus DNA are produced. If it crosses a certain threshold, the test is positive. If the virus isn't present, no DNA copies are made and the threshold isn't reached. In this case, the test is negative.



ISSUES WITH TESTING REAGENT ISSUES High demand and issues with reagents have delayed testing in some countries. TIME-CONSUMING It takes a few hours to get results from the test, limiting how many tests can be done. FALSE POSITIVES AND NEGATIVES In some cases sample degradation or contamination can affect the results. **FUTURE TESTS** The current tests are good for diagnosing an infection - but they can't tell us if someone has had it and then recovered. Tests that look for antibodies against the virus can do this.



Tests that look for proteins on the surface of the virus are also in development. These tests are faster, but less accurate.

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3

Fluorescent dye molecules bind to the virus DNA as it is copied. Binding makes them give off more light, which is used to confirm the presence of the virus in the sample.



PCR: Polymerase Chain Reaction





POSITIVE AND NEGATIVE TESTS

The fluorescence increases as more copies of the virus DNA are produced. If it crosses a certain threshold, the test is positive. If the virus isn't present, no DNA copies are made and the threshold isn't reached. In this case, the test is negative.





ISSUES WITH TESTING



REAGENT ISSUES

High demand and issues with reagents have delayed testing in some countries.



TIME-CONSUMING

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8

FALSE POSITIVES AND NEGATIVES

In some cases sample degradation or contamination can affect the results.

ANTIBODY TESTS PART 1: WHAT THE TESTS TELL US



----- Constant region of antibody Identical in antibodies of the same type

Once we've recovered from an infection, our immune cells "remember" the antigen. If we are reinfected, antibodies are rapidly made to remove it. This is immunity; it's life-long for some diseases and fades over time for others.

Ci

WHAT ANTIBODY TESTS TELL US

Antibody tests usually test for the presence of two different types of antibody: IgM and IgG. IgG is the most common antibody produced in the body in response to an infection.



*Antibodies don't appear until someone has had an infection for several days, so this doesn't guarantee they're not infected.

Having antibodies against an antigen isn't a guarantee of immunity. Levels of antibodies and their effectiveness are also important.

ANTIBODY TEST ACCURACY



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ANTIBODY TESTS PART 2: HOW DO THE TESTS WORK?

RAPID DIAGNOSTIC TESTS



How effective are the antibodies?

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NEUTRALISATION ASSAY TEST

This test is lab-based and takes several days. It can tell us how effectively patient antibodies can neutralise a virus.



Serial dilutions of the patient sample are mixed with a suspension of the virus (the concentration of which remains constant).

The combination of patient samples and virus suspensions are incubated then added to host cells in a petri dish. The dishes are covered in agar and incubated.

A plaque forms on the dish contents over several days. Antibodies to the virus in the patient sample reduce plaque formation. Results at different dilutions help us know how effectively the patient antibodies block the replication of the virus.

The test usually takes 3-5 days.





The patient sample is added to a microplate well coated with deactivated antigen or a protein from the antigen, then incubated.

2 If the patient sample has antibodies to the antigen, they bind to the antigen or protein. Enzyme-labelled antibodies are then added which bind to the patient antibodies.

The enzyme substrate is added.

3

The substrate changes colour when it binds to the enzyme. The intensity of the colour links to the level of antibodies in the sample.

The test usually takes 2–5 hours.

WHAT CAN THIS TEST TELL US?





The test usually takes under an hour.

OTHER TYPES

1

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Other types of immunoassay include fluorescence and microsphere immunoassays.

WHAT CAN THESE TESTS TELL US?

How effective are the antibodies?

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The patient sample is added here. The sample and any antibodies it contains then flows down the strip.

This part of the test strip contains the antigen attached to gold nanoparticles. If there are antibodies in the patient sample for the antigen they bind to it, carrying the antigen (and the gold particles) with them.

3

At the test lines, antibodies from the sample are captured. The gold nanoparticles they carried with them make the test line turn red to indicate a positive test. The control line shows the test has worked correctly.

The test usually takes 10-30 minutes.



RDT

WHAT CAN THIS TEST TELL US?

Are antibodies present in the sample?



The level of antibodies in the sample

How effective are the antibodies?





WORLD ASSOCIATION FOR DISASTER AND EMERGEN

3

The patient sample is added to a microplate well coated with deactivated antigen or a protein from the antigen, then incubated.

2

1

If the patient sample has antibodies to the antigen, they bind to the antigen or protein. Enzyme-labelled antibodies are then added which bind to the patient antibodies.



4

The enzyme substrate is added.

The substrate changes colour when it binds to the enzyme. The intensity of the colour links to the level of antibodies in the sample.

The test usually takes 2-5 hours.

<u>ELISA</u>

WHAT CAN THIS TEST TELL US?

Are antibodies present in the sample?

The level of antibodies in the sample

How effective are the antibodies?



CHEMILUMINESCENT IMMUNOASSAY (CLIA)



Similar to ELISA, but the substrate added causes a light-producing chemical reaction. The amount of light produced links to the sample antibody levels.



The test usually takes 1-2 hours to run.

ELECTROCHEMILUMINESCENCE IMMUNOASSAY



Uses electrochemiluminescent labels, which produce light when an electric current is applied. The amount of light produced links to the sample antibody levels.



The test usually takes under an hour.

OTHER TYPES

Other types of immunoassay include fluorescence and microsphere immunoassays.



Some Other Immuno-Assays

WHAT CAN THESE TESTS TELL US?

Are antibodies present in the sample?



The level of antibodies in the sample

How effective are the antibodies?





RDT: At least 3 Big Clinical Take Aways:

- 1. NOT resource, time, or technology intense.
- 2. + Asymptomatic (if in window and if seroconverter)
- 3. Treatment (a. as CareGiver Ebola, b. Convalescent Plasma)



Serum....CPP and BBB are ALWAYS the Right Answer....so, What About CSF?

"A case series of three patients attending an inner city US hospital who had severe, laboratory-confirmed COVID-19 and encephalitis shows that while only one had abnormal white blood cells or protein present in CSF, all had evidence of immunoglobulin (IgM) antibodies.

"What was novel about our study was that we were able to show IgM, the acute phase reactant against COVID, in the spinal fluid of these patients, which is a direct indicator they had COVID in their brain," lead author Karima Benameur, MD...

"The only reason we could actually diagnose them with COVID encephalitis is because we were able to measure the IgM in their spinal fluid," said Benameur. Neurologists ordering spinal taps on patients may incorrectly assume there's no brain involvement if the spinal fluid is normal, said Benameur. Benameur emphasized, "just because the PCR in CSF is negative, this does not mean that the virus has not made it into the brain." The PCR test is a good test for some viruses, including the herpes virus, but is a poor test for this new coronavirus, she said. "

TAKE HOME: Consider Testing CSF for IgM in suspected COVID encephalitis.





A recent study showed that csall recent studies are false.



K+, pH, Mg++, PO4----





We'll Be Exploring / Referencing The Molecular Biology of Selected Examples of Each of These:

Anti-Viral Therapy Immune Modulators Antithrombotic Concomitant Rx / "Others" (HCQ, Steroids, etc)



Why Chloroquine and various permutations? Proposed effects: May interfere with SARS-CoV-2 binding of ACE-2 receptors and subsequent host cell entry. Alters cellular pH with concomitant effects Interferes with viral assembly Immunomodulatory effect by down regulating TNF-alpha (a cytokine)

Anti Viral Example: Remdesivir: Adenosine nucleoside triphosphate analog. Interferes with RNA polymerase, to terminate chain, stop synthesis, and decrease viral RNA production.

We'll Be Exploring / Referencing The Molecular Biology of Selected Examples of Each of These:

Famotidine : aka "Pepcid AC" Serum Protease Inhibitor. Interferes with Viral Replication. Low Cost outpatient, etc. Small observational study, but potentially encouraging.

Other Anti Virals: Act against various targets. Research in process but not particularly promising to date. Please see NIH website.

VACCINE ACTIVE IMMUNITY Specificity, Mutation, Equitable Access, etc.

NB: WADEM Has an excellent podcast specifically on Vaccines and SARS-CoV-2. Please visit our website and YouTube channel for an in depth discussion from Prof. Jerome Hauer.

- Concomitant Rx / "Others" : CONVALESCENT PLASMA
- **PASSIVE Immunity** Direct Transfer of Antibodies. Limits COVID-19 progression/load.
- Passive Transfer Of Other Plasma Components. Possible anti-inflammatory and anti-coagulation benefits.

A. CONVALESCENT PLASMA

B. ANTIVIRAL EFFECTS

Immune Modulators:

COVID-19 illness in native and immunosuppressed states: A clinical-therapeutic staging proposal Hasan K. Siddiqi, MD, MSCR, and Mandeep R. Mehra, MD, MSc

Image Follows.

Immune Modulators: Examples and Theory. "Cytokine Storm" IL-1,IL-6, Interferons, Janus Kinase: Involved Multitude of Inflammatory/Immune Responses

Modulate These, Modulate the Inflammatory Response.

NIH Guidelines: "There are insufficient data to recommend either for or against the use of the following agents for the treatment of COVID-19 (AIII):

Interleukin-1 inhibitors (e.g., anakinra)

Interleukin-6 inhibitors (e.g., sarilumab, siltuximab, tocilizumab)

Except in the context of a clinical trial, the Panel **recommends against** the use of other immunomodulators, such as:

Interferons (AIII), because of the lack of efficacy in treatment of severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) and toxicity.

Janus kinase inhibitors (e.g., baricitinib) (AIII), because of their broad immunosuppressive effect."

Please Reference SCCM Lecture on Same. (Later Slide)

Immune Modulators:

NB: Also "Filtration" systems to reduce bioburden of Immune Modulators...Inflammatory Mediators...Cytokines.

Steroids NB: Dynamic Differences between CURRENT reported research and NIH guidelines. **Dexamethasone Takeaways.** Seriously ill COVID-19 patients see 20-30% reduction in mortality, depending on exact demographic. (nonvent vs vent dependent.) Good news but different studies show varying efficacy with different doses/ timing and jury still out. Literally.

If Real and Reproducible= Readily Accessible Rx/Tx.)

Antithrombotics: COVID-19 Hypercoagulable Pathology. Two Clinical Questions:

VTE Prophylaxis: Standard vs Increased?
2. EMPIRIC Anticoagulation?

Smith's Law: The Yo Mama Principle. Cookie Cutter Medicine vs Evidence Driven Proactive Clinical Management. Right Treatment. Right Patient. Right Time. Please Reference SCCM Lecture on Same. (Later Slide) Spoiler Alert: They Agree w/ Smith's Law.

Question & Answer IMMUNE MODULATORS

COVID-19 Resources

Speaker: Andrea Nei Pharm.D., BCPS, BCCCP

Speaker: Brian Dee BCCCP, PharmD

Moderator: Lewis J. Kaplan MD, FACS, FCCM

pharmacists advancing healthcare

Debate 2 – Approaches to anticoagulation in patients with COVID-19

COVID-19 Resources

CON

William E Dager, Pharm.D., BCPS, MCCM, FASHP, FCCP, FCSHP Pharmacist Specialist UC Davis Medical Center Clinical Professor of Pharmacy UC San Francisco School of Pharmacy Clinical Professor of Medicine, UC Davis School of Medicine

Nothing to disclose

Allison E. Burnett, PharmD, PhC, CACP Antithrombosis Stewardship Pharmacist University of New Mexico Hospital Adjunct Associate Professor University of New Mexico College of Pharmacy Albuquerque, New Mexico, USA

Nothing to disclose

Society of Critical Care Medicine

Multi-System Inflammatory Syndrome in Children (MIS-C): Multifaceted Proactive Supportive Care

<u>Thank You</u> <u>To WADEM</u> <u>Our Support Staff</u> <u>My Molecular Biology Mentors</u> <u>Drs. Freeman,Burris,Roux,Easton, etc</u> <u>My M.Sc. Forensic Science Professors</u> <u>GO GATORS!</u> <u>Abacus Pharma International (RDT)</u>

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Questions, Comments, Concerns? THANK YOU...VERY MUCH Linked In Facebook <u>CriticalCareProfessionals@Gmail.com</u> Twitter Instagram

