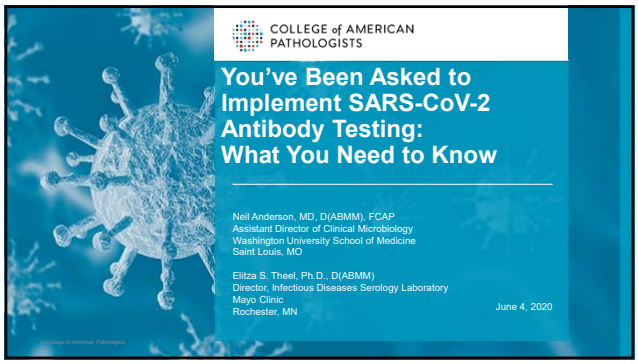
COLLEGE of AMERICAN PATHOLOGISTS
You've Been Asked to Implement SARS-CoV-2 Antibody Testing: What You Need to Know
Neil Anderson, MD, D(ABMM), FCAP
Assistant Director of Clinical Microbiology
Washington University School of Medicine
Saint Louis, MO
Elitza S. Theel, Ph.D., D(ABMM)
Director, Infectious Diseases Serology Laboratory
Mayo Clinic
Rochester, MN
June 4, 2020
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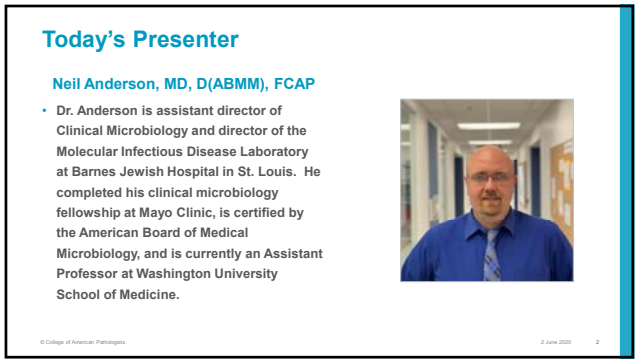
Today's Presenter
Neil Anderson, MD, D(ABMM), FCAP
• Dr. Anderson is assistant director of Clinical Microbiology and director of the Molecular Infectious Disease Laboratory at Barnes Jewish Hospital in St. Louis. He completed his clinical microbiology fellowship at Mayo Clinic, is certified by the American Board of Medical Microbiology, and is currently an Assistant Professor at Washington University School of Medicine.
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Today's Presenter
Elitza S. Theel, Ph.D., D(ABMM)
• Dr. Theel is the director of the Infectious Diseases Serology laboratory and co-director of the vector borne diseases service line at Mayo Clinic, in Rochester MN. She completed her Clinical Microbiology fellowship at Mayo Clinic, is certified by the American Board of Medical Microbiology, and is currently an Associated Professor of Laboratory Medicine and Pathology.
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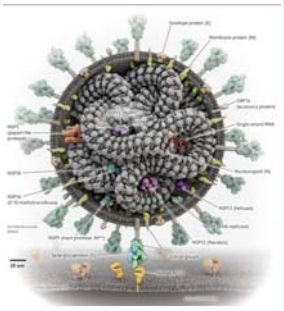
SARS-CoV-2: The Virus

- Enveloped, with a ssRNA genome
- 4 Coronavirus genera
  - *Alphacoronavirus (Mammals)*
    - 229E and NL63
  - *Betacoronavirus (Mammals)*
    - OC43 and HKU1
    - SARS-CoV (2002-2003)
    - MERS-CoV (2012)
    - SARS-CoV-2 (2019-?)
  - *Gammacoronavirus (Birds)*
  - *Deltacoronavirus (Birds)*
- Bats are the natural reservoir for SARS-CoV-2
  - Pangolins and/or turtles as intermediate hosts?



Parks JM and Smith JC. NEJM. 2020. DOI: 10.1056/NEJMcibr2007042

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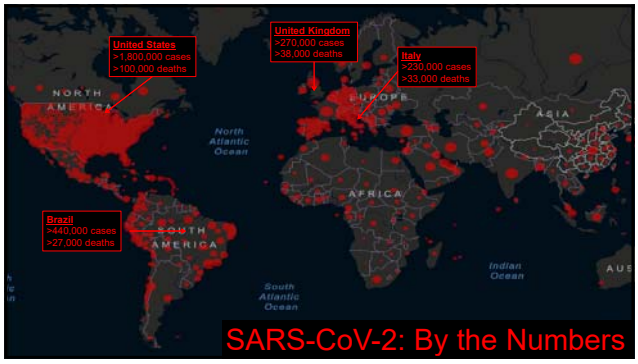

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Testing Methods for SARS-CoV-2

- Molecular methods to detect viral RNA
  - Preferred method for direct diagnosis of COVID-19
  - Numerous molecular tests with EUA
    - Target combination of genes: Nucleocapsid (N), Open reading frame 1ab (Orf), Envelope (*E*), or the RNA dependent RNA polymerase (RdRp)
    - Performed on upper or lower respiratory tract samples
    - *Many* challenges associated with collection device and reagent supply chain issues
- Antigen Detection
  - 1 EUA assay available
    - Detects nucleocapsid protein (most abundant viral protein) from nasal or nasopharyngeal swabs
  - 15 minute, lateral flow immunofluorescent assay
  - Reported performance characteristics:
    - 80% sensitivity – confirm negatives with a molecular assay, "if necessary for patient management."
    - 100% specificity
  - Independent evaluations of accuracy needed

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Antibody Testing for SARS-CoV-2:
So much hype…

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Serologic Tests for SARS-CoV-2:
The Regulatory Perspective

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SARS-CoV-2 Serologic Test Regulations in the USA:
Where we started and where we are now

- Initially, the Food and Drug Administration did not require emergency use authorization (EUA) for SARS-CoV-2 serologic tests because:
  - Antibody tests were not meant to be diagnostic
  - Intended to be used to answer the question of prevalence
  - Intended to limit antibody testing to CLIA-certified high-complexity labs
  - Indicated that this policy would be re-visited
- Manufacturers were encouraged to apply for EUA
- Serologic tests fell under FDA's 'Pathway D' for COVID-19 tests:

> A: As stated in Section IV.D of the FDA's *Policy for Diagnostic Tests for Coronavirus Disease-2019*, the FDA does not intend to object to the development and distribution by commercial manufacturers, or development and use by laboratories, of serology tests to identify antibodies to SARS-CoV-2, where the test has been validated, notification is provided to FDA, and information along the lines of the following is included in the test

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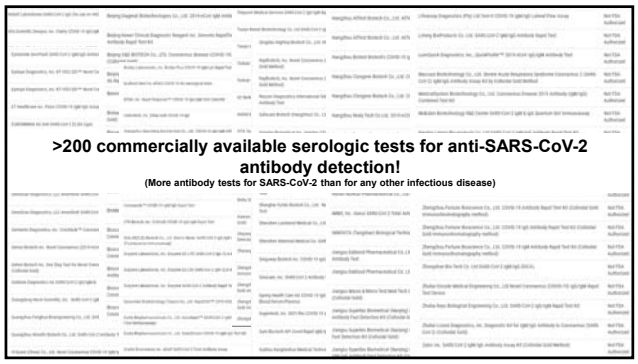

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Updated FDA Guidance for SARS-CoV-2 Serologic Tests

- May 4th, 2020 new guidance:
  - Manufacturers *must* submit validation data for EUA w/in 10 days from the date of FDA notification
  - FDA has provided specific performance threshold requirements
  - LDT's can still be developed and validated in high-complexity, CLIA-certified labs
    - Lab should notify FDA, follow labeling recommendations and are *encouraged* to seek EUA
- Streamlined processes for EUA submission:
  - Serology EUA template available
  - Independent assay evaluation through NIH's National Cancer Institute (NCI)
    - *NEW* 'Umbrella' Route

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'Umbrella' EUA Route for SARS-CoV-2 Serologic Tests (April, 28th 2020)

- Manufacturer's voluntarily submit their assay for independent evaluation by the NCI
  - LFAs or ELISAs for anti-SARS-CoV-2 IgM, IgG or IgM/IgG assays (IgA tests not eligible)
  - Plasma/serum only
- FDA approved evaluation panel and acceptance criteria performed at NCI:
  - 30 confirmed SARS-CoV-2 Ab positive samples/Ab type
  - 80 Ab negative and/or pre-COVID-19 samples (10 must be HIV positive)
  - Acceptance criteria:
    - Total Ab tests: ≥90% PPA and 95% NPA
    - IgM specific tests: ≥70% PPA
    - IgG specific tests: ≥90% PPA
    - NO cross-reactivity in HIV positive samples
- Manufacturer must supply or adhere to:
  - Antibody class specificity data if IgM and IgG are detected separately
  - Any additional validation data to support their claims
  - Must follow specific test labeling recommendations

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Current SARS-CoV-2 Antibody Test Status

- Currently: ~190 commercially available serologic tests for SARS-CoV-2
  - 15 with emergency use authorization (EUA) granted by the FDA
  - Remaining have submitted for EUA
- 31 serologic test manufacturers either did not receive or submit for EUA
  - Test should not be distributed or used
- No antibody tests are approved for at-home or point-of-care use
  - Alternative, non-venipuncture collection methods are increasingly being investigated
  - Do not require separate EUA
  - Do require bridging validation study

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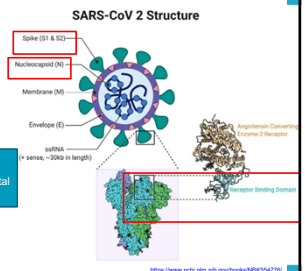
Variations in SARS-CoV-2 Serologic Test Designs

- Format
  - Lateral flow assays
  - Enzyme immunosorbent assays
  - Chemiluminescent immunoassays
- Specimen type
  - Serum, Plasma,
  - Finger stick/venous whole blood (LFAs)
- Immunoglobulin class detected
  - IgM
  - IgG
  - IgA
  - Total Ab

CDC COVID-19 Guidelines (May 23, 2020):
- No advantage testing for IgG, IgM & IgG or Total
- Testing for IgA *not recommended!*

- SARS-CoV-2 antigen used
  - S1 and/or S2 of Spike protein
  - Receptor binding domain (RBD)
  - Nucleocapsid – most abundant viral protein



https://www.ncbi.nlm.nih.gov/books/NBK554776/

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12 Serologic Assays with FDA Emergency Use Authorization

Manufacturer	Specimen Type	Ab Class Detected	SARS-CoV-2 Protein Target	Method
Wadsworth Center (NY)	Serum (S)	Total	Nucleocapsid (NC)	CLIA
Bio-Rad Laboratories	S, Plasma (P)	Total	NC	ELISA
Ortho-Clinical Diagnostics	S, P	Total	S1	CLIA
Roche Diagnostics	S, P	Total	NC	CLIA
Autobio Diagnostics	S, P	IgM & IgG	Spike	LFA
Chembio Diagnostics	Finger/venous Whole Blood, S, P	IgM & IgG	NC	LFA
Cellex Inc.	S, P, venous WB	IgM & IgG	?	LFA
Abbott Laboratories	S, P	IgG	NC	CLIA
DiaSorin Inc.	S, P	IgG	S1/S2	CLIA
Ortho-Clinical Diagnostics	S	IgG	S1	CLIA
Mount Sinai Laboratory	S, P	IgG	RBD	ELISA
Euroimmun US Inc	S, P	IgG	S1	ELISA
Siemens Healthcare Diag.	S, P	Total	RBD	CLIA
Healgen	WB, S, P	IgM & IgG	S1	LFA

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

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Timing of Antibody Response to SARS-CoV-2

- New virus = no pre-existing antibodies or immunity
- We are *still* learning about our immune response to SARS-CoV-2
  - Many develop Abs ~1-2 weeks after symptoms
    - Due to delay in seroconversion, Abs do not play a routine role in diagnosis
  - >95% of patients are Ab positive after 2 weeks
    - Some patients may not seroconvert
      - Immunostatus
      - Assay dependent?
      - Severity of illness?
  - IgM declines 5-7 weeks post onset
  - IgG remains positive for ≥10 weeks post onset

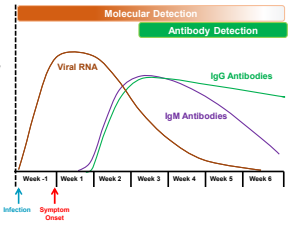

Figure modeled after Sethuraman N,et.al. JAMA. 2020; 814-63

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Verification of Emergency Use Authorized Serologic Tests for SARS-CoV-2

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Verification Requirements

CAP treats EUA assays similar to FDA cleared assays

Test Method Verification (COM.40300/COM.40325)

- Analytical Interferences
- Precision
- Reportable Range
- Accuracy

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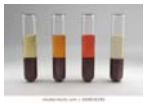
Analytical Interferences

- Effect that a compound other than the analyte has on the accuracy of measurement
- Ideally should be performed at the limit of detection (LOD)
- Typical substances include hemoglobin, bilirubin, and triglycerides
- Consider other exogenous inhibitors as well
- May determine whether or not you accept a certain sample type or add a comment to the result



Laboratory may use data from manufacturer in lieu of performing own study

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Precision

- Closeness of agreement between independent test measures
  - "Reproducibility/repeatability"
- Typical sources of imprecision include differences in timing, temperature, mixing, pipetting, etc.

Two aspects should be tested

- Intra-assay precision
  - Measurements collected under very similar conditions (i.e. same run)
- Inter-assay precision
  - Measurements collected under very different conditions (i.e. different operators, different instruments, different days, etc.)

Ideal to test concentrations at or near the level of detection

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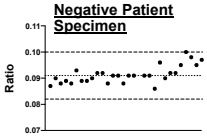
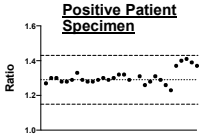
Sample Precision Data

Negative Patient Specimen



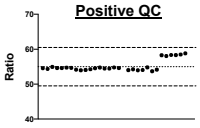
Positive Patient Specimen



Positive patient near the limit of detection

CV=SD/Mean=<20%

Positive QC



Qualitative Analysis

Positive Percent Agreement: 60/60=100%

Negative Percent Agreement: 30/30=100%

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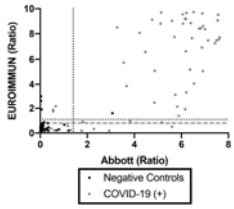
Reportable Range

- Does NOT apply to any SARS-CoV-2 assays at this time
  - All are currently designated as qualitative
- Reportable range MUST be determined if laboratories report results quantitatively
- Need to demonstrate quantitative accuracy and quantitative precision across reportable range

Comparison of Quantitative Results



Quantitative results may not correlate well between assays and no "standard" exists making this evaluation challenging

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Accuracy

- Extent to which a particular test is in agreement with a reference method or comparator
  - "Trueness"
- Ideal "comparator": Specimens from patients with known COVID-19 infection (established through molecular testing)
  - With the increasing prevalence of COVID-19 infections, most laboratories should be able to obtain these
- Secondary "comparator": Specimens with known positive and negative antibody status tested using another validated/verified antibody test

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Determination of Accuracy

- Qualitative test data typically analyzed in a 2x2 table

Specimen type	Reference Method		
		Positive	Negative
Method being evaluated	Positive	True positives	False positives
	Negative	False negatives	True negatives

Sensitivity: TP/(TP and FN) x100
Specificity: TN/(TN + FP) x100
Set goals for each prior to experiments (typically 95%)

Laboratory Determines:

-Reference method

-How many positive and negative samples to include

-Thresholds for acceptable sensitivity and specificity

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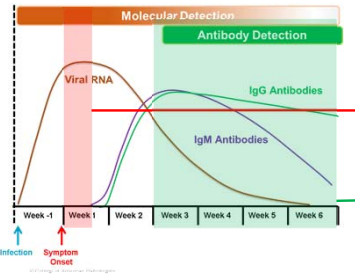
Defining Reference Method for Accuracy Studies



- If using serum from positive patients one must consider timing of serum collection

Specimen type	Reference Method		
		Positive	Negative
Method being evaluated	Positive	1	0
	Negative	9	110

Sensitivity: 10%
Specificity: 100%

Specimen type	Reference Method		
		Positive	Negative
Method being evaluated	Positive	32	0
	Negative	2	110

Sensitivity 94%
Specificity: 100%

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Example Accuracy Study

Determination of Sensitivity:

- 89 "positive" samples
  - PCR positivity used as comparator
- Serum drawn at a variety of times post symptom onset
  - Used remnant CBC samples
- Overall sensitivity: 56% (50/89 positive)
- Data analyzed at different time points

Example Sensitivity Data

	<3	3 to 7	8 to 13	>14
n=	10	22	23	34
Positive	1	7	10	32
Negative	9	14	13	2
Sensitivity	10% (0.3-44.5)	31.8% (13.8-54.9)	43.5% (23.2-65.5)	94.1% (80.3-99.3)
Specificity				

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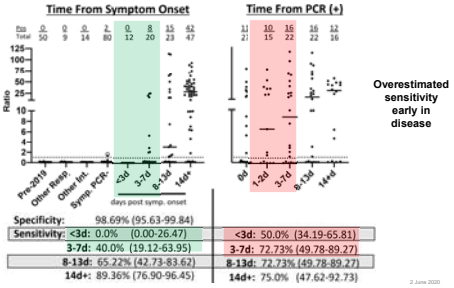
Sensitivity Varies Based on Analysis Strategy

Expected low sensitivity early in disease



Overestimated sensitivity early in disease

	Time From Symptom Onset	Time From PCR (+)
Specificity:	98.69% (95.63-99.84)	
Sensitivity: <3d:	0.0% (0.00-26.47)	50.0% (34.19-65.81)
3-7d:	40.0% (19.12-63.95)	72.73% (49.78-89.27)
8-13d:	65.22% (42.73-83.62)	72.73% (49.78-89.27)
14d+:	89.36% (76.90-96.45)	75.0% (47.62-92.73)

Tang MS, Hock KG, Logsdon NM, et al. Clinical Performance of Two SARS-CoV-2 Serologic Assays [published online ahead of print, 2020 May 13]. Clin Chem. 2020;hvaa120. doi:10.1093/clinchem/hvaa120

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Determination of Specificity

- Formal and exhaustive cross-reactivity studies are NOT needed for evaluation of an EUA assay
- Accuracy studies SHOULD take into account common cross-reacting targets
- Laboratories should try to include samples from patients with
  - Documented seasonal coronavirus positivity
  - Disease processes similar to COVID-19 (i.e. other respiratory viruses)
  - Common conditions that can lead to cross reacting antibodies (i.e. lupus or infectious mononucleosis)

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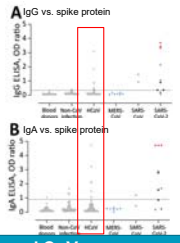
Cross Reactivity with Seasonal Coronaviruses

- SARS-CoV-2 has high amino acid homology with SARS, less so with seasonal CoVs (21-34%)
- Some studies have shown no cross-reactivity, others have shown some

Seroprevalence studies for seasonal coronaviruses suggest:

- 65%-75% of young kids have Abs to ≥1 cCov
- >90% of adults ≥50 years have Abs to all 4 cCoVs



False-positive results due to antibodies to seasonal CoVs may occur
(FDA required comment on positive reports)

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Manufacturer Specificity Studies

Assay	Seasonal Coronaviruses Included in Evaluation per "Instructions for Use"	Cross-reactivity
Abbott Alinity i SARS-CoV-2 IgG	None	Not applicable
Abbott Architect SARS-CoV-2 IgG	None	Not applicable
Autobio Anti-SARS-CoV-2 Rapid Test	18 (OC43, 229E)	None
Bio-Rad Platelia SARS-CoV-2 Total Ab	29 (229E, NL63, OC43, HKU1)	None
Cellex qSARS-CoV-2 IgG/IgM Rapid Test	"Human coronavirus panel"	None
Chembio Diagnostic Systems DPP Covid-19 IgM/IgG System	9 (229E, NL63, OC43, HKU1)	2/9, 22% (IgG cross-reactivity only)
DiaSorin LIAISON SARS-CoV-2 S1/S2 IgG	8 (OC43, HKU1, and "unknown strains")	None
EUROIMMUN SARS-COV-2 ELISA (IgG)	16 (229E, NL63, OC43, HKU1)	None
Ortho-Clinical Diagnostics VITROS Anti-SARS-CoV-2 IgG test	None	Not applicable
Roche Elecsys Anti-SARS-CoV-2	40 (229E, NL63, OC43, HKU1)	None

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https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance, Accessed 5/29/20

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How well do these serologic tests perform?

Manufacturer	Method	Ab Class	Sensitivity	Specificity	PPV (5% prevalence)	NPV (5% prevalence)
Wadsworth Center (NY)	CLIA	Total	88%	98.8%	79.4%	99.4%
Bio-Rad Laboratories	ELISA	Total	92.2%	99.6%	91.7%	99.6%
Ortho-Clinical Diagnostics	CLIA	Total	100%	100%	100%	100%
Roche Diagnostics	CLIA	Total	100%	99.8%	96.5%	100%
Autobio Diagnostics[1]	LFA	IgM/IgG	85.4%/86.2%	99.7%/99.4%	82.9%	99.4%
Chembio Diagnostics[1]	LFA	IgM/IgG	77.4%	87.1%	46.8%	99.6%
Cellex Inc.[1]	LFA	IgM/IgG	93.8%	96.0%	55.2%	99.7%
Abbott Laboratories	CLIA	IgG	100%	99.6%	92.9%	100%
DiaSorin Inc.	CLIA	IgG	97.6%	99.3%	88%	99.9%
Ortho-Clinical Diagnostics	CLIA	IgG	87.5%	100%	100%	99.3%
Mount Sinai Laboratory	ELISA	IgG	92.5%	100%	100%	99.6%
Euroimmun US Inc	ELISA	IgG	90%	100%	100%	99.5%

[1]Results are combined
Note: Data submitted by manufacturer to FDA for EUA

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

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Example Specificity Study

Determination of Specificity:

- 110 "negative" samples
  - 50 pre-COVID-19 outbreak
  - 9 with other respiratory illnesses
    - 2 FluA, 2 FluB, and 5 seasonal CoV
  - 14 with other interferents
    - 5 CMV IgG, 5 EBV IgG, 3 EBV IgM, 1 Rheumatoid Factor
- Overall specificity: 100% (110/110)

Sample Specificity Data

	Pre nCOV	Other Resp	Other Interferent	PCR(-), Symptoms
n=	50	9	14	37
Positive	0	0	0	0
Negative	50	9	14	37
Sensitivity				
Specificity				100% (96.7-100)

Sample Sensitivity Data

	<3	3 to 7	8 to 13	>14
n=	10	22	23	34
Positive	1	7	10	32
Negative	9	14	13	2
Sensitivity	10% (0.3-44.5)	31.8% (13.8-54.9)	43.5% (23.2-65.5)	94.1% (80.3-99.3)
Specificity				

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Sensitivity and Specificity Thresholds

- Determined by laboratory, dependent upon proposed use

Questions to consider:

- Are my providers going to want to test earlier than day 14 post symptom onset?
  - Consider a high sensitivity threshold early in disease course
- What patient population will be tested?
  - Symptomatic patients for diagnostic purposes?
  - Asymptomatic patients for screening/surveillance purposes?

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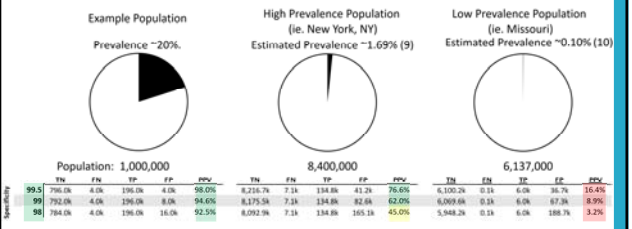
Population Screening and Specificity



Specificity	TN	FN	TP	FP	PPV
99.5	796.0k	4.0k	196.0k	4.0k	98.0%
99	792.0k	4.0k	196.0k	8.0k	94.6%
98	784.0k	4.0k	196.0k	16.0k	92.5%

TN	FN	TP	FP	PPV
8,216.7k	7.1k	134.8k	41.2k	76.6%
8,175.5k	7.1k	134.8k	82.6k	62.0%
8,092.9k	7.1k	134.8k	165.1k	45.0%

TN	FN	TP	FP	PPV
6,100.2k	0.1k	6.0k	36.7k	16.4%
6,069.6k	0.1k	6.0k	67.3k	8.9%
5,948.2k	0.1k	6.0k	188.7k	3.2%

Screening of asymptomatic populations MUST be performed using a high specificity approach

Fenwoorth CW, Anderson NW. SARS-CoV-2 Serology: Much Hype, Little Data [published online ahead of print, 2020 Apr 28]. Clin Chem. 2020;hvaa107. doi:10.1093/clinchem/hvaa107

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How Specific is My Test?

What is the specificity of this assay?

		Gold Standard	
		Positive	Negative
Assay being evaluated	Positive	20	0
	Negative	0	20

Specificity: TN/(TN + FP) x100 = 20/(20 + 0) = 100%
(95% CI 83.16-100%)

What is the specificity of this assay?

		Gold Standard	
		Positive	Negative
Assay being evaluated	Positive	200	0
	Negative	0	200

Specificity: TN/(TN + FP) x100 = 200/(200 + 0) = 100%
(95% CI 98.17-100%)

Assays to be used for population screening require more rigorous verification to prove acceptable specificity

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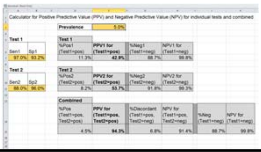
Alternative Approaches for Population Screening

- Updated CDC recommendations state population based screening should only be performed with verified HIGH SPECIFICITY assays

If laboratories cannot achieve this they can

1) Avoid testing low pretest probability populations
2) Use a combination of assays in an algorithmic fashion

PPV Calculator Available at:
https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance



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Implementing SARS-CoV-2 Serologic Testing

Test Method Verification (COM.40300/COM.40325)

- Analytical Interferences
- Precision
- Reportable Range
- Accuracy

→

Physician Communication and Result Reporting

What do providers need to know about these results???

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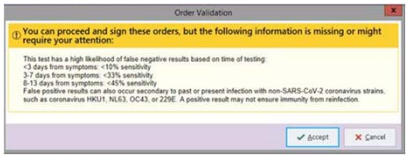
Examples of Physician Education

- Information can be communicated in laboratory newsletters or FAQ documents
  - Useful to use a form of communication that is centralized and can be updated frequently
- Information can be communicated at the point of physician ordering

Example of Clinical Decision Support Tool



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Secondary Benefit of Clinical Decision Support

- Depending on how they are built, CDS tools can be used to monitor appropriateness of use
  - Insight into effectiveness of education
  - Insight into need for more education
- At Barnes Jewish Hospital providers are asked to answer the following prior to ordering:

Ordering Patterns over Thirty Days of Testing

Time From Symptom Onset	N (%)
<3 days	18 (3%)
3-7 days	21 (4%)
8-13 days	8 (1%)
>14 days	423 (76%)
Never symptomatic	87 (16%)
Total	**557**

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Mandatory Education: Interpretation of Positive SARS-CoV-2 Serology Results

- Very important to include interpretation of positive results in any educational material
- Many misconceptions!

Immune from Reinfection???

Immunity Passport???

Less Viral Shedding???

Safe to Discontinue Infection Prevention Precautions???

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Protective Immunity Against SARS-CoV-2: What do we know?

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The Role of Neutralizing Antibodies in Protective Immunity

- Protective immunity is multifaceted!
- Antibodies can be *binding* or *neutralizing*
  - Binding (non-neutralizing) Abs
    - Produced at high levels, but unable to independently prevent infection
    - Bind and flag pathogen as 'invader'
    - Good markers of prior infection
  - Neutralizing Abs (NAbs)
    - NAbs bind virus leading to loss of infectivity and blocking viral entry into host cells
    - Function *independent* of other immune system components
- Commercially available assays do not distinguish NAbs from non-NAbs
- Testing for NAbs is challenging
  - Classically detected using plaque reduction neutralization tests (PRNTs) with live virus
    - SARS-CoV-2 requires BSL-3 for culture
  - Increasingly, BSL-2 methods are being developed using pseudotyped Vesicular Stomatitis Virus (VSV) expressing SARS-CoV-2 spike protein

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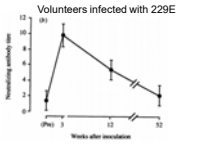
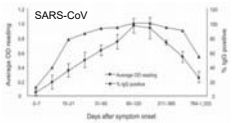
What Do We Know About NAbs and Immunity From Other CoVs?

- Common CoVs (volunteer studies):
  - IgG peaks ~2 wks post infection and decline over 1 yr
  - Re-challenge at 1 yr
    - 66% shed virus, none developed colds
  - Protective antibody levels thought to drop off at ~2 yrs
- SARS-COV:
  - Abs max out ~3-4 months post infection
  - Decline to undetectable by 6 to 7 yrs
- MERS-CoV:
  - Neutralizing antibodies remain at 3 yrs
- The unknown: what level of NAbs is protective?





Wu LP, *et. al. EID.* 2007;13(10):1562-1564
Tang F, *et. al. J Immuno.* 2011;186(12):7264-7268
Callow KA, *et. al. Epidemiol. Infect.* 1990;105:435-436

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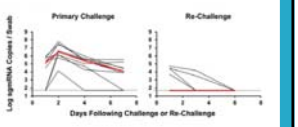
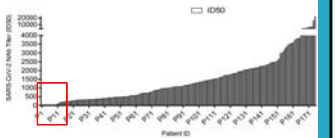
Protective immunity post-SARS-CoV-2 initial infection?

- Rhesus macaques studies
  - Initial infection led to binding and neutralizing antibodies to spike protein in all animals
  - Re-challenged on day 35 post-initial infection
    - Subgenomic mRNA levels significantly lower and no recoverable virus post day 2
    - Little to no clinical disease observed
- NAbs in 175 recovered patients
  - Titers peaked 10-15 days after symptom onset and were variable
    - 5.7% did not develop NAbs (<1:40)
    - 30% developed low NAbs (<1:500)
- The Unknowns:
  - What NAb titer is clinically significant?
  - How long do NAbs persist?





Wu F, *et. al.* medRxiv preprint https://www.medrxiv.org/content/10.1101/2020.03.30.20047365v2
Chandrashekar A, *et. al. Science.* 2020. DOI: 10.1126/science.abc4776

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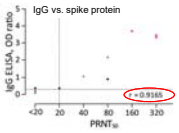
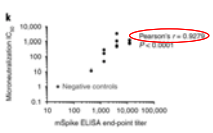
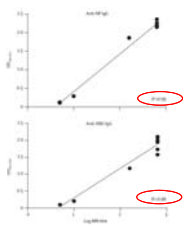
Do high throughput immunoassays correlate with NAb titers?

- Commercial immunoassays are all qualitative
- Few studies published to date
  - Most compared to BSL-2 pseudotype virus neutralization assays
  - Methods are highly variable
- Published studies do suggest correlation…
  - R^2 values >0.9







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SARS-CoV-2 Serologic Test Result Reporting and Test Utilization Recommendations

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Interpretation of Results from Antibody Tests for SARS-CoV-2

- **Negative Result:**
  - ***Likely* no prior infection or exposure to the virus**
    - **Individuals tested too soon following infection or immunosuppressed patients may be negative**
    - **Small percentage of individuals may not seroconvert**
- **Positive Result:**
  - **Suggests recent or past infection**
    - **May be impacted by the local/regional prevalence**
  - **What these results do not (yet) tell us:**
    - **When the patient was infected**
    - **Whether they are shedding virus (live or dead)**
    - **Whether patients/individuals are protected against re-infection**
  - **Cannot use positive results to guide decisions regarding adherence to social distancing recommendations or use of personal protective equipment**

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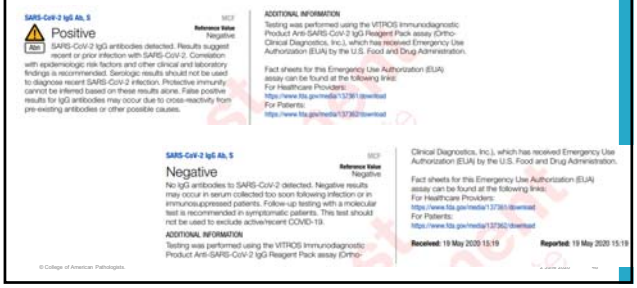
Example Reporting Comments at Mayo Clinic



SARS-CoV-2 IgG Ab, S

Positive

Reference Value: Negative

SARS-CoV-2 IgG antibodies detected. Results suggest recent or prior infection with SARS-CoV-2. Correlation with epidemiologic risk factors and other clinical and laboratory findings is recommended. Serologic results should not be used to diagnose recent SARS-CoV-2 infection. Protective immunity cannot be inferred based on these results alone. False positive results for IgG antibodies may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

ADDITIONAL INFORMATION

Testing was performed using the VITROS Immunodiagnostic Product Anti-SARS-CoV-2 IgG Reagent Pack assay (Ortho-Clinical Diagnostics, Inc.), which has received Emergency Use Authorization (EUA) by the U.S. Food and Drug Administration.

Fact sheets for this Emergency Use Authorization (EUA) assay can be found at the following links:
For Healthcare Providers:
For Patients:

SARS-CoV-2 IgG Ab, S

Negative

Reference Value: Negative

No IgG antibodies to SARS-CoV-2 detected. Negative results may occur in serum collected too soon following infection or in immunosuppressed patients. Follow-up testing with a molecular test is recommended in symptomatic patients. This test should not be used to exclude active/recent COVID-19.

ADDITIONAL INFORMATION

Testing was performed using the VITROS Immunodiagnostic Product Anti-SARS-CoV-2 IgG Reagent Pack assay (Ortho-Clinical Diagnostics, Inc.), which has received Emergency Use Authorization (EUA) by the U.S. Food and Drug Administration.

Fact sheets for this Emergency Use Authorization (EUA) assay can be found at the following links:
For Healthcare Providers:
For Patients:

Received: 19 May 2020 15:19 Reported: 19 May 2020 15:19

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How Should Patients with Positive Results be Managed?

Interim Guidelines for COVID-19 Antibody Testing (CDC, May 23rd, 2020)

- "…it cannot be assumed that individuals with a truly positive antibody test result are protected from future infection."
- Asymptomatic w/o recent history of COVID-19
  - Follow general recommendations to prevent infection with SARS-CoV-2 and otherwise continue with normal activities, including work
- Symptomatic patient with compatible or confirmed COVID-19
  - Follow previous guidance regarding resumption of normal activities, including work
- No change in clinical practice or use of personal protective equipment (PPE) by health care workers who test positive for SARS-CoV-2 antibody
- Additional Considerations:
  - Serologic tests should not be used to make decisions about:
    - Admitting persons to congregate settings (*e.g.*, schools, correctional facilities, etc.)
    - Returning persons to the workplace

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Proposed Uses For SARS-CoV-2 Serologic Testing

- Diagnosis?
  - Limited utility. Can be offered as an adjunct for those who present late or have suspected false negative upper respiratory samples *and* a lower respiratory sample cannot be collected
- Epidemiologic Studies?
  - Useful, *if:*
    - Assay has adequate specificity (>99.5%)
    - Used to screen high pretest probability populations
    - Used as part of a two assay algorithm
- Identification of Convalescent Plasma Donors?
  - Yes
  - FDA: Ideally, donors will have a NAb titer of ≥ 1:160
- Evaluation of immune response to candidate vaccines?
  - Yes

CDC. Interim Guidelines for COVID-19 Antibody Testing. https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html
IDSA. COVID-19 Antibody Testing Primer. https://www.idsociety.org/globalassets/idsa/practice-guidelines/covid-19/idsa-covid-19-antibody-testing-primer.pdf

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Implementation of SARS-CoV-2 Serologic Testing: Key Points

- Wide variety of commercial assays with EUA available for SARS-CoV-2 serology
- CAP treats EUA assays similar to FDA cleared assays, requiring full verification (COM.40300/COM.40325)
  - Analytical Interferences, Precision, Reportable Range, and Accuracy
- Verification studies should be performed to interrogate assay pitfalls and proposed use
  - Sensitivity across disease duration
  - Specificity in pre-outbreak samples and those w/ antibodies to other respiratory infections (*e.g.*, common CoVs)
  - High specificity required for population screening
- Testing should not be offered without providing education regarding pitfalls and utility
  - Should not be used as a standalone diagnostic test
  - Positivity does not necessarily equate to immunity

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Questions?

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