Serologic Testing for IgG Antibodies Against SARS-CoV-2

Elli Theel, Ph.D, D(ABMM)
Director, Infectious Diseases Serology

MAYO CLINIC LABORATORIES

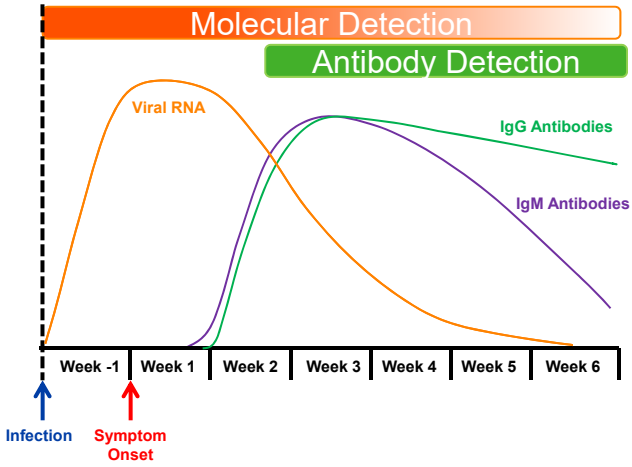
Disclosures

- None

MAYO CLINIC LABORATORIES

Timing of Antibody Development After SARS-CoV-2 Infection

- New virus = no pre-existing antibodies or immunity

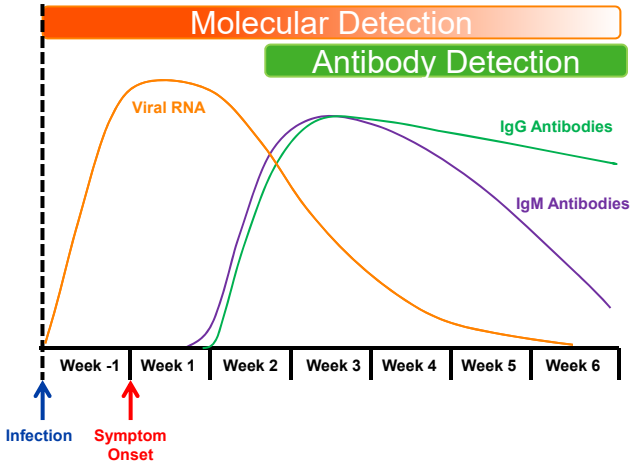


MAYO CLINIC LABORATORIES

Figure modeled after Sethuraman N, Jeremiah SS, and Ryo A. Interpreting diagnostic tests for SARS-CoV-2. *[published online ahead of print, 2020, May 6] JAMA*. 2020;10.1001/jama.2020.8259.

Timing of Antibody Development After SARS-CoV-2 Infection

- 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
  - >95% of patients are Ab positive after 2 weeks

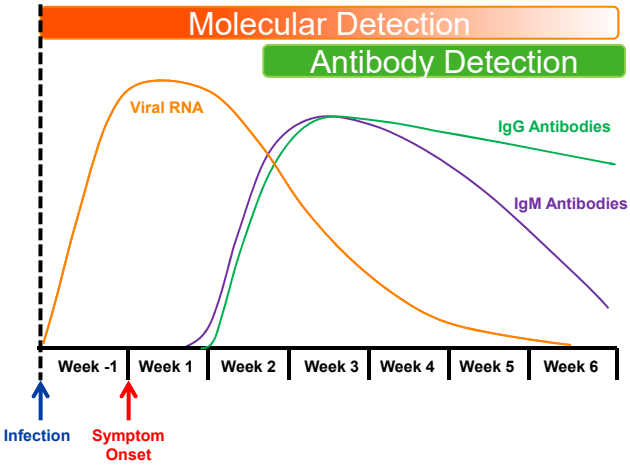


MAYO CLINIC LABORATORIES

Figure modeled after Sethuraman N, Jeremiah SS, and Ryo A. Interpreting diagnostic tests for SARS-CoV-2. *[published online ahead of print, 2020, May 6] JAMA*. 2020;10.1001/jama.2020.8259.

Timing of Antibody Development After SARS-CoV-2 Infection

- 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
  - >95% of patients are Ab positive after 2 weeks
  - IgM declines rapidly 5-7 weeks post onset
  - IgG remains positive for ≥10 weeks post onset

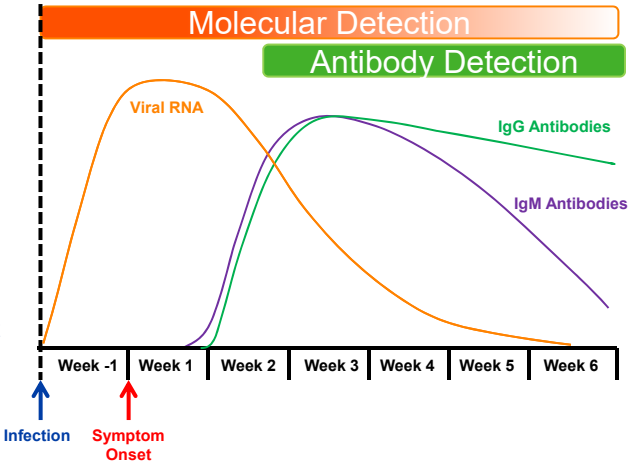


MAYO CLINIC LABORATORIES

Figure modeled after Sethuraman N, Jeremiah SS, and Ryo A. Interpreting diagnostic tests for SARS-CoV-2. *[published online ahead of print, 2020, May 6] JAMA*. 2020;10.1001/jama.2020.8259.

Timing of Antibody Development After SARS-CoV-2 Infection

- 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
  - >95% of patients are Ab positive after 2 weeks
  - IgM declines rapidly 5-7 weeks post onset
  - IgG remains positive for ≥10 weeks post onset
- What role do serologic tests for SARS-CoV-2 play?



MAYO CLINIC LABORATORIES

Figure modeled after Sethuraman N, Jeremiah SS, and Ryo A. Interpreting diagnostic tests for SARS-CoV-2. *[published online ahead of print, 2020, May 6] JAMA*. 2020;10.1001/jama.2020.8259.

©MFMER | 3793435-6

What Role Does Serology Play for SARS-CoV-2?

- Utilization consensus among multiple organizations (ASM, IDSA, etc.):
  - Epidemiology/prevalence studies and/or to facilitate contact tracing
  - Identification of potential convalescent plasma donors

MAYO CLINIC LABORATORIES

What Role Does Serology Play for SARS-CoV-2?

- Utilization consensus among multiple organizations (ASM, IDSA, etc.):
  - Epidemiology/prevalence studies and/or to facilitate contact tracing
  - Identification of potential convalescent plasma donors
  - Evaluation of immune response to candidate vaccines

MAYO CLINIC LABORATORIES

What Role Does Serology Play for SARS-CoV-2?

- Utilization consensus among multiple organizations (ASM, IDSA, etc.):
  - Epidemiology/prevalence studies and/or to facilitate contact tracing
  - Identification of potential convalescent plasma donors
  - Evaluation of immune response to candidate vaccines
  - *Potential* aid for the diagnosis of COVID-19 in PCR negative patients presenting later in their disease course

MAYO CLINIC LABORATORIES

What Role Does Serology Play for SARS-CoV-2?

- Utilization consensus among multiple organizations (ASM, IDSA, etc.):
  - Epidemiology/prevalence studies and/or to facilitate contact tracing
  - Identification of potential convalescent plasma donors
  - Evaluation of immune response to candidate vaccines
  - *Potential* aid for the diagnosis of COVID-19 in PCR negative patients presenting later in their disease course
- Recommendations against use for:
  - Diagnosis of acute/recent COVID-19
  - Determination of whether or not a patient has developed protective immunity
  - Guide Personal Protective Equipment use or adherence for social distancing practices

MAYO CLINIC LABORATORIES

An Unprecedented Influx of Serologic Tests for SARS-CoV-2

- Currently: ~190 commercially available serologic tests for SARS-CoV-2

An Unprecedented Influx of Serologic Tests for SARS-CoV-2

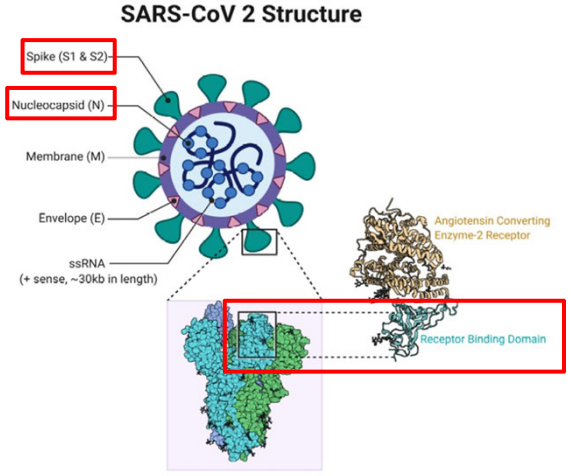
- Currently: ~190 commercially available serologic tests for SARS-CoV-2
  - 12 with emergency use authorization (EUA) granted by the FDA
  - Remaining have submitted for EUA

An Unprecedented Influx of Serologic Tests for SARS-CoV-2

- Currently: ~190 commercially available serologic tests for SARS-CoV-2
  - 12 with emergency use authorization (EUA) granted by the FDA
  - Remaining have submitted for EUA
- 29 serologic test manufacturers either did not receive or submit for EUA
  - Test should not be distributed or used

Differences in SARS-CoV-2 Serologic Test Designs

- Format
  - Lateral Flow Assays
  - Enzyme Linked Immunosorbent Assays (ELISAs)
  - Chemiluminescent Immunoassays (CLIAs)
- Type of Antibody Detected
  - IgM
  - IgG
  - IgA
  - Total Abs
- SARS-CoV-2 Protein Used in the test
  - Spike protein – Subunit 1 and/or 2 (S1/S2)
  - Receptor Binding domain (RBD)
  - Nucleocapsid



MAYO CLINIC LABORATORIES

Cascella M, Rajnik M, Cuomo A, et al. Features, Evaluation and Treatment Coronavirus (COVID-19 [Updated 2020 Apr 6]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554776/

12 Serologic Assays with FDA Emergency Use Authorization

Manufacturer	Specimen Type	Ab Class Detection	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

MAYO CLINIC LABORATORIES

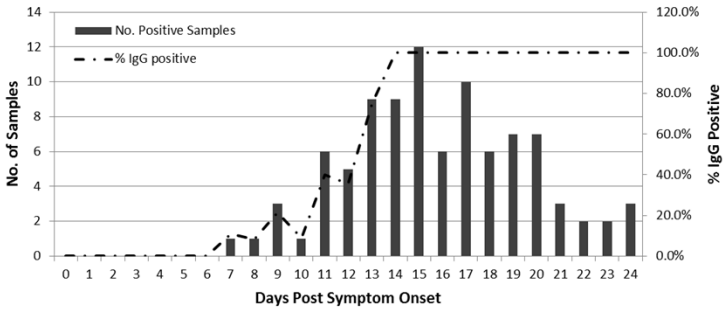
12 Serologic Assays with FDA Emergency Use Authorization

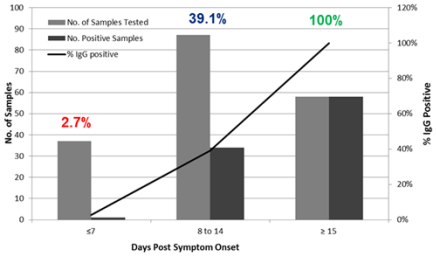
Manufacturer	Specimen Type	Ab Class Detection	SARS-CoV-2 Protein Target	Method
Wadsworth Center (NY)	Serum (S)	Total	Nucleocapsid (NC)	CLIA
Bio-Rad Laboratories	S, Plasma (P)	Total	NC	ELISA
Ortho-Clinical Diagnostics	S, P	Total	S1	CLIA
Roche Diagnostics	S, P	Total	NC	CLIA
Autobio Diagnostics	S, P	IgM & 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

MAYO CLINIC LABORATORIES

Performance Characteristic: Sensitivity

- Serial serum samples were collected from COVID-19 RT-PCR confirmed hospitalized patients and tested by the Ortho-Clinical anti-SARS-CoV-2 IgG CLIA





MAYO CLINIC LABORATORIES

Performance Characteristic: Specificity

- Serum collected from 149 healthy donors pre-outbreak and 105 sera with antibodies to other pathogens:

	% Negative		
	2018 Normal Donor Sera	Cross-Reactivity Panel	Overall
Ortho-Clinical IgG CLIA	99.3% (149/150)	100% (105/105)	99.6%

- Positive predictive value: Probability that individuals that test positive truly has antibodies to the virus
  - Impacted by test specificity *and* prevalence of the disease
  - The lower the prevalence, the higher the risk of a false positive result

MAYO CLINIC LABORATORIES

Performance Characteristic: Specificity

- Serum collected from 149 healthy donors pre-outbreak and 105 sera with antibodies to other pathogens:

	% Negative		
	2018 Normal Donor Sera	Cross-Reactivity Panel	Overall
Ortho-Clinical IgG CLIA	99.3% (149/150)	100% (105/105)	99.6%

- Positive predictive value: Probability that individuals that test positive truly has antibodies to the virus
  - Impacted by test specificity *and* prevalence of the disease
  - The lower the prevalence, the higher the risk of a false positive result

	1% Prevalence of COVID-19	5% Prevalence of COVID-19
Sensitivity	100%	100%
Specificity	99.6%	99.6%
PPV	71.6%	92.9%

MAYO CLINIC LABORATORIES

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 who are significantly immunosuppressed may be negative

MAYO CLINIC LABORATORIES

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 who are significantly immunosuppressed may be negative
- **Positive Result:**
  - Suggests infection at some point in the recent or more distant past
    - May be impacted by the local/regional prevalence

MAYO CLINIC LABORATORIES

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 who are significantly immunosuppressed may be negative
- **Positive Result:**
  - Suggests infection at some point in the recent or more distant past
    - May be impacted by the local/regional prevalence
  - What these results **do not** (yet) tell us:
    - **Whether patients/individuals are protected against re-infection**

MAYO CLINIC LABORATORIES

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 who are significantly immunosuppressed may be negative
- **Positive Result:**
  - Suggests infection at some point in the recent or more distant past
    - May be impacted by the local/regional prevalence
  - What these results **do not** (yet) tell us:
    - **Whether patients/individuals are protected against re-infection**
  - Cannot use positive antibody results to guide decisions regarding adherence to social distancing recommendations, use of masks or other personal protective equipment

MAYO CLINIC LABORATORIES

What Do We Know About Protective Immunity Against Re-Infection with SARS-CoV-2?

- Protective immunity is a multi-faceted

MAYO CLINIC LABORATORIES

Chandrashekar A, Liu J., Martinot A, *et. al. Science. 20 May 2020.* 10.1126/science.abc4776(2020)
Wu F, Wang A, Liu M, *et. al.* medRxiv preprint 20 April 2020 https://www.medrxiv.org/content/10.1101/2020.03.30.20047365v2

What Do We Know About Protective Immunity Against Re-Infection with SARS-CoV-2?

- Protective immunity is a multi-faceted
- IgG antibodies can be binding or neutralizing antibodies (NAbs)
  - NAbs independently block viral entry into host cells
  - Commercially tests do not distinguish NAbs from non-NAbs

MAYO CLINIC LABORATORIES

Chandrashekar A, Liu J., Martinot A, *et. al. Science. 20 May 2020.* 10.1126/science.abc4776(2020)
Wu F, Wang A, Liu M, *et. al.* medRxiv preprint 20 April 2020 https://www.medrxiv.org/content/10.1101/2020.03.30.20047365v2

What Do We Know About Protective Immunity Against Re-Infection with SARS-CoV-2?

- Protective immunity is a multi-faceted
- IgG antibodies can be binding or neutralizing antibodies (NAbs)
  - NAbs independently block viral entry into host cells
  - Commercially tests do not distinguish NAbs from non-NAbs
- Testing for NAbs is challenging and requires specialized facilities

MAYO CLINIC LABORATORIES

Chandrashekar A, Liu J., Martinot A, *et. al. Science. 20 May 2020.* 10.1126/science.abc4776(2020)
Wu F, Wang A, Liu M, *et. al.* medRxiv preprint 20 April 2020 https://www.medrxiv.org/content/10.1101/2020.03.30.20047365v2

What Do We Know About Protective Immunity Against Re-Infection with SARS-CoV-2?

- Protective immunity is a multi-faceted
- IgG antibodies can be binding or neutralizing antibodies (NAbs)
  - NAbs independently block viral entry into host cells
  - Commercially tests do not distinguish NAbs from non-NAbs
- Testing for NAbs is challenging and requires specialized facilities
- Initial studies indicate:
  - Most individuals develop NAbs after SARS-CoV-2 infection
  - Re-infection does not occur in rhesus macaques following primary SARS-CoV-2 infection

MAYO CLINIC LABORATORIES

Chandrashekar A, Liu J., Martinot A, *et. al. Science. 20 May 2020.* 10.1126/science.abc4776(2020)
Wu F, Wang A, Liu M, *et. al.* medRxiv preprint 20 April 2020 https://www.medrxiv.org/content/10.1101/2020.03.30.20047365v2

What Do We Know About Protective Immunity Against Re-Infection with SARS-CoV-2?

- Protective immunity is a multi-faceted
- IgG antibodies can be binding or neutralizing antibodies (NAbs)
  - NAbs independently block viral entry into host cells
  - Commercially tests do not distinguish NAbs from non-NAbs
- Testing for NAbs is challenging and requires specialized facilities
- Initial studies indicate:
  - Most individuals develop NAbs after SARS-CoV-2 infection
  - Re-infection does not occur in rhesus macaques following primary SARS-CoV-2 infection
- Unknowns:
  - What level of NAbs are protective?
  - How long do they last (6 months, 1 year, 2 years)?

MAYO CLINIC LABORATORIES

Chandrashekar A, Liu J., Martinot A, *et. al. Science. 20 May 2020.* 10.1126/science.abc4776(2020)
Wu F, Wang A, Liu M, *et. al.* medRxiv preprint 20 April 2020 https://www.medrxiv.org/content/10.1101/2020.03.30.20047365v2

Summary and Outstanding Questions

- Recently increased regulation and oversight of serologic tests by the FDA

Summary and Outstanding Questions

- Recently increased regulation and oversight of serologic tests by the FDA
- Primary role for antibody testing (currently) is for:
  - Epidemiologic studies, convalescent plasma donor screening, vaccine clinical trials

Summary and Outstanding Questions

- Recently increased regulation and oversight of serologic tests by the FDA
- Primary role for antibody testing (currently) is for:
  - Epidemiologic studies, convalescent plasma donor screening, vaccine clinical trials
- Serologic assays vary in design and performance characteristics
  - IgG antibodies persists for at least 8 weeks post symptom onset
  - Performance characteristics are generally good for EUA tests
    - Potentially limited utility due to low PPV in low prevalence areas, despite high assay specificity

MAYO CLINIC LABORATORIES

Summary and Outstanding Questions

- Recently increased regulation and oversight of serologic tests by the FDA
- Primary role for antibody testing (currently) is for:
  - Epidemiologic studies, convalescent plasma donor screening, vaccine clinical trials
- Serologic assays vary in design and performance characteristics
  - IgG antibodies persists for at least 8 weeks post symptom onset
  - Performance characteristics are generally good for EUA tests
    - Potentially limited utility due to low PPV in low prevalence areas, despite high assay specificity
- Neutralizing antibody assays detect antibodies able to inactivate/inhibit virus

MAYO CLINIC LABORATORIES

Summary and Outstanding Questions

- Recently increased regulation and oversight of serologic tests by the FDA
- Primary role for antibody testing (currently) is for:
  - Epidemiologic studies, convalescent plasma donor screening, vaccine clinical trials
- Serologic assays vary in design and performance characteristics
  - IgG antibodies persists for at least 8 weeks post symptom onset
  - Performance characteristics are generally good for EUA tests
    - Potentially limited utility due to low PPV in low prevalence areas, despite high assay specificity
- Neutralizing antibody assays detect antibodies able to inactivate/inhibit virus
- **Unknowns: Level and duration of protective immunity against reinfection**

MAYO CLINIC LABORATORIES

THANK YOU

MAYO CLINIC LABORATORIES