Clinical Laboratory COVID-19 Response Call

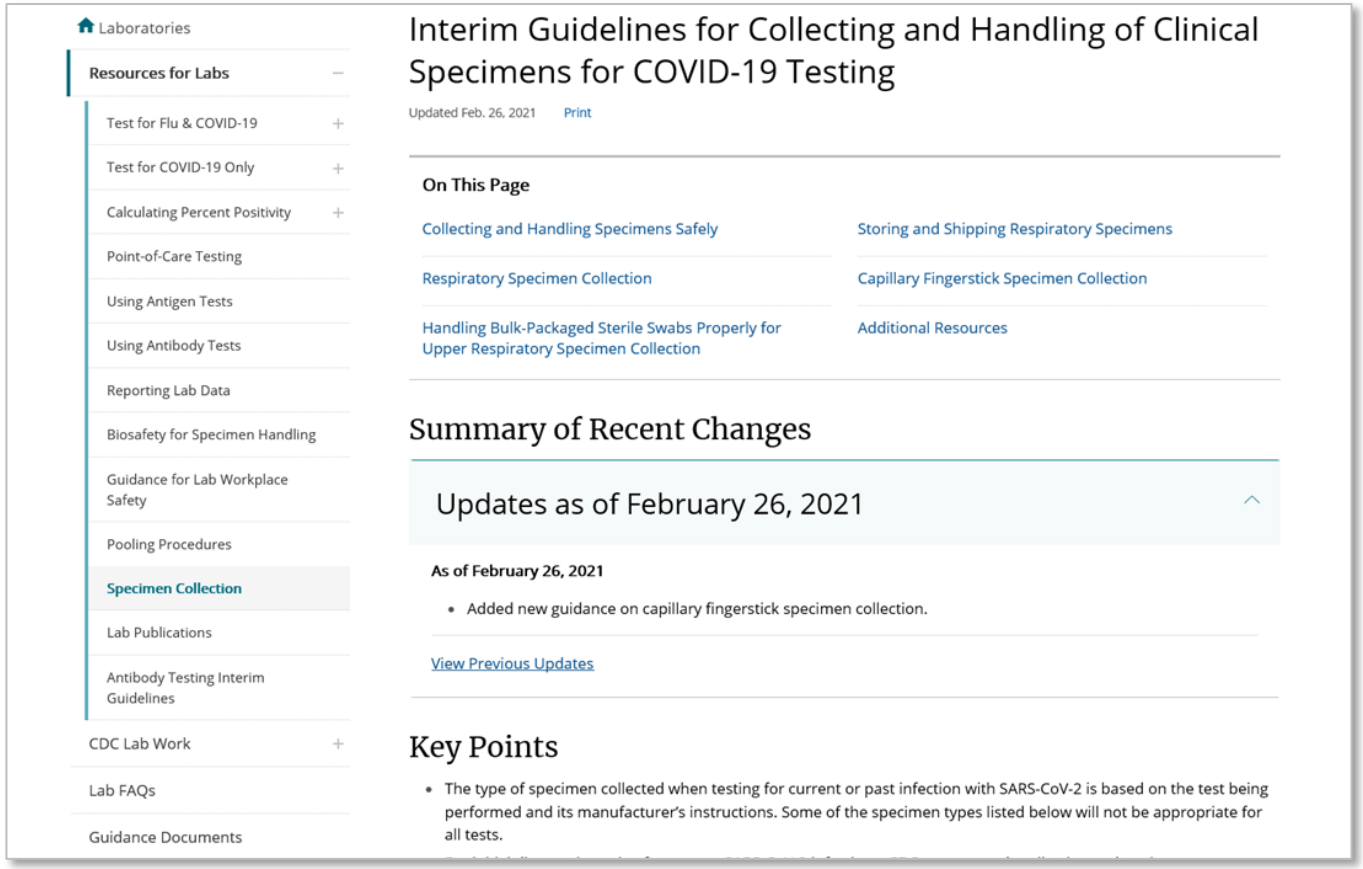
Monday, March 8, 2021 at 3:00 PM ET

- **Welcome**
  - Jasmine Chaitram, Division of Laboratory Systems, CDC
- **SARS-CoV-2 Variants Update**
  - Vivien Dugan, CDC Laboratory and Testing Task Force for the COVID-19 Response
- **Updates from the CDC Infectious Diseases Pathology Branch**
  - Julu Bhatnagar, CDC Division of High-Consequence Pathogens and Pathology
- **FDA Update**
  - Tim Stenzel, U.S. Food and Drug Administration

Slide decks may contain presentation material from panelists who are not affiliated with CDC. Presentation content from external panelists may not necessarily reflect CDC's official position on the topic(s) covered.

Specimen Collection Guidance Update

https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html



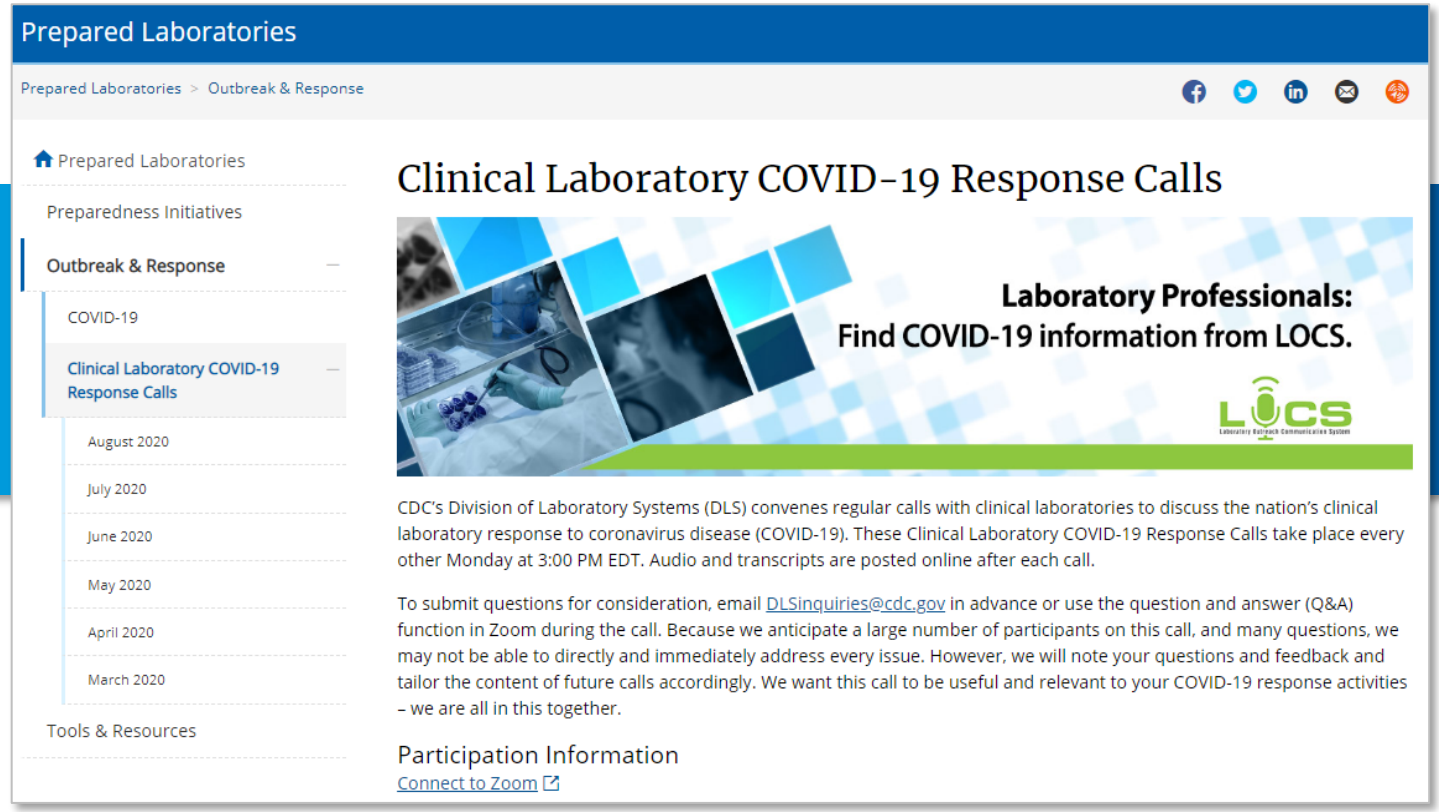
COVID-19 Resources for Laboratories

- **LOINC In-Vitro Diagnostic (LIVD) Test Code Mapping for SARS-CoV-2 Tests**
  https://www.cdc.gov/csels/dls/sars-cov-2-livd-codes.html
- **IVD Industry Connectivity Consortium**
  https://ivdconnectivity.org/livd/
- **Antigen Testing Guidance**
  https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antigen-tests-guidelines.html
- **Frequently Asked Questions about COVID-19 for Laboratories**
  https://www.cdc.gov/coronavirus/2019-ncov/lab/faqs.html
- **Interim Guidance for Collecting, Handling, and Testing Clinical Specimens**
  https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html
- **Diagnostic Tools and Virus**
  https://www.cdc.gov/coronavirus/2019-ncov/lab/tool-virus-requests.html
- **Emergency Preparedness for Laboratory Personnel**
  https://emergency.cdc.gov/labissues/index.asp
- **CDC Laboratory Outreach Communication System (LOCS)**
  https://www.cdc.gov/csels/dls/locs/

CDC Preparedness Portal

https://www.cdc.gov/csels/dls/preparedlabs/covid-19-clinical-calls.html

Find CLCR call information, transcripts, and audio recordings on the CDC Preparedness Portal



Schedule for Clinical Laboratory COVID-19 Response Calls

The next call will be on **Monday, March 22** from

3:00 PM to 4:00 PM ET

We Want to Hear From You!

Training and Workforce Development

Questions about education and training?

Contact LabTrainingNeeds@cdc.gov

How to Ask a Question

- **Using the Zoom Webinar System**
  - Click the **Q&A** button in the Zoom webinar system
  - Type your question in the **Q&A** box and submit it
  - Please do not submit a question using the chat button

- For media questions, please contact CDC Media Relations at media@cdc.gov
- If you are a patient, please direct any questions to your healthcare provider

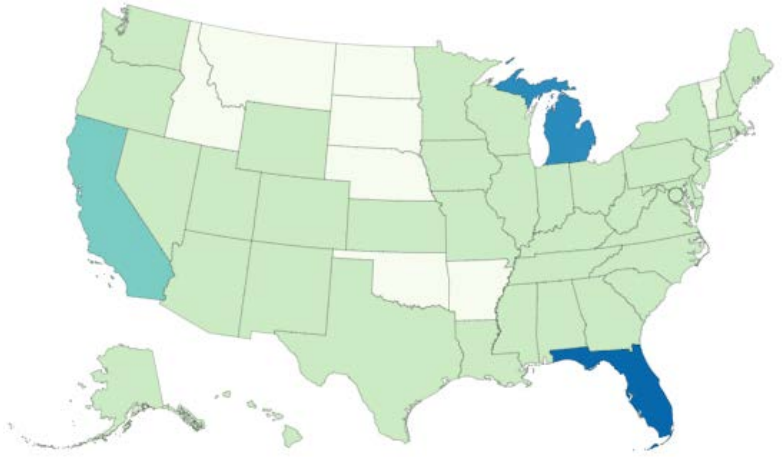
SARS-CoV-2 Variants Update

Vivien Dugan
CDC Laboratory and Testing Task Force for the COVID-19 Response

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

Emerging Variant Cases in the United States

B.1.1.7 variant



B.1.351 variant

P.1 variant

Territories AS GU MH FM MP PW PR VI

Total US Variant Cases	B.1.1.7		B.1.351		P.1	
	Total B.1.1.7	US Jurisdictions	Total B.1.351	US Jurisdictions	Total P.1	US Jurisdictions
1688	1661	44	22	10	5	4

Number of Cases

0 to 0
1 to 100
101 to 200
201 to 300
401 to 500

Numbers reflect the number of jurisdictions with > 1 case that have been reported to CDC as of February 21, 2021 and may be higher than what is shown on the US COVID-19 Cases Caused by Variants webpage. Numbers will be updated on Sunday, Tuesday and Thursday by 7pm and final case counts may be higher.
US COVID-19 Cases Caused by Variants | CDC

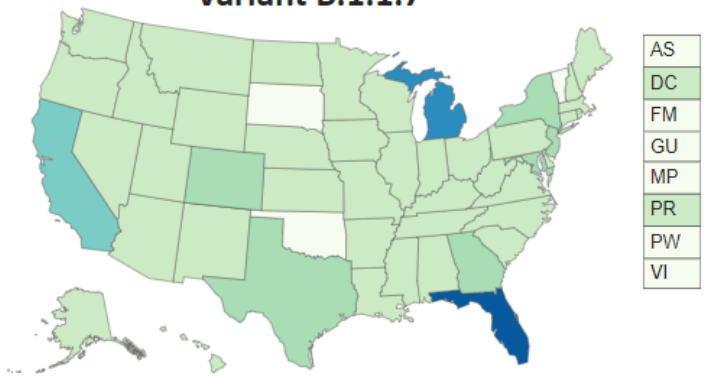
U.S. COVID-19 Cases Caused by Variants

March 7, 2021

SARS-CoV-2 Variant	Cases	# of Reporting States
Variant B.1.1.7	3037	49
Variant B.1.351	81	20
Variant P.1	15	9
Total	**3133**	

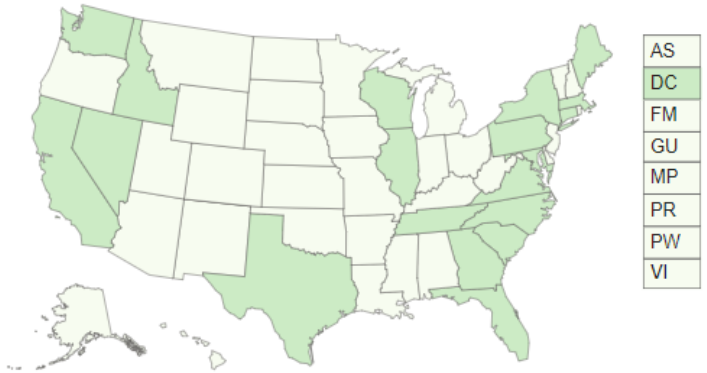
Cases Reported
- 0
- 1 to 100
- 101 to 200
- 201 to 300
- 301 to 400
- 401 to 500
- 501+

Variant B.1.1.7



Variant P.1

Variant B.1.351

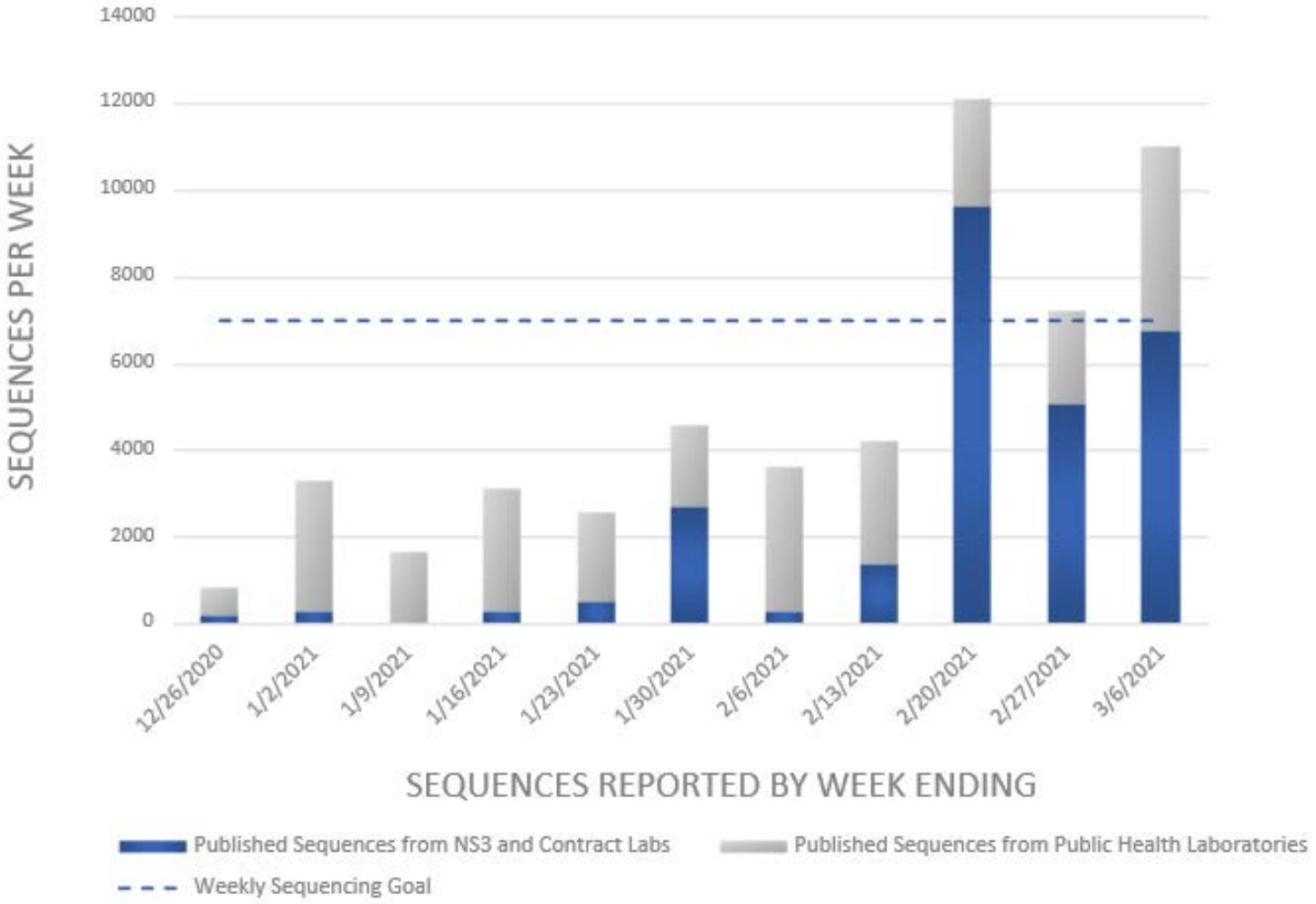


Last Updated Mar 7, 2021

Data Source: CDC National Center for Immunization and Respiratory Diseases, Division of Viral Diseases; Visualization: CDC CPR DEO Situational Awareness Public Health Science Team

Numbers reflect the number of jurisdictions with > 1 case that have been reported to CDC as of March 07, 2021 and may be higher than what is shown on the US COVID-19 Cases Caused by Variants webpage. Numbers will be updated on Sunday, Tuesday and Thursday by 7pm and final case counts may be higher.
[US COVID-19 Cases Caused by Variants | CDC]

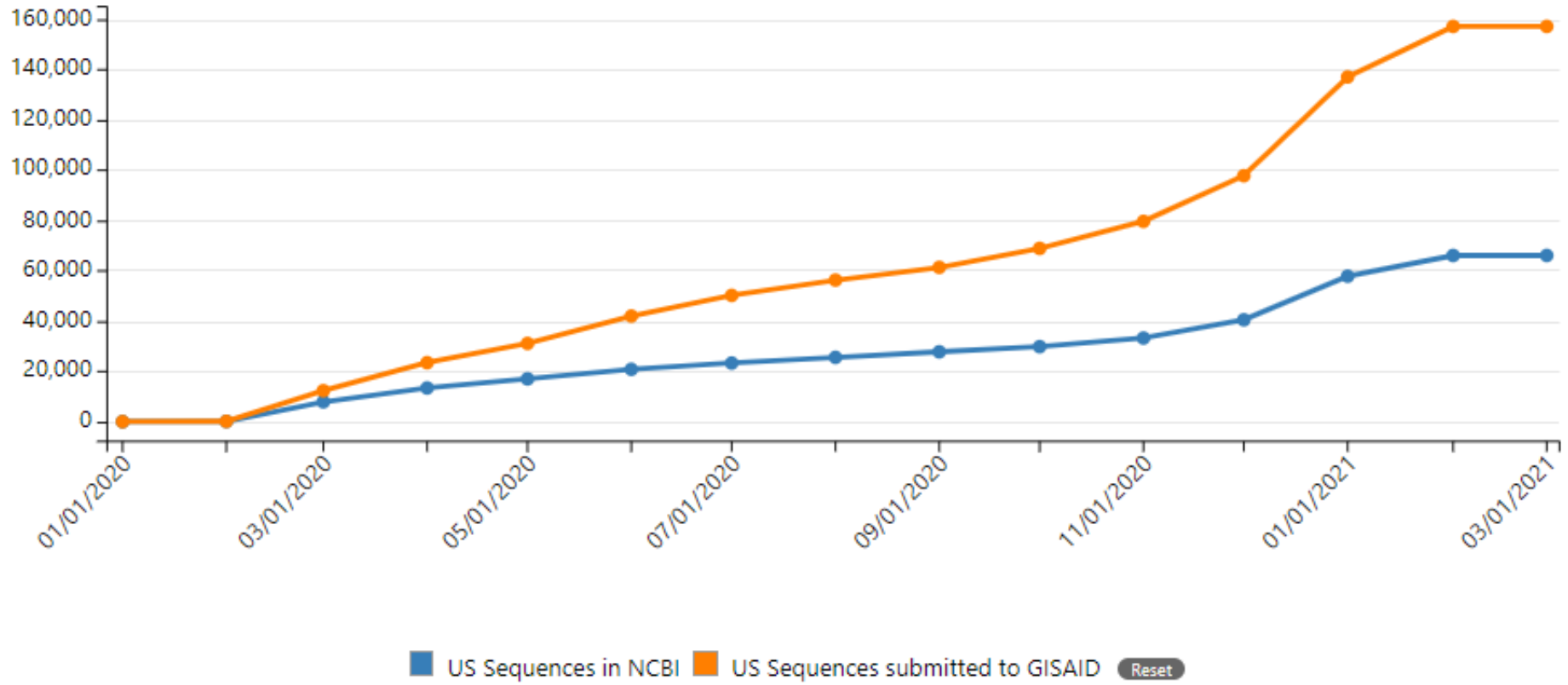
CDC Genomic Dashboard: Published Sequences



- Reported by week ending date
  - Sequences published in NCBI and GISAID, deduplicated
  - Includes data from CDC National SARS-CoV-2 surveillance, contracts and public health laboratories
- Data available to inform public health actions before being published
- Delays in processing may impact displayed results
- Weekly totals reflect date of submission and may change over time

https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/genomic-surveillance-dashboard.html

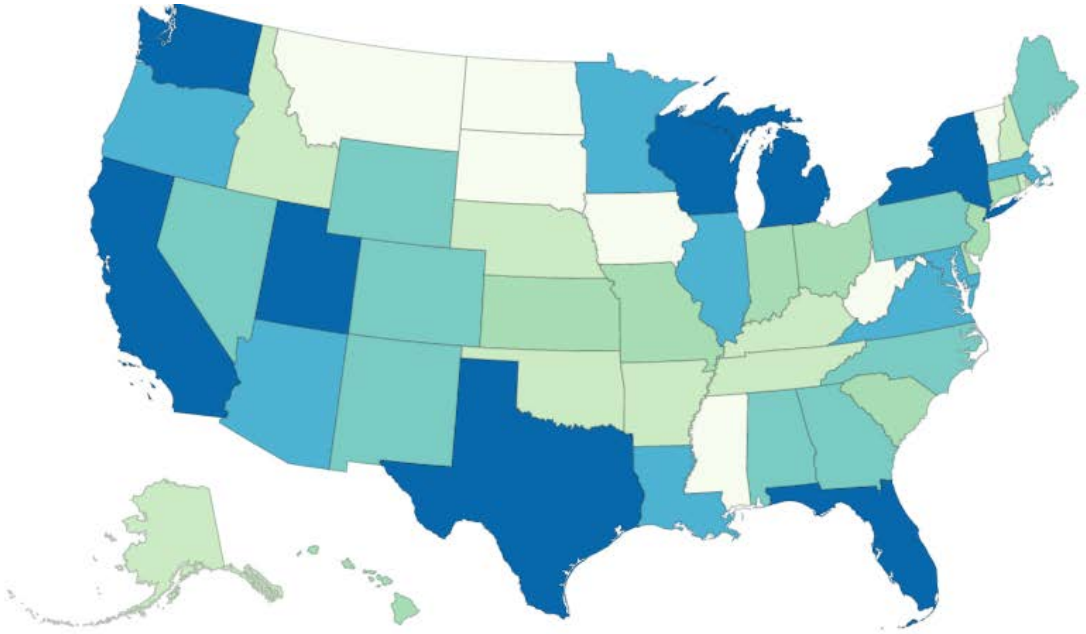
U.S. Sequences Available in Public Repositories



This line chart captures the cumulative number of published SARS-CoV-2 sequences by collection date from laboratories in states and territories across the US from January 2020 to the present. The blue line represents US sequences available in NCBI, the National Center for Biotechnology Information, and the orange represents sequences available in GISAID, a global initiative that maintains a repository of virus sequence data.

[National SARS-CoV-2 Genomic Surveillance Dashboard | CDC]

Total Sequences Submitted (GISAID)

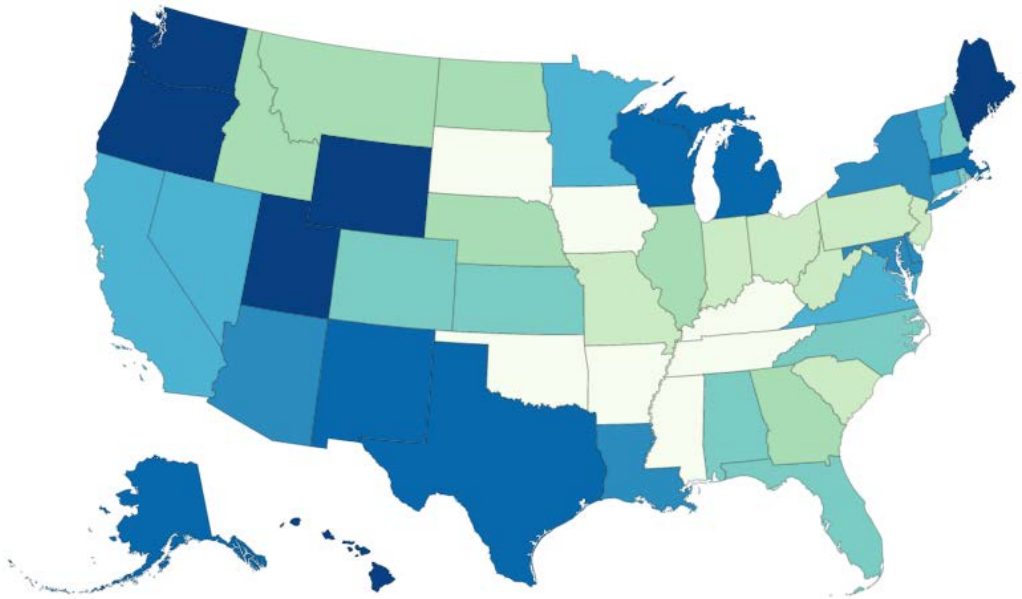


The map shows the percentage of SARS-CoV-2-positive cases by state that have been sequenced and published in public repositories from Jan 2020 to the present.

National SARS-CoV-2 Genomic Surveillance Dashboard | CDC

https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/genomic-surveillance-dashboard.html

Percentage of Cumulative Cases Sequenced (%)



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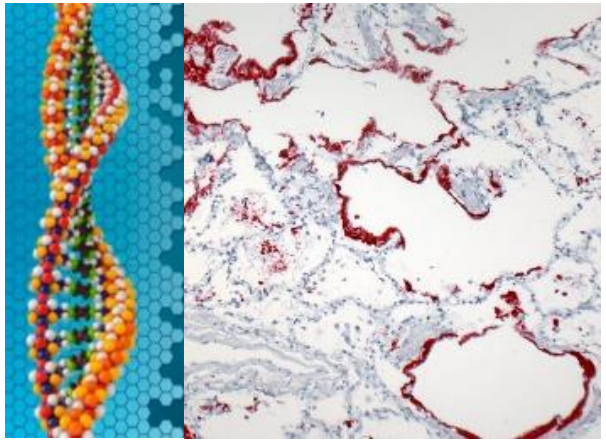
National SARS-CoV-2 Genomic Surveillance Dashboard | CDC

https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/genomic-surveillance-dashboard.html

Clinical Laboratory COVID-19 Response Call

Updates from the CDC Infectious Diseases Pathology Branch

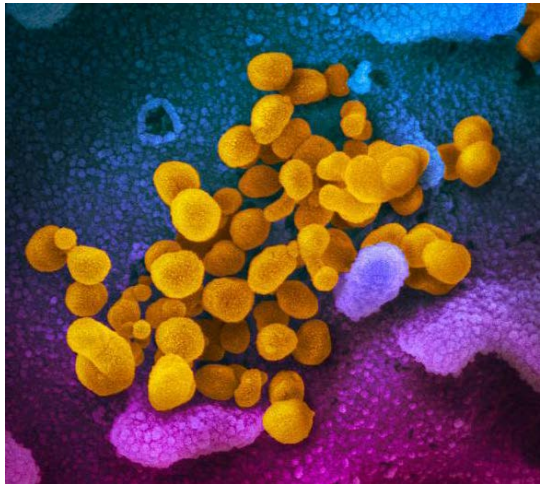
Molecular Identification of SARS-CoV-2 from Formalin-fixed, Paraffin Embedded Tissues



Julu Bhatnagar, Ph.D.
Molecular Pathology Team Lead
Infectious Diseases Pathology Branch (IDPB)
Division of High-Consequence Pathogens and Pathology
NCEZID, Centers for Disease Control and Prevention

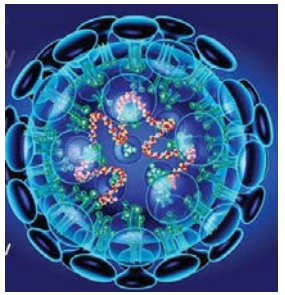
Outline

- Background
- Tissue-based molecular assays for SARS-CoV-2
- Testing of fixed autopsy tissue specimens from suspected and confirmed COVID-19 case-patients
- Summary of initial findings
- Instructions for fixed tissue submission to the CDC's Infectious Diseases Pathology Branch (IDPB)



Background

Rationale



Molecular analysis of formalin-fixed, paraffin embedded (FFPE) tissues—

- Expands diagnostic opportunities for SARS-CoV-2 in fatal cases with suspected COVID-19
  - Cases in which no prior testing of SARS-CoV-2 was performed
  - No other specimens, except autopsy tissues, were available
- Improves specificity and sensitivity of tissue-based analysis
  - Viral RNA generally persists longer in tissues (e.g., Zika, Influenza viruses, SARS-CoV)
- Provides important sequencing information for genetic characterization of strains
  - Helpful for retrospective epidemiologic and phylogenetic analysis
- Can localize SARS-CoV-2 RNA directly in the tissues and help to reveal-
  - Sites of viral tropism and replication
  - Mechanism of severe disease outcome and pathogenesis

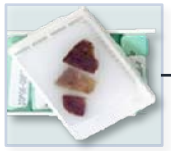
Challenges with FFPE tissues

- Fragmented nucleic acids
- Presence of PCR inhibitors
- Real-time assays generally do not work well
- Cross-linking between nucleic acids and proteins
- Presence of excessive host tissue DNA

Tissue-based molecular assays for detection of SARS-CoV-2

- **Primary molecular diagnostic assays—Conventional RT-PCR, followed by Sanger sequencing**

  As part of COVID-19 response efforts, new primers were designed, and conventional RT-PCR (cRT-PCR) assays were developed for the identification of SARS-CoV-2 from FFPE tissues*



FFPE tissue blocks

16 µm section

RNA Extraction
2-days manual extraction process

SARS-COV-2 N-gene RT-PCR
Nucleocapsid (N); Product sizes: 150 bp

SARS-CoV-2 S-gene RT-PCR
Spike (S); Product size: 162 bp

Validation controls included:
FFPE culture controls and confirmed cases of various viral and bacterial pathogens

Sanger sequencing of PCR amplicons

*Primers and the details of the procedures are recently published:

Bhatnagar J. et al., J Infect Dis. 2021, jiab039. doi: 10.1093/infdis/jiab039. PMID: 3350247

SARS-CoV-2 in-situ hybridization (ISH) assay

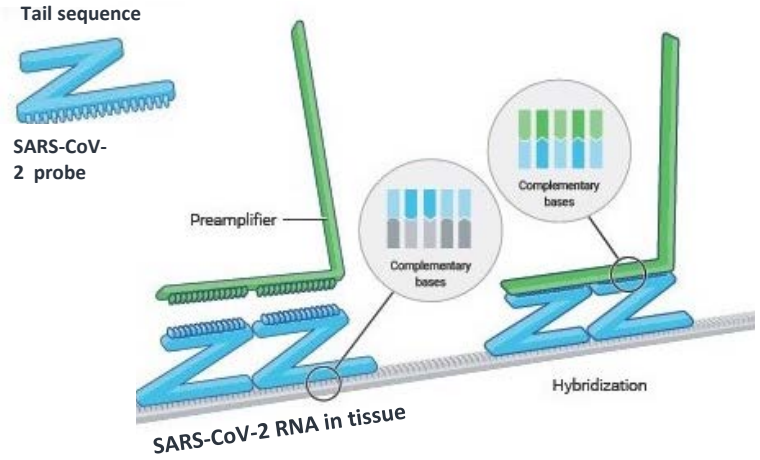
To localize SARS-CoV-2 RNA directly in the tissues

Gene targets for the ISH probes:

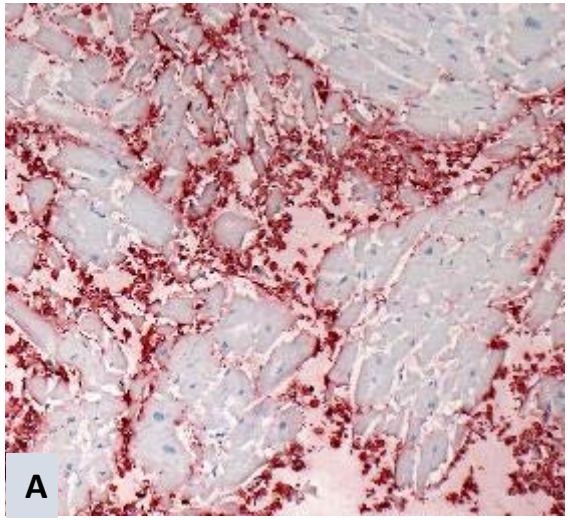
S-gene of SARS-CoV-2

N-gene of SARS-CoV-2

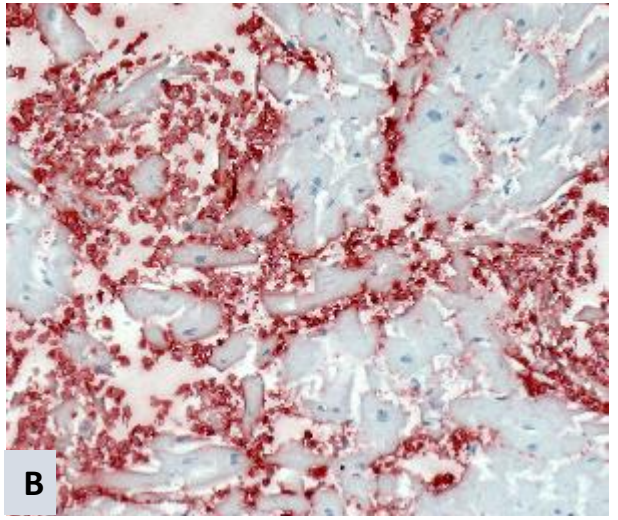
RNAscope In-Situ Hybridization



SARS-CoV-2 ISH on FFPE SARS-CoV-2 cell culture control

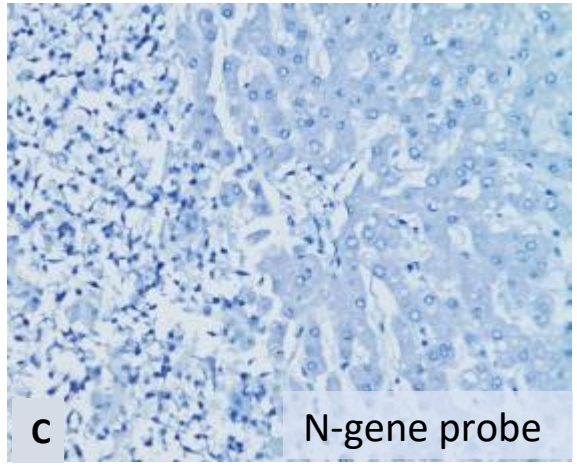


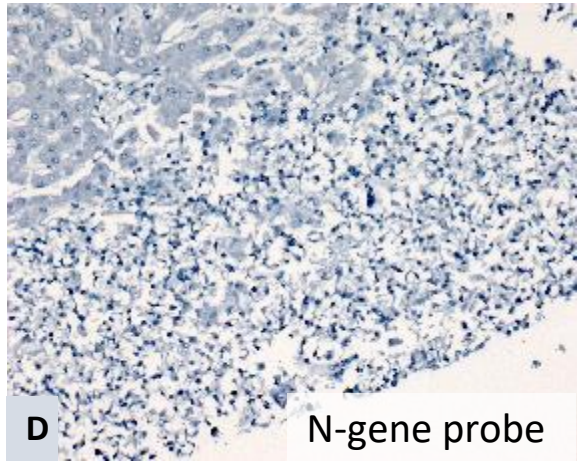
N-gene probe



S-gene probe

SARS-CoV-2 ISH on FFPE MERS-CoV and CoV-NL63 controls





Testing of autopsy tissues from confirmed and suspected COVID-19 case-patients

(Summary of work published in Bhatnagar J. et al., J Infect Dis. 2021, jiab039. doi: 10.1093/infdis/jiab039)

➢ Specimens Tested

FFPE autopsy tissues from **64 case-patients** (age range 1 month to 84 years), including-

COVID-19 confirmed case-patients: n=21

COVID-19 suspected case-patients: n=43

➢ Case definitions

Confirmed cases: Cases with prior laboratory evidence of SARS-CoV-2 by respiratory swab RT-PCR

Suspected cases: Cases with clinical or epidemiologic suspicion of COVID-19, but prior SARS-CoV-2 testing was not performed or negative

➢ Submitted to the IDPB from local and state public health departments, medical examiners, and pathologists

Between January 23 to August 4, 2020 - from 23 US states for diagnostic consultation

Tissues tested and algorithm of testing

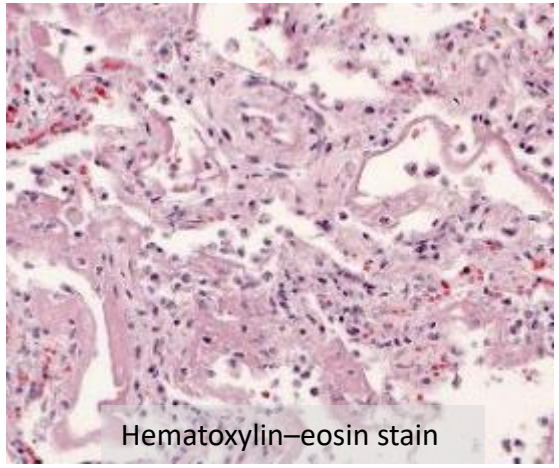
➢ Tissues Tested

- FFPE respiratory tissues (lung, trachea, or bronchi) from all case-patients
- Additional FFPE non-respiratory tissue, including-
  - Heart, brain, kidney, lymph nodes, liver, spleen, pancreas, gastrointestinal (GI), and urogenital tissues, as available

➢ Testing algorithm

- **All cases** were tested by-
  - **Hematoxylin–eosin** (H & E) to identify histopathological changes
  - **Conventional tissue-based SARS-CoV-2 RT-PCR**
- **Tissue SARS-CoV-2 cRT-PCR positive cases** tested by-
  - **ISH assays** (to localize the viral RNA in tissues)
  - **Subgenomic RT-PCR** (for detection of active viral replication)
  - **NGS analysis** (Illumina MiSeq or MinION)

  In collaboration with Respiratory Viruses Branch, NCIRD, CDC


Hematoxylin–eosin stain

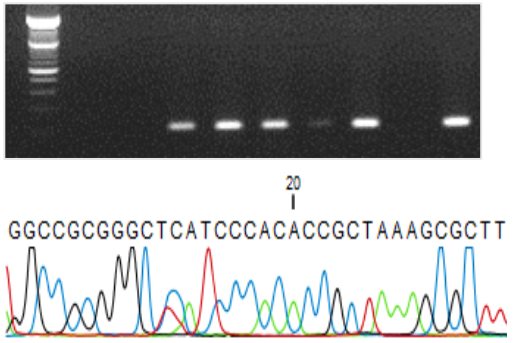
- **RT-PCR/PCR testing for other viral and bacterial pathogens** performed on respiratory tissues.

Results

- SARS-CoV-2 was identified by cRT-PCR (both N and S-gene) in respiratory tissues of 32/64 (50%) case-patients
  Sequencing of positive amplicons showed 99%–100% nucleotide identity with SARS-CoV-2

- All previously confirmed COVID-19 cases (n=21) were also positive by tissue cRT-PCR
- SARS-CoV-2 tissue cRT-PCR was positive in 11/43 (26%) suspected cases
  - provided the evidence of infection retrospectively, including in the first 2 U.S. COVID-19 deaths

Tissue-based SARS-CoV-2 Assay	Total Number of Case-patients (n=64)	
	Number Tested	Number Positive (%)
SARS-CoV-2 Conventional RT-PCR	64	32 (50%)
Subgenomic RNA RT-PCR	32	17 (53%)
SARS-CoV-2 In-Situ Hybridization	32	20 (63%)
Whole Genome Sequencing (full/partial)	27	26 (96%) D614G variant in 9/26 (35%)



Results

ISH and histopathological findings in respiratory tissues

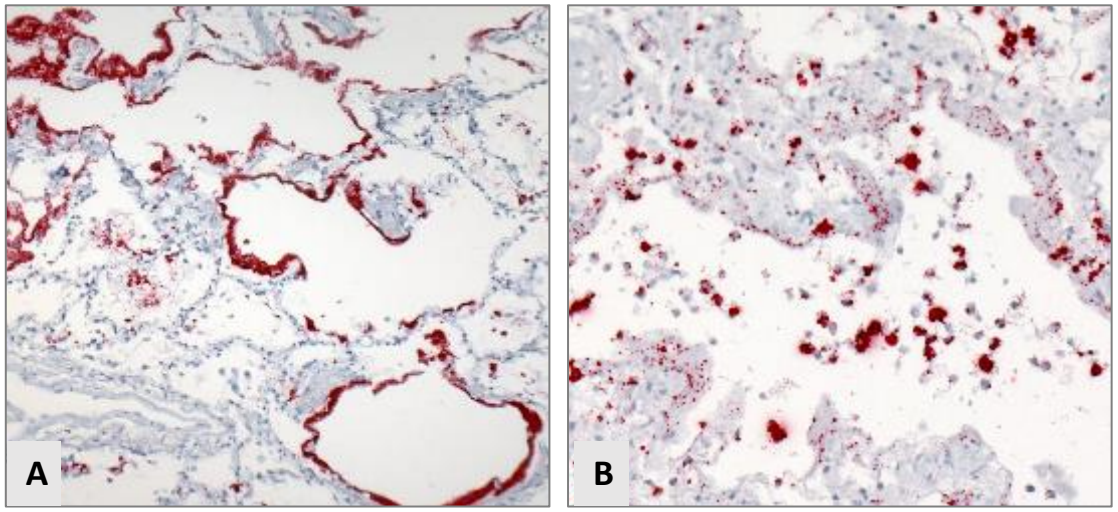
- Predominant histopathologic findings:

  - Diffuse alveolar damage (DAD) in the lung (21/32; 66%)
  - Tracheitis or tracheobronchitis in the airways (19/22; 86%)

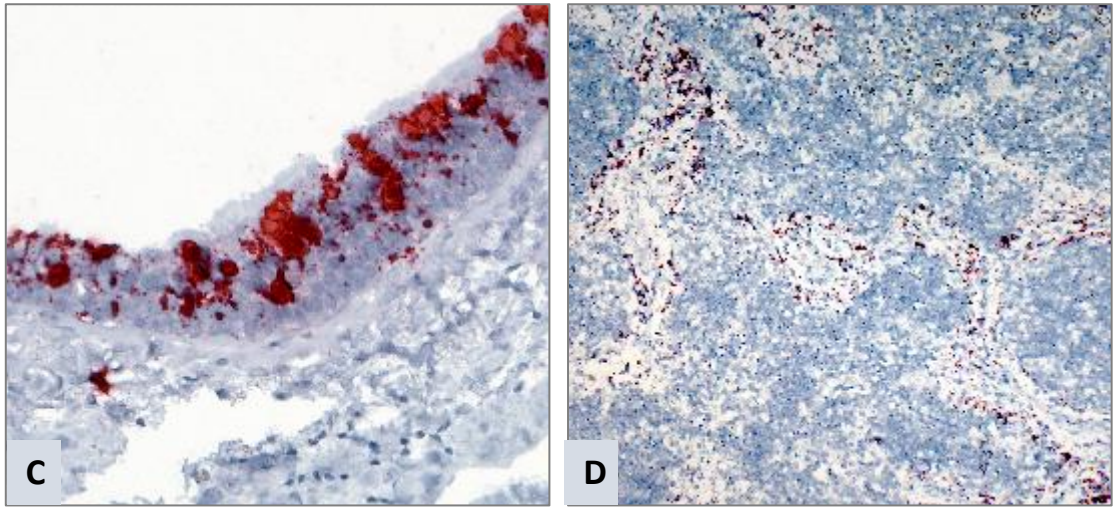
- SARS-CoV-2 RNA was localized by ISH:
  - **Lung** (hyaline membranes, pneumocytes and macrophages)
  - **Airways** (epithelial cells and goblet cells)
  - **Lymph nodes**
  - **Submucosal glands of trachea**

- Of 32 SARS-CoV-2 cRT-PCR-positive case-patients, pulmonary thrombi or emboli were detected in the lungs of 8 (25%).

SARS-CoV-2 ISH on lung tissues (A-B)



SARS-CoV-2 ISH on trachea (C) and lymph nodes (D)



Results

Additional Findings

- SARS-CoV-2 RNA was detected within the endothelial cells of blood vessels and blood vessel wall of 2 cases:

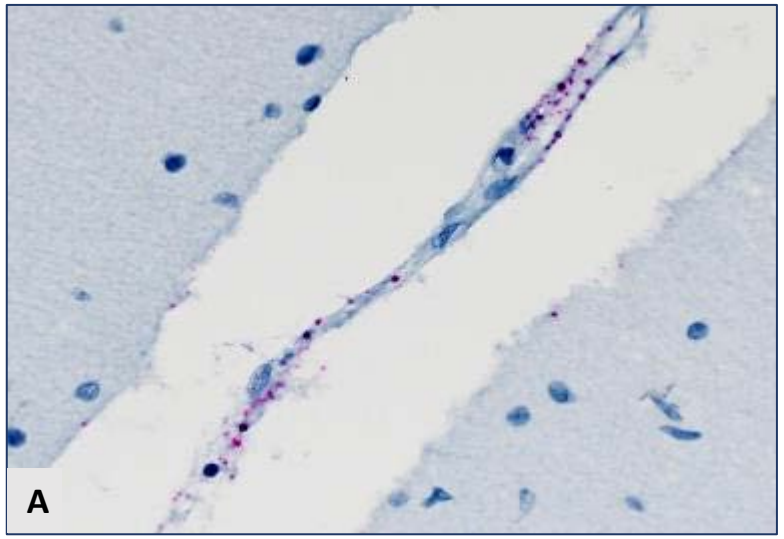
  - meninges (shown), brain stem
  - kidney
  - heart
  - pancreas
  - liver
  - lung (in both cases)

- No ISH staining was observed directly in any non-respiratory tissues

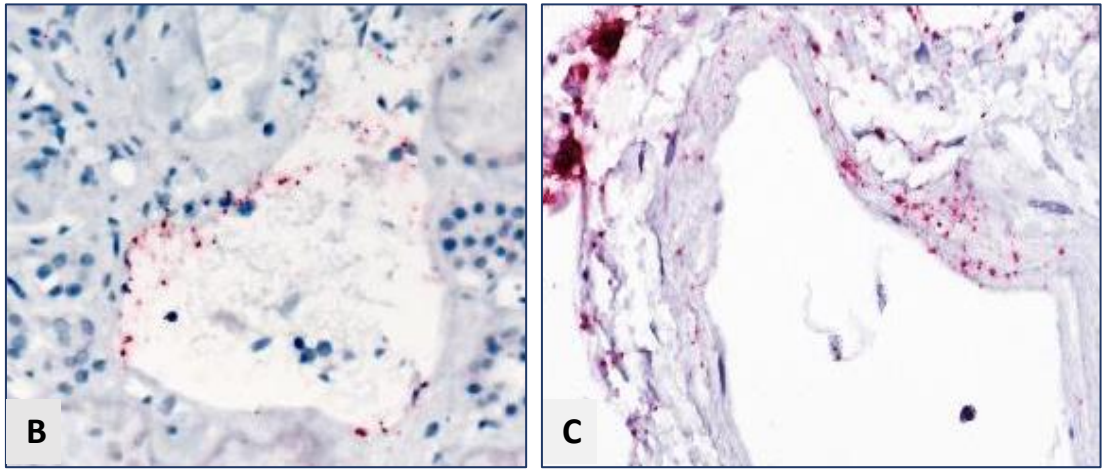
- Both cRT-PCR and rRT-PCR were positive for heart, GI, kidney, brain, liver or pancreas tissues for 14 (44%) case-patients

- SARS-CoV-2 real-time RT-PCR Ct values were higher in non-respiratory tissues, in comparison to respiratory tissues

ISH staining within the endothelial cells of blood vessels and blood vessel wall in the meninges of the cerebellum (A)



ISH staining within vessels in the kidney (B) and within vessel wall of the lung (C)



Results

- **Co-infection** of SARS-CoV-2 with other viral or bacterial pathogens were also identified in respiratory tissues of 10 of 32 (31%) SARS-CoV-2 cRT-PCR-positive case-patients

  These pathogens included:
  - Influenza B virus
  - Human parainfluenza virus (HPIV)-3
  - *Streptococcus* spp.
  - *Staphylococcus aureus*

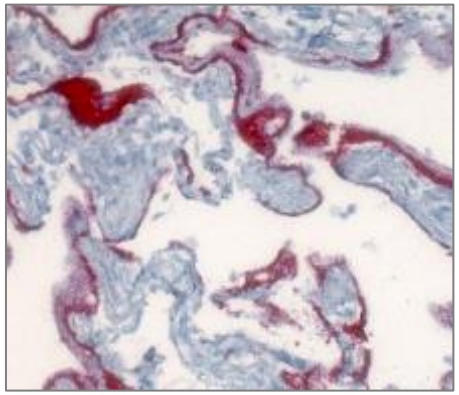
- **Other non-SARS-CoV-2 respiratory pathogens** were identified in respiratory tissues of 14 of 32 (44%) SARS-CoV-2 cRT-PCR-negative case-patients

  These pathogens included- influenza virus, HPIV, RSV, *S. aureus* and *S. pyogenes*

Summary and Conclusions

Tissue analysis:

- Is a valuable tool for retrospective diagnosis and genetic characterization of SARS-CoV-2 in fatal cases
- It also helps in detection of co-infections and infection with other etiologic pathogens
- Provides important insights into pathogenesis and mechanism of severe outcomes of COVID-19
  This work showed-
  - Direct evidence of SARS-CoV-2 RNA replication in lungs and trachea of COVID-19 patients
  - Cellular targets of SARS-CoV-2 tropism and replication were identified
  - Replicative viral RNA was detected in lungs and trachea within the areas of histopathological changes, suggesting direct virus-induced injury and inflammation
  - Cellular localization of SARS-CoV-2 RNA in endothelial cells provides strong evidence of endothelial (blood vessels and vessel wall) infection



Instructions for fixed autopsy tissue specimen submission to CDC IDPB

- Contact pathology@cdc.gov
- Healthcare providers, pathologists, medical examiners, and coroners should first contact your health department.

- If any questions regarding this presentation, please contact:

Julu Bhatnagar, PhD
Team Lead, Molecular Pathology,
Infectious Diseases Pathology Branch
Email: zrn1@cdc.gov
Phone: 404-639-2826/404-984-5507

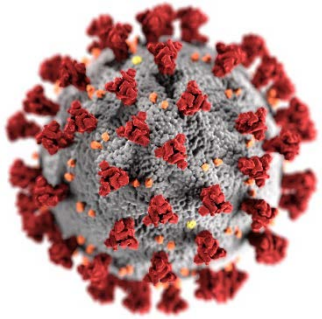
Submission of Fixed Autopsy Tissue Specimens to CDC

Fixed Autopsy Tissue Specimen Pre-Approval and Submission Instructions

For cases meeting the above criteria, follow the steps outlined below to obtain pre-approval from CDC's Infectious Diseases Pathology Branch to submit specimens for evaluation:

1. **Reminder–Healthcare providers, pathologists, medical examiners, and coroners—please first contact your state, tribal, local, or territorial health department for approval for specimen submission to CDC.**
2. **Contact CDC's Infectious Diseases Pathology Branch** at pathology@cdc.gov for pre-approval. Include the following information in the email**:**
   a. Brief clinical history
   b. Description of gross or histopathologic findings in the tissues to be submitted
   c. Listing of available formalin-fixed tissues

In your email correspondence, **do not** include patient identifiers such as name, date of birth, or medical record number. You must follow all applicable federal, state, tribal, local, and territorial regulations to adhere to patient confidentiality and privacy protections.



https://www.cdc.gov/coronavirus/2019-ncov/hcp/guidance-postmortem-specimens.html#submission-specimens

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

FDA Update

Tim Stenzel
U.S. Food and Drug Administration (FDA)

U.S. Food and Drug Administration (FDA)

- **COVID-19 Emergency Use Authorization (EUA) Information for Medical Devices**
  https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations
- **COVID-19 In Vitro Diagnostic EUAs**
  https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas
- **COVID-19 Frequently Asked Questions**
  https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/coronavirus-disease-2019-covid-19-frequently-asked-questions
- **COVID-19 Updates**
  https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization#2019-ncov
- **FDA Townhall Meetings**
  https://www.fda.gov/medical-devices/workshops-conferences-medical-devices/virtual-town-hall-series-immediately-effect-guidance-coronavirus-covid-19-diagnostic-tests-06032020
- **Independent Evaluations of COVID-19 Serological Tests**
  https://open.fda.gov/apis/device/covid19serology/

FDA U.S. FOOD & DRUG ADMINISTRATION

U.S. Food and Drug Administration (FDA)

- **COVID-19 Diagnostic Development**
  CDRH-EUA-Templates@fda.hhs.gov

- **Spot Shortages of Testing Supplies: 24-Hour Support Available**
  1. Call 1-888-INFO-FDA (1-888-463-6332)
  2. Then press star (*)

- **FDA MedWatch**
  https://www.fda.gov/safety/medwatch-fda-safety-information-and-adverse-event-reporting-program

U.S. FOOD & DRUG ADMINISTRATION

CDC Social Media

https://www.facebook.com/CDC

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Thank You For Your Time!

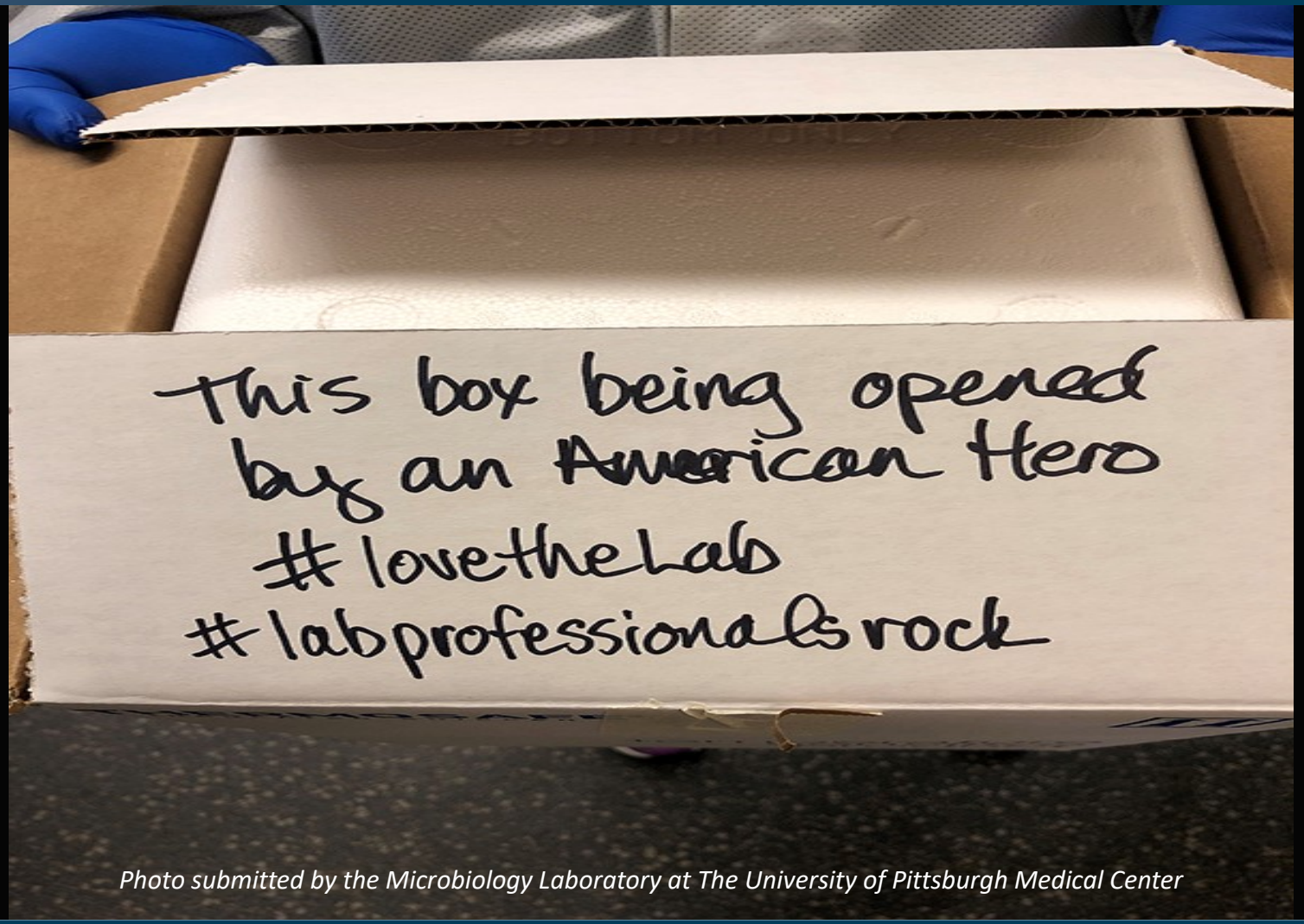

Photo submitted by the Microbiology Laboratory at The University of Pittsburgh Medical Center