• 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

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

- Frequently Asked Questions about COVID-19 for Laboratories

- Interim Guidance for Collecting, Handling, and Testing Clinical Specimens

- Diagnostic Tools and Virus

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

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](mailto: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
Emerging Variant Cases in the United States

B.1.1.7 variant

B.1.351 variant

P.1 variant

<table>
<thead>
<tr>
<th>Total US Variant Cases</th>
<th>B.1.1.7</th>
<th>B.1.351</th>
<th>P.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total B.1.1.7 US Jurisdictions</td>
<td>1661</td>
<td>22</td>
<td>5</td>
</tr>
<tr>
<td>Total B.1.351 US Jurisdictions</td>
<td>44</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Total P.1 US Jurisdictions</td>
<td>1688</td>
<td></td>
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</tr>
</tbody>
</table>

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

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

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.
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.

The map shows the cumulative number of SARS-CoV-2 sequences by state that have been published in public repositories from January 2020 to the present.
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**
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
  - **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.

<table>
<thead>
<tr>
<th>Tissue-based SARS-CoV-2 Assay</th>
<th>Total Number of Case-patients (n=64)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number Tested</td>
</tr>
<tr>
<td>SARS-CoV-2 Conventional RT-PCR</td>
<td>64</td>
</tr>
<tr>
<td>Subgenomic RNA RT-PCR</td>
<td>32</td>
</tr>
<tr>
<td>SARS-CoV-2 In-Situ Hybridization</td>
<td>32</td>
</tr>
<tr>
<td>Whole Genome Sequencing (full/partial)</td>
<td>27</td>
</tr>
</tbody>
</table>

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%).
**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
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.


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)
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

COVID-19 Frequently Asked Questions

COVID-19 Updates

FDA Townhall Meetings

Independent Evaluations of COVID-19 Serological Tests
https://open.fda.gov/apis/device/covid19serology/
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
Thank You For Your Time!

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