Laboratory Clinical Specimen Collection and Storage Guidance for Lung Injury Associated with E-Cigarettes, or Vaping

Updated 9/27/2019

As this investigation continues, CDC encourages clinicians to report possible cases of lung injury associated with e-cigarette use, or vaping to their local or state health department for further investigation.

If e-cigarette or vaping product use is suspected as a possible cause for a patient's symptoms, a detailed history of the substances used, the sources, and the devices used should be obtained, as outlined in the Health Alert Network (HAN), and efforts should be made to determine if any remaining product, devices, and liquids are available for testing.

While testing of vaping liquids and devices is ongoing, CDC has been asked to provide guidance on collection and storage of clinical specimens. This may include retention of residual clinical specimens collected for patient care or specimens collected and stored from early in a patient's presentation to look for markers of exposure.

Healthcare providers and state public health authorities should work together to make decisions about the collection and storage of clinical samples. Healthcare providers should initiate communication promptly with their local or state public health laboratory to retain samples if further analysis is anticipated. CDC will update this guidance on the basis of the ongoing investigation.

Scope

The purpose of this document is to provide **general specimen collection and storage guidance** for healthcare providers and public health laboratory personnel involved in the care of patients who meet, or are highly suspected of meeting, the case definition for lung injury associated with e-cigarette use, or vaping. These recommendations are not intended to direct laboratory guidance for patient care. Empiric collection instructions are limited to collection and storage of blood and urine. For clinicians treating patients who have undergone bronchoalveolar lavage (BAL), instructions are provided for preservation of BAL fluid. This guidance is provided in case a useful marker specific to respiratory disease caused by e-cigarette products is identified in the future.

To date, there are no identified chemical or biological markers that would warrant specific recommendations for analysis of blood or urine.

Retention and storage of residual specimens that were collected for other types of diagnostic screening and testing can also be considered. Healthcare providers should contact their hospital laboratories to identify and retain such specimens before disposal. Healthcare providers should also coordinate long-term storage of specimens with state public health authorities and state public health laboratories.

NOTE: The decision to collect samples is at the discretion of healthcare providers, and the decision to store samples is at the discretion of healthcare providers and local or state public health authorities. CDC recommends that healthcare providers consult their local or state health department for the department's recommendations on collection and storage of samples.

Specimen collection – guidance for healthcare providers and clinical laboratory personnel

A. Blood samples

1) For each patient, collect up to 12 mL of blood in two (3) 4-mL **PURPLE**-top (K_2 -EDTA) glass or plastic tubes. If only 3-mL tubes are available, four (4) 3-mL tubes may be collected. (Note: **DO NOT** use gel separators.)
2) Mix contents of tubes by inverting them 8 -10 times.
3) Label tubes in order of collection. Example: #1, #2, #3, #4.
4) Place a barcode label on each tube so that the barcode looks like a ladder when the tube is upright.
5) Store blood samples at 1 ^{o}C to 10 ^{o}C. **DO NOT FREEZE**.
6) Keep one PURPLE- top (K2 – EDTA blood tube). Store this whole blood sample at 1 ^{o}C to 10 ^{o}C. DO NOT FREEZE or ship this blood sample.
7) If the samples will take longer than 6 hours to reach the state lab, centrifuge the other purple tops for 15 minutes at 1000 to 1300 g-force to separate the plasma from whole blood cells within 6 hours of collection. Check with the centrifuge rotor manual (or RCF to RPM table) for the proper RPM (e.g. 2400 RPM) to use with your specific rotor.
8) Aliquot plasma into cryotubes.

B. Urine samples

1) For each patient, store 40 to 60 mL of urine in a screw-cap urine cup.
2) Place barcoded label on the cup when the cup is upright; the barcode will look like a ladder.
3) Indicate on the cup how the sample was collected if the method was other than “clean catch” (example: catheterization).
4) **Store urine samples in the freezer**. Freezer temperatures of -20 ^{o}C or lower are recommended.

Following the above collection protocol, contact your state public health laboratory for specimen transport and long-term storage.

C. Bronchoalveolar lavage fluid (BAL fluid) samples

1) Due to the increased technical skill and equipment needed and invasive nature of sampling, the decision and timing for a patient to undergo BAL should be left to the treating clinicians.
2) **Optimal timing**. These specimens may be obtained at any time during the clinical course, but ideally prior to initiation of antimicrobial or steroid therapy. If antibiotics or steroids have been initiated, course and duration should be noted.
3) **Specimen collection**. Collect specimens in sterile containers. BAL fluid should undergo culture and routine centrifugation followed by cellular analyses and cytopathology, including lipid and other staining, as clinically indicated at the local institution.

Lipoid pneumonia associated with inhalation of lipids in aerosols generated by e-cigarettes has been reported based on the detection of lipid-laden alveolar macrophages obtained by bronchoalveolar lavage (BAL) and lipid staining (e.g., Oil Red O).

The following guidance is primarily for retaining BAL fluid samples left over from routine clinical evaluation:

Remaining uncentrifuged BAL fluid and supernatant from centrifuged BAL fluid should be labeled as such and be retained. Up to 10 unstained cytology slides should be briefly fixed in formalin and retained for future evaluation.

Excess cell pellet after cytopathologic evaluation can be divided in half, with half being fixed in formalin and stored at room temperature for further cytopathologic evaluation and half frozen at -20 ^{o}C or lower for future chemical or lipid analysis.

Place remaining uncentrifuged fluid and centrifuged supernatant from centrifuged fluid into sterile vials with external caps and internal O-ring seals. If there is no internal O-ring seal, then seal tightly with the available cap and secure with Parafilm®. Label each specimen container with the patient's name, ID number, the specimen type, subsection of lung lavaged, and the date the specimen was collected.

Long-term storage – guidance for public health laboratory personnel

1) Establish communication protocols with local hospitals and other medical providers regarding appropriate specimen collection guidance (see above).
2) Plan to transport collected specimens on at least a twice-weekly (or more frequently if needed) basis.
3) Follow your local in-house chain of custody protocols (i.e., shipping manifests).
4) Collect specimens from local health providers.
5) Separate plasma from whole blood cells within 6 hours of collection. Centrifuge the purple tops for 15 minutes at 1000 to 1300 g-force to separate the plasma from whole blood cells. Check with the centrifuge rotor manual (or RCF to RPM table) for the proper RPM (e.g. 2400 RPM) to use with your specific rotor.
6) Aliquot plasma into cryotubes. **FREEZE** these specimens (urine, plasma, BAL fluid) at freezer temperatures of -20 ^{o}C or lower.
7) Any formalin-fixed specimens (BAL pellet, cytology slides) should be stored at **room temperature NOT FROZEN.**
8) Contact your state epidemiologist or outbreak principal investigator regarding next steps.

Note: Consult your local or state health department about the outbreak of lung disease associated with e-cigarette use (vaping) as soon as possible. State health department officials seeking technical assistance with case reporting or epidemiologic issues can contact CDC at VapingAssocIllness@cdc.gov. State health department officials seeking technical assistance with laboratory testing can discuss with their state health department laboratories or contact CDC at VapingAssocIllness@cdc.gov.

Shipping instructions for chemical analyses by CDC's Division of Laboratory Sciences

Shipments are accepted from state public health laboratories or local and state health departments. Healthcare providers who would like to submit specimens should coordinate with health officials.

Before shipping specimens, please email NCEHSampleLogistics@cdc.gov and wvg4@cdc.gov with the shipping tracking information and a spreadsheet with the following fields:

> CDC Case ID; State Case ID; Specimen ID; Matrix; Box #; Position in Box; Volume (mL); Collection Date; and Any Pertinent Comments.

Please list “E-cigarette Lung Injury Response” in all correspondence with the sample logistics laboratory.

Do not include patient identifiers such as name, date of birth, or medical record number. All applicable federal, state, and local regulations must be followed to adhere to patient confidentiality and privacy protections.

After you receive approval by email to submit the specimens to CDC’s Division of Laboratory Sciences, package frozen specimens with adequate dry ice to remain frozen until receipt and ship to:

CDC Warehouse
3719 N Peachtree Road
Chamblee, GA 30341
ATTN: Sample Logistics--Sina De Leon
Chamblee Building 109, Room 1312B

Tel: 770-488-4227, Fax: 770-488-4301, Email: NCEHSampleLogistics@cdc.gov