



RICIN TOXIN

I. Protocol Overview

Ricin is a potent biological toxin that is derived from castor beans. Its mechanism of action in the body is inhibition of protein synthesis. Clinical manifestations are dependent on the route of exposure. Ingestion of ricin typically leads to profuse vomiting and diarrhea followed by multi-system organ failure and possibly death within 36 to 72 hours of exposure. Inhalation of ricin typically leads to respiratory distress, fever, and cough followed by the development of pulmonary edema, hypotension, respiratory failure, and possibly death within 36 to 72 hours. Weakness and influenza-like symptoms, fever, myalgia, and arthralgia, might also be reported. For all suspected chemical exposures, consult the Poison Control Center (800-222-1222) located at Children's Hospital of Philadelphia. Information and treatment advice is available to the public and healthcare professionals at no charge.

Mass spectrometry methods are used to detect the urinary biomarker L-ricinine which shares a common plant source to the toxin. Samples are extracted and L-ricinine is quantified via HPLC separation followed by isotopic dilution mass spectrometry analysis. Ricin toxin is detected qualitatively by Time Resolved Fluorescence ImmunoAssay (TRFIA) and polymerase chain reaction (PCR) in environmental and food samples (e.g., filters, swabs, or wipes) or by Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS).

II. Contact Information

- a. Delaware Public Health Laboratory (DPHL) Director: 302-223-1520. If the DPHL Director is not available, please request the DPHL Deputy Director, or Chemical Terrorism Coordinator.
- b. During non-business hours, DPHL utilizes an answering service at the same number. Indicate the nature of the call to reduce delay in notification of DPHL.

III. Authorization for Sample/Specimen Submittal: (coordinate with Epi/EHEB)

- a. Contact the DPHL Lab Director, DPHL Deputy Director, or DPHL Chemical Terrorism Coordinator for Authorization to submit each laboratory sample/specimen. Samples/specimens received without Authorization will be rejected.
- b. The event nature may require samples be shipped directly to another laboratory.

IV. Samples/specimens

Please reference the CT Sample Collection Chart for more detailed information.

- a. Clinical testing for MS/MS: urine.
 - DPHL is a Qualified Ricinine Biomarker in Urine by LC/MS/MS Laboratory.
 - Collect at least 50 mL in a screw-capped plastic container, preferably clean catch. Do not overfill. Note on documentation any collection method other than clean catch.
 - Freeze as soon as possible (-70°C or dry ice preferred).
 - Ship clinical samples frozen on dry ice in a cooler, store at -70°C.
 - For each lot number of containers used, provide two empty unopened containers.

Refer to DPHL's "CDC Sample Collection Protocol for a Chemical-Exposure Event" for more detailed directions on clinical collection, handling, packaging and shipping.

- b. Environmental or food testing for TRF: variable (consult referral laboratory for details).
 - DPHL does perform Ricin toxin testing using TRF. Samples may be referred to a FERN-C (food) or ERLN (environmental) reference laboratory for further testing.
 - Collect minimum 2 teaspoons of liquid into a 50 mL plastic centrifuge tube.
 - Collect a minimum 2 teaspoons of solid into a 50 mL plastic centrifuge tube.
 - For each lot number of containers used, provide two empty unopened containers.

Refer to DPHL's "Environmental Sample Collection Protocol for a Potential Exposure Events" for more detailed directions on collection, handling, packaging and shipping.

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- c. Label samples/specimens with labels generated by your facility or organization, including a sample identification number, collector's initials, and date and time of collection.
- d. Handle samples in accordance with biosafety practices. Gloves should be worn while collecting samples/specimens. Use other personal protective equipment as necessary.

V. Packaging Instructions

- a. Clinical Specimens: Package as Biological Substance, Category B (Packing Instruction 650) utilizing three layer packaging. Freeze urine specimens on collection, ship on dry ice.
- b. Environmental Samples: Place containers in a secondary unbreakable secondary container (DO NOT USE GLASS!). Place secondary containers into leak-proof, sealed transport device. Decontaminate the outer container exterior with 10% bleach.
- c. All: Seal two layers of packaging with tamper-proof, waterproof forensic evidence tape with collector's initials, date, and time of collection. Do not to cover the sample labels.

VI. Documentation

All forms are located at <http://www.dhss.delaware.gov/dhss/dph/lab/labs.html>

- a. Clinical: Complete DPH Chain of Custody, DPH Request for Preparedness Testing, LIMS Test Requisition (each patient)
- b. Environmental and Food: Complete DPH Chain of Custody, DPH Request for Preparedness Testing, DPH Field Screening Form (each sample)

VII. Receiving Samples/specimens at DPHL

- a. Clinical specimens may be transported utilizing DPHL couriers or by submitters. Authorized samples/specimens are received at the rear dock entrance of DPHL.
- b. Environmental and Food samples are transported by submitters to the apron of the red building at the rear of DPHL. DPHL performs an interview prior to sample acceptance.
- c. All submitters must present ID and have proper forms completed.

VIII. Testing Methods

- a. **LC/MS/MS**: Testing involves analyte isolation from the urine matrix and pre-concentration using solid phase extraction and monitoring of specific analyte ions abundance and fragmentation products produced. The comparison of relative response factors of native analyte area divided by labeled internal standard area of known standard concentrations yields individual analyte concentrations. Positive or negative results may take up to 8 hours after the sample is received.
- b. **TRF/TRFIA**: Testing involves sample preparation and addition of a Europium fluorescent tag to the sample with high specificity and affinity for Ricin followed by detection using a plate reader with multi-label counter. If ricin toxin is present, an antigen antibody complex will form. The reader detects absorbance and the results are analyzed. Positive or negative TRF test result may take up to 24 hours after the sample is received.

IX. Reporting Results

- a. Preliminary: A preliminary positive or negative test using PCR may be issued upon completion of PCR testing for environmental samples and may take up to 4 – 8 hours.
- b. Confirmation: A positive or negative test result may be issued upon completion of LC/MS/MS analysis for clinical specimens and may take up to 12 hours. A positive or negative test result may be issued upon completion of TRF analysis for environmental or food samples referred to other laboratories. TRF may take up to 24 hours.

Notification procedure: Laboratory Director, Deputy Laboratory Director or Chemical Terrorism Coordinator will notify the submitter of test results.

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