

Testing Raw Milk Samples That May Contain Highly Pathogenic Avian Influenza



Introduction

This guide, developed by the Association of Public Health Laboratories (APHL), provides a framework for laboratories to assess their **risk of exposure** to Highly Pathogenic Avian Influenza (HPAI) A (H5N1) when testing raw milk samples. If HPAI creates higher risk in any tasks, safety practices may need to be enhanced—even if only temporarily.

Raw milk is known to contain infectious pathogens such as *Salmonella*, *E. coli*, *Listeria* and *Campylobacter*, so it should always be handled with safety in mind. In light of the 2024 cattle-based outbreak of HPAI, laboratories receiving raw or pre-processed milk, or other cattle-derived samples for testing should re-examine related safety considerations. Use this guide to help evaluate if current procedures could expose personnel to HPAI—such as handling sample containers, approach to the selection of **personal protective equipment (PPE)** and the formation of **aerosols**—and how to adjust accordingly.

Each laboratory must assess its own level of risk regarding raw milk testing to determine if enhanced safety precautions are warranted, and should treat this guide as a supplement to its existing approach to **risk assessment**. Of note, analysts who perform dairy regulatory testing have already been certified by the US Food and Drug Administration (FDA) Laboratory Proficiency Evaluation Team. Further, this guidance is not intended to supersede the NCIMS 2400 Form requirements.

Using this Guide

- Words from the glossary are **bold and red** in the text. Click on them to jump to their definitions.
- Hyperlinks to external sources are teal and underlined.
- Visit the Additional Resources section to find links to HPAI- and biosafety-related reference materials and websites from APHL and other partners.

Biological Safety Cabinets Alternatives

Ideally, raw milk samples should be handled within a certified Class II **biological safety cabinet (BSC)**, following the US Centers for Disease Control's (CDC) [Fundamentals of Working Safely in a BSC](#).

However, **access to a BSC may not be feasible for every laboratory**. Applying CDC's [guidance](#) of using a combination of PPE and **containment** devices (e.g., bench top safety shield, centrifuge safety cups or sealed rotor) can help keep analysts safe by creating additional **barriers** between the **hazard** and personnel.



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Processing Raw Milk Samples

#	Procedure(s)	Potential Hazard(s)	Mitigation Control(s)/Protection(s)	Additional Information
A	- Receiving raw milk samples. - Performing temperature checks.	- Leaking raw milk samples; raw milk aliquots mislabeled as pasteurized. - Droplets and aerosols from mixing samples; taking temperature of the temperature control.	- During sample receipt, open coolers and look for damaged contents before removing any samples. - Transfer and open inside a Class II BSC, when possible, using CDC's Fundamentals of Working Safely in a BSC. If a BSC is not available, apply CDC's guidance of using a combination of PPE and containment devices (e.g., bench top safety shield, centrifuge safety cups or sealed rotor). - When performing temperature checks, attempt to minimize aerosol formation. - Disinfect the temperature probe with a US Environmental Protection Agency (EPA)-approved disinfectant after use. See: Additional Information > PPE for specific PPE requirements	- Review spill decontamination procedures to assess HPAI risk and update as necessary. For large raw milk spills (volume greater than 100 mL) outside a BSC, alert employees and evacuate the area. - Sample receiving staff should be trained or refreshed on decontamination procedures and spill response for biological hazards, such as HPAI. - Disinfect all surfaces with EPA-approved disinfectant prior to moving samples to the testing area. - Replace soiled PPE with clean PPE. Carefully remove contaminated PPE and wash your hands. Contaminated, single-use PPE is discarded and placed in the biohazard waste, - Autoclave all single-use PPE and laboratory consumables used in sample handling and testing. If autoclaving is not possible (e.g., reusable laboratory coats) decontaminate and then clean for re-use. - Contact the biosafety officer or laboratory manager immediately if known exposure occurs.
B	Moving samples between testing areas	Damaged, broken and leaking raw milk samples	Move or store raw milk samples in a secondary container that can contain spills or leaks.	- Place the secondary container of samples on a cart to move samples around the laboratory. This keeps hands free to open doors, etc. - Decontaminate the secondary container and cart after use. - Secondary containers should be autoclaved, if possible.

#	Procedure(s)	Potential Hazard(s)	Mitigation Control(s)/Protection(s)	Additional Information
C	Regulatory dairy testing: • Temperature checks • Plate counts • Somatic cell count (electronic and/or manual) • Antibiotic testing	• Spill, splash, or splatter that also results in aerosol formation. • Accidental exposure while transferring raw material between containers and testing supplies or apparatus.	• When using a centrifuge that does not have a removable rotor, enhanced PPE is recommended. • Minimize the number of analysts handling the raw milk samples. • Use disposable testing supplies, when possible. • Decontaminate exposed materials with EPA-approved disinfectant. • Perform work in a BSC with appropriate PPE, if possible.	• If tubes, cartridges or other sample vessels leak within an analytical instrument or centrifuge, allow the samples to sit for 15 minutes so that aerosols can settle. • Sample preparation involves shaking milk samples 25 times in seven (7) seconds in a one-foot movement before diluting or pipetting. To mitigate aerosols and other risks, consider bringing the sample into the BSC after shaking, for dilution and plating. • When selecting and using disinfectant: ○ Use disinfectant that is both EPA-approved and permissible for use in dairy testing. ○ When selecting a disinfectant that is effective against HPAI, consider also any applicable 2400 form requirements or SMEDP guidance (i.e., to prevent false positive results from residues of certain agents). ○ Read and follow label instructions to inactivate the HPAI virus. Wipe down all materials with the disinfectant. Allow appropriate contact time to elapse before cleaning. Follow up with cleaning and sterilization, where required, prior to reuse of materials.

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D	Additional testing: - Pathogen screening - Molecular - Immunoassay (e.g., VIDAS) - Pathogen confirmation	- Spill, splash or splatter that also results in aerosol formation. - Accidental exposure while transferring raw material between containers and testing supplies or apparatus.	- See Mitigation Controls/Protections outlined in Section C, “Regulatory Dairy Testing.” - Refer to the assay manufacturer’s safety precautions. - If the laboratory has the resources, centrifuge sample tubes inside the BSC using a sealed head rotor.* If the centrifuge is outside BSC, follow the instructions described in “Regulatory Dairy Testing.”	- Adopt the Additional Information outlined in Section C, “Regulatory Dairy Testing.” - Decontaminate exposed materials with EPA-approved disinfectant. If applicable, follow up with nucleic acid decontamination according to laboratory practice.
E	Utilization of sharps for dairy testing (regulatory and pathogen screening/contamination)	Injuries from sharps in the presence of potentially-infectious material (both a physical and biological hazard).	- Minimize the use of sharps. - Use disposable sharps, when possible. - Discard sharps in a biohazardous waste container (minimum requirement).	
F	Post-analysis decontamination of BSC/workspace	Contamination of BSC/workspace surfaces, leading to the possibility of future exposures	- Wipe the inside of the BSC/workspace with appropriate/approved disinfectant. - Remove PPE and decontaminate or dispose as described in Section A, “Receiving Samples/Temperature Checks,” Additional Information.	- Disinfect surfaces with EPA-approved disinfectant; allow appropriate contact time to elapse before wiping. - When applicable, wipe down all work and testing surfaces with 70% alcohol and allow to air dry.

* CDC’s [Fundamentals of Centrifuge Safety Course](#) can assist with identifying potential risks associated with centrifuge use and identify safe practices for centrifuge use.

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G	Waste Disposal	- Exposure to contaminated waste, laboratory materials and waste containers. - Contaminated waste from electronic somatic cell count (ESCC) and direct microscopic somatic cell count (DMSCC).	- Twist and secure (with tape) the opening of the biohazard waste bags. Some looseness is recommended so that steam from autoclave can enter the bag. - Disinfect outside of waste containers with EPA-approved disinfectant - Treat waste fluids from ESCC and/or DMSCC methods prior to their disposal. - To prevent adverse chemical reactions from waste treatment, review chemical compatibility of EPA-approved disinfectant with solutions, buffers, rinses, stains, dyes or other reagents used to perform ESCC or DMSCC on raw milk.	- Autoclave raw milk samples and associated waste prior to disposal. - Follow all applicable regulations for waste disposal, which may vary by location.

Additional Information

Personal Protective Equipment

PPE Level	Regulatory Dairy Testing	Pathogen Testing
Minimum Required	• Buttoned or snapped lab coat/uniform • Protective eye wear • Lab coats/uniforms remain on-site	• Gloves
Recommended	• Liquid-impermeable: ○ Closed front gown ○ Gloves ○ Face protection (face shield) • NIOSH-approved® N95® filtering facepiece respirator when: ○ BSC is unavailable; perform testing away from doors and away from other workers in the area. ○ Mixing samples in quantities greater than 100 mL outside BSC.	
Enhanced	• If the samples are known to have HPAI, wear a powered air purifying respirator (PAPR), if available. In this situation, access to the testing area should be restricted to essential personnel. • Replace soiled PPE with clean PPE. Carefully remove contaminated PPE and wash your hands. Contaminated, single-use PPE is discarded and placed in the biohazard waste, • Autoclave all single-use PPE and laboratory consumables used in sample handling and testing. If autoclaving is not possible (e.g., reusable laboratory coats) decontaminate and then clean for re-use.	

Laboratory Waste Management

Follow local, regional, state, national and international regulations for waste disposal, as policies will vary by location. For more information, see:

- [EPA Resource Conservation and Recovery Act Regulations](#)
- [State Universal Waste Programs in the United States](#)
- US Department of Transportation's [Managing Solid Waste | F-2 pages 94–97](#)

Glossary

- **Aerosols**

Very fine droplets or particles that can form during sample handling activities like shaking, mixing, pipetting and centrifuging. Care should be taken to minimize the formation of aerosols and to avoid inhaling aerosols while handling raw milk samples.

- **Barriers**

Device used to physically separate analysts from biohazardous materials; includes primary and secondary barriers.

- **Biohazardous Materials**

Infectious agents or hazardous biologic materials that present a risk or potential risk to the health of humans, animals, or the environment.

- **Biological Risk Assessment**

Process that includes the identification of risks, determining the likelihood of their occurrence, and the severity of their effects on human health or the environment associated with a specific infectious agent or material.

- **Biological Safety Cabinet (BSC)**

A BSC is an enclosed, ventilated workspace that protects analysts while working with biohazardous materials. Most BSCs use high efficiency particulate air (HEPA) filters in the exhaust and air return system to prevent exposure to biohazards.

- **Biological Safety Level-1 (BSL-1)**

Describes laboratory setting in which analysts work with low-risk microbes that pose little to no threat of infection in healthy adults.

- **Biological Safety Level-2 (BSL-2)**

Laboratory setting is suitable for work involving agents that pose moderate hazards to personnel and the environment.

- **Biological Safety Level-3 (BSL-3)**

Laboratory setting where work is performed with infectious agents that may cause serious or potentially lethal infections through inhalation.

- **Containment**

Methods/devices used to shield or protect analysts and the immediate work environment from exposure to biohazardous materials.

- **Decontamination**

The reduction or removal of biohazardous contamination from a person, object or area. Any process for removing and/or killing microorganisms.

- **Disinfectant**

A chemical or mixture of chemicals used to kill microorganisms, but not necessarily spores. Disinfectants are usually applied to inanimate surfaces or objects.

- **Disinfection**

A physical or chemical process of reducing or eliminating microorganisms from a surface or space, but not necessarily spores.

- **Exposure**

Contact of skin, mucous membranes (eyes, nose, mouth), open wounds to biohazardous material. A low exposure risk determination may result in the need for symptom monitoring only whereas a moderate and/or high exposure risk determination may result in the need treatment.

- **Hazard**

Anything that can cause harm.

- **Infection**

Invasion of the body tissues of a host by an infectious agent, whether or not it causes disease.

- **Mode of Transmission (of Infection)**

Any mode or mechanism by which an infectious agent is spread to a susceptible host. Airborne transmission is the transfer of an agent through the air. Direct transmission is by direct contact or droplet spread. Indirect transmission is transfer to the analyst through the air, by contact with an inanimate object or carried by other means (i.e., a vector).

- **Mitigate**

Action to make a hazard less severe.

- **Mucous membranes**

The moist, inner lining of some organs and body cavities, such as the mouth, nose and eyes. Mucous membrane transmission is a known route of transmission for HPAI and many other organisms.

- **Personal Protective Equipment (PPE)**

Items worn by laboratory workers to prevent direct exposure to workplace materials. Examples include: gloves, gowns, aprons, coats, containment suits, shoe covers, eye and face shields, respirators and masks.

- **Risk**

Likelihood or adverse health effect if exposed to a hazard.

- **Risk Assessment**

A process to evaluate the probability and consequences of exposure to a hazard and identifying ways to control it.

- **Sharps**

Items capable of cutting or piercing human skin. Examples include hypodermic needles, syringes (with or without attached needles), Pasteur pipettes, scalpel blades, . Also includes broken or unbroken glassware that has been in potential contact with infectious agents (e.g., used microscope slides and cover slips).

- **Sterilization**

The use of physical or chemical process to completely destroy or eliminate all microorganisms, including spores.

- **Universal Precautions**

Standard laboratory practice to treat all samples as though they are infectious, including the containers, testing supplies and work surfaces that come into contact with samples.

- **Waste Disposal**

Waste disposal requirements vary among jurisdictions. Adhere to state, local, or applicable requirements for waste disposal.

Additional Resources

The following are helpful resources when determining if safety practices should be enhanced, or to inform risk assessments and increase safety:

- APHL:
 - [Biosafety and Biosecurity website](#)
 - [HPAI A \(H5N1\) Response webpage](#)
- [Biosafety in Microbiological and Biomedical Laboratories 6th Edition](#)
- [Canadian Pathogen Safety Data Sheets](#)
- CDC:
 - [Guidelines for Laboratory Biosafety: Handling and Processing Specimens Associated with Novel Influenza A Viruses, Including Potential A\(H5N1\) Virus](#)
 - [Biological Risk Assessment: General Considerations for Laboratories website](#)
- FDA: [Interim HPAI Virus Biosafety Recommendations and Resources for Industry Laboratories and State Dairy & Dairy Product Laboratories](#)
- Information on dairy testing* that supports the Pasteurized Milk Ordinance (PMO):
 - [National Conference on Interstate Milk Shipments \(NCIMS\) 2400 forms](#)
 - [Standard Methods for the Examination of Dairy Products \(SMEDP\)](#)

* Refers to dairy testing that supports PMO and rapid screening for foodborne pathogens.



Association of Public Health Laboratories

The Association of Public Health Laboratories (APHL) works to strengthen laboratory systems serving the public's health in the US and globally. APHL's member laboratories protect the public's health by monitoring and detecting infectious and foodborne diseases, environmental contaminants, terrorist agents, genetic disorders in newborns and other diverse health threats.

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