

# Decontamination of Select Agents Isolated in the Clinical Laboratory

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Select agents are regulated by the United States (US) Department of Health and Human Services (HHS), Centers for Disease Control and Prevention (CDC) and Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS). These regulations governing the possession, use and transfer of select agents are detailed in 7 CFR 331, 9 CFR 121 and 42 CFR 73, which require that material containing an identified select agent must either be **destroyed or transferred** to a select agent registered facility within seven days from confirmation (unless an extension is granted from CDC and/or USDA). Select agents may only be held more than seven days from confirmation by facilities that are registered and approved by CDC and/or USDA to possess those specific select agents. Once an isolate from a patient specimen in a non-select agent registered clinical lab has been confirmed by a registered Laboratory Response Network (LRN) reference laboratory as a select agent, within seven days the non-registered clinical lab must either **destroy** all other relevant patient specimens and cultures remaining in their possession **or obtain permission from CDC to transfer** them to the nearest LRN reference laboratory that is registered to possess the specific select agent.

If a clinical lab chooses to **transfer the relevant specimens and cultures** after organism confirmation, the lab personnel will need to work with their LRN reference laboratory to ensure the proper paperwork (e.g., [APHIS/CDC Form 2](#))<sup>3</sup> and transfer protocols are followed in compliance with all applicable local, state and federal shipping regulations, and carrier/courier requirements **prior to transport**. Transfer considerations should be discussed between clinical laboratories and LRN reference laboratories **before** LRN reference testing is conducted to avoid some potential shipping restrictions or dilemmas. If a facility does not have an autoclave on-site and chooses not to chemically decontaminate the cultures, all positive cultures including blood culture bottles must be transferred to an appropriate select agent registered laboratory approved and willing to accept the specific select agent material.

If a non-registered clinical lab decides to **destroy the relevant specimens and cultures** in-house, inactivation using an on-site autoclave or chemical decontamination method must be performed before final disposal or transferring the items to a contracted medical waste hauler for destruction and final disposal. Specimens associated with an identified select agent cannot be directly discarded into the biohazardous waste stream like other regulated infectious medical waste materials because the material would be classified as Category A waste and restricted according to both the select agent regulations and the US Department of Transportation Hazardous Material Regulations (49 C.F.R., Parts 171-180). Autoclaving is the preferred method of destruction, however when an autoclave is not available, chemical decontamination may be the only feasible option. For both chemical inactivation decontamination procedures below, the clinical laboratory should note the date, amount/quantity of material being destroyed, method of destruction, and the laboratorian(s) performing the procedures for record keeping purposes.

**Non-registered clinical labs are not required to have a validated select agent inactivation protocol but may use these decontamination and destruction procedures as a recommended best practice.**<sup>4</sup>

## Chemical Inactivation Decontamination Process for Samples and Cultures

1. Prepare a fresh (daily) 10% (1:10) solution of household bleach in a receptacle large enough to submerge all containers/plates containing the select agent specimen(s).
2. Working in a biological safety cabinet (BSC), **slowly** and completely immerse open sample/culture containers in the bleach solution.
3. Leave the open and submerged containers in the bleach solution overnight in the BSC and post a warning/safety sign for it.
4. Once overnight inactivation is complete, turn the sink faucet on and discard the bleach solution down the drain with running tap water.
5. Place the inactivated sample/culture plates and containers in a biohazard bag and discard them with the other biohazardous waste that is transported off site by a medical waste management contractor for final treatment and disposal.

## Chemical Inactivation Decontamination Process for Blood Culture Bottles

If an organism is subcultured from a blood culture bottle and a LRN reference laboratory confirms the organism as a select agent, or if the patient is diagnosed with a select agent such as viral hemorrhagic fever (VHF), the associated blood culture bottles and any additional bottles or cultures that would contain the organism, must be decontaminated before transport off site within seven days from confirmation. Autoclaving is the preferred destruction method since the contents in these bottles cannot be easily decontaminated using chemical inactivation decontamination.

- Bring all needed materials into a BSC including the blood culture bottle(s), a syringe, and a small amount of undiluted household bleach (e.g., ~50mL per blood culture bottle to decontaminate).
- Working in a BSC, the blood culture bottles can be chemically decontaminated by adding straight (not diluted) household bleach to the bottle to obtain a final concentration of 1-2% sodium hypochlorite (20–40% household bleach or ~10,000 parts per million (PPM) available chlorine) within the bottle. The higher undiluted bleach concentration works well for inactivation and accounts for the large amount of organic material present.
- Cover the top of the bottle with a disinfectant soaked gauze pad (e.g., 10% bleach) to contain any splashes and slowly inject the undiluted bleach into the bottle(s) through the gauze pad.
- Discard the used syringe in the sharps container inside the BSC.
- Let the bottle(s) sit overnight in the BSC and post a warning/safety sign for it.
- Package the inactivated bottle(s) with other biohazardous waste that is transported off site by a medical waste management contractor for final treatment and disposal.

## Toxin Inactivation

For specimens to which there may be a suspected or confirmed select agent toxin present, the clinical lab should consult with their LRN reference laboratory about specific concerns and inactivation methods. In general, most toxins associated with biological specimens can be easily inactivated or denatured by steam sterilization, dry heat or chemical means such as sufficient contact time with a fresh (daily) 10% solution of prepared bleach, or another chemical such as sodium hydroxide (NaOH, 0.1N). Consult the [Biosafety in Microbiological and Biomedical Laboratories \(BMBL\)](#), section VIII-G for specific toxin information and recommended inactivation methods.

**Table: Preparation of Bleach Solutions Containing 5.25–6.15% Sodium Hypochlorite (NaOCl)**

Dilution	Chlorine (PPM)
None / straight, concentrated bleach	52,500–61,500
(10% bleach) 1:10, or 1½ cup:1 gallon, or 100mL:1000mL	5,250–6,150
(5% bleach) 1:20, or ¾ cup:1 gallon	2,625–3,075
(1% bleach) 1:100, or ¼ cup:1 gallon	525–615

**Notes:** Bleach is usually between 5.25–6.15% sodium hypochlorite (52,500–61,500 ppm available chlorine), but will vary depending on the manufacturer and if it is “regular” strength (typically 5.25%) vs. “ultra” strength. “Ultra” strength products are typically about 6.15%, but the germicidal Clorox brand can be up to ~8.25%. It is important to know the concentration of the bleach being used to ensure the desired final concentration will be obtained when preparing the solution. Different bleach products may have different concentrations of hypochlorite.

Hypochlorite concentrations will degrade over time and with storage conditions. Bleach will last up to one year from date of manufacture when properly stored away from direct sunlight and heat. Working bleach solutions should be changed daily and will also be affected and have a decreased efficacy by the amount of organic material that may be present in the material intended to be decontaminated. Follow all manufacturer product specific instructions. Some manufacturers use a code for the product expiration date, such as Clorox, whose expiration date can be interpreted from the production code.<sup>11</sup> Clorox bleach production codes always start with a letter and number, which indicates the

plant where the bottle was made. After the plant code comes the date on which the bottle was made, which starts with the last two digits of the year, followed by the Julian date. Julian dates give the numbered day out of 365 total days in a year. For example, a bottle stamped A822 13019: 48CA3 was made in 2022 on the 130th day of the year, or May 10. Use a [Julian date calculator](#) to determine the date. Check with your manufacturer if you are using a product other than Clorox.

## Decontamination of Material That May Contain Select Agent Spores

If there is a concern that select agent spores (e.g., *Bacillus anthracis*) may be present, or if there is a need to decontaminate material that may contain spores, stronger disinfectants than those used routinely may be required. Clinical labs should consult with their LRN reference laboratory about specific concerns and decontamination methods. The pH of a bleach solution may also need to be checked and amended in order to efficiently decontaminate spores by chemical inactivation methods. Consult the EPA list of approved disinfectants<sup>10</sup> for additional info.

## References

1. [www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html#Chlorine](http://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html#Chlorine)
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