

# Growsafe: A Chemical Method To Deactivate Cultivated Microorganisms Using Low-Cost Kitchen Supplies<sup>‡</sup>

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**One limitation to engaging K–12 students and the public with microorganisms is the inability to cultivate and dispose of bacterial and fungal samples safely without expensive equipment or services. This barrier has been amplified with remote learning modalities and laboratory closures driven by safety precautions due to the COVID-19 pandemic. At-home lab kits are being used to bring hands-on experience in microorganism cultivation to students learning remotely, but these kits often fail to take into full consideration the safety aspects or the costs associated with microorganism disposal, limiting which experiments can be performed at home. Here, we outline a method that makes cultivating and deactivating microorganisms accessible to the public through low-cost and readily available equipment. This method reduces exposure to microorganisms by forgoing the need to open petri plates for chemical deactivation with sanitizing reagents. This technique may benefit remote K–12 and postsecondary students, students wishing to get hands-on microbiology research experience, and members of the public interested in cultivating microorganisms to contribute to citizen science efforts or for creative art applications.**

## INTRODUCTION

Despite the importance microorganisms have to our fundamental understanding of ecology, biodiversity, nutrient cycling, and our own health, K–12 students have limited exposure to microbiology or cultivating microorganisms (1, 2). With the COVID-19 pandemic and the shift to remote learning for K–12 and postsecondary students due to school and laboratory dedensification, even fewer opportunities exist for hands-on microbiology experience. Despite the relative ease of acquiring tools and reagents for cultivation (e.g., at-home lab kits available commercially or through educators) and the increasing amount of educational material to assist teachers with integrating microorganisms into the curriculum (1, 3), safety concerns and limited equipment for aseptic technique and disposal of cultivated microorganisms prevent incorporation of hands-on microbiology experience (2, 4).

Research universities often have access to autoclave equipment or professional biohazard waste-removal services. However, these measures are prohibitively expensive for many K–12 school systems or for people who wish to cultivate microbes at home, and few alternative equipment and practices are available. Some methods for at-home microbial deactivation include using kitchen pressure cookers (5) or submerging open petri plates in a chemical disinfectant solution (6). Petri plates are usually left open during the addition of sodium hypochlorite (bleach), leading to potential microbe exposure [particularly to spores and/or aerosols (7)], putting educators, their students, and/or the custodial staff at risk. Additional effective and cost-efficient methods for deactivating microorganisms growing on solid media would therefore aid in education efforts, as well as science and engagement efforts that require at-home microorganism cultivation [e.g., citizen scientist–assisted research (8), course-based research opportunities (9), or creative “agar art” initiatives (10)].

We have devised a method to chemically deactivate cultured microorganisms in plastic petri plates that reduces the chance of microorganism exposure. This protocol is cost-effective, uses readily available household items and reagents, and allows for the safe disposal of deactivated microorganisms in standard household waste. We provide a protocol for this “Growsafe” method and further test the efficacy of this method in deactivating a variety of bacterial and fungal species. This method is appropriate for labora-

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atories, classrooms, and homes, provided that the area is well-ventilated and sufficient safety measures are taken.

## PROCEDURE

### Materials

A list of materials and reagents and detailed instructions are available in Appendix I. The materials needed are low-cost and are available from common grocery or retail stores.

### Protocol

Briefly, petri plates containing viable microorganisms on a solid nutrient medium are deactivated with a chemical disinfectant applied through a created portal in the petri plate. The petri plate is sealed with electrical tape and placed in a glass baking dish (or other fire-resistant container). In a well-ventilated space, the wide diameter hole of a metal Bismarck-style frosting tip is covered with aluminum foil to create a barrier (Fig. 1); this barrier is necessary to reduce exposure to aerosolized particles. Holding the covered tip with kitchen tongs, the narrow end of the frosting tip is heated with a kitchen lighter and is then immediately pressed down into the plastic top of the petri plate, forming a pore into the plate. After allowing the tip to cool completely,

a condiment squeeze-bottle containing 20% bleach (1.5% sodium hypochlorite) is used to pierce the foil barrier and slowly dispense the bleach until the plate is filled with bleach solution. The bottle, foil, and metal tip are sequentially removed from the petri plate, leaving a ~4-mm-diameter hole. The plate is then left in a well-ventilated area (using a fan or with windows and doors open) for 24 hours and can then be disposed in household trash; municipal guidelines for bleach disposal should be consulted. The foil, metal tip, and the condiment bottle should all be disinfected prior to disposal or being reused.

### Efficacy testing

We tested the microbial deactivation efficacy of this method on petri plates containing confluent growth of a gram-positive bacterium, a gram-negative bacterium, a fungus with a yeast morphology, or a spore-forming filamentous fungus. After implementing the method on each sample in duplicate, we used replica plating methods to transfer microorganisms from the bleach-exposed petri plate to a fresh petri plate and growth medium. We then incubated these plates and checked for growth. Our testing revealed the Growsafe method is effective at deactivating the microorganisms tested after 24-hour exposure to the chemical disinfectant. See Appendix 2 for full methods, results, and discussion.

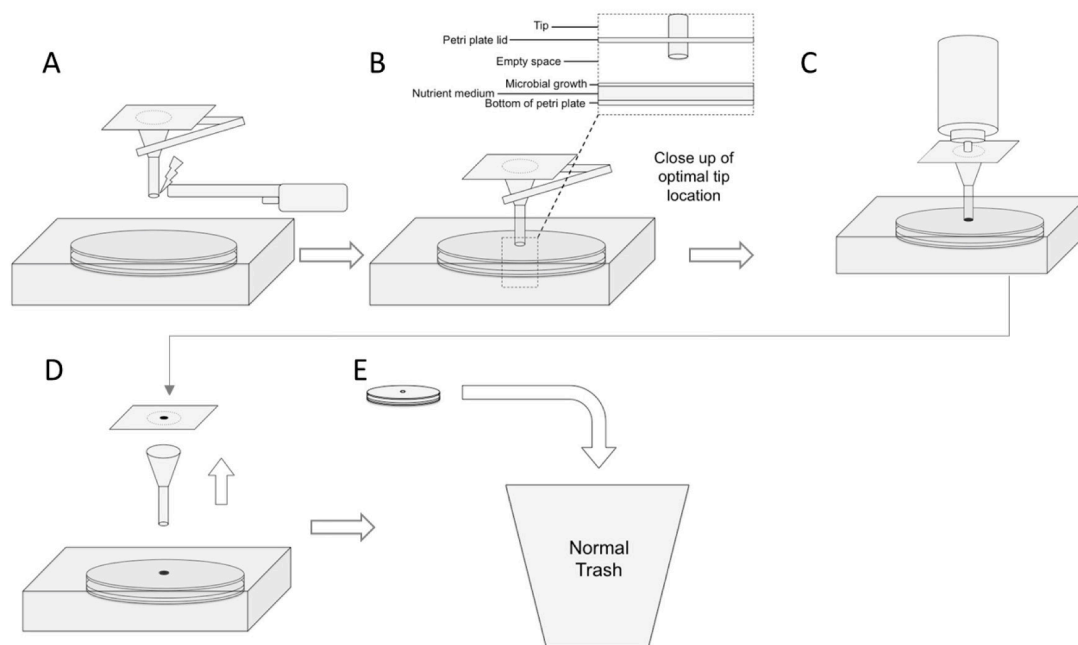


FIGURE 1. Brief schematic of Growsafe method to deactivate cultivated microorganisms through a sealed petri plate. (A) A metal Bismarck frosting tip with a foil barrier is heated, (B) gently pushed through the lid of a sealed petri dish, and (C) filled with a 20% bleach solution through the foil barrier using a condiment bottle. (D) Frosting tip can be removed and reused. (E) After 24 hours in a well-ventilated area, the dish can be disposed in a standard trash bin without any additional biohazard concerns.

## Safety issues

When performed as written, this method aligns with the American Society for Microbiology (ASM) Guidelines for Biosafety in Teaching Laboratories (6) and OSHA safety guidelines on minimizing chemical and burn risk. Proper safety measures should be taken throughout the culturing process, as well; educators and home microbiologists should review the ASM supplemental considerations for at-home microbiology kits (11). To prevent exposure to volatilized compounds from melting plastic or bleach vapor, this method should be performed by an adult in a well-ventilated area, and the metal tip should be completely cooled before addition of bleach solution. Care should be taken when using this method with alternative media (especially those with high concentrations of ammonium) where chemical reactions between the media and sodium hypochlorite may produce unwanted byproducts. While we have sought to reduce the risks of fire and burns, the frosting tip should be carefully examined for flammable plastic carry-over if it is to be reused. This method is recommended for biohazard level I microorganisms.

## CONCLUSION

We describe a method for at-home microbial deactivation that uses readily available kitchen supplies. This method can be used in conjunction with activities in the classroom or at home that include observing growing microorganisms. It can also be used to teach associated STEM skills such as dilution plating, plate spreading, proper handling and cultivation techniques, isolation from mixed cultures, and even creative endeavors such as microbial agar art initiatives (10). It may also be useful in remote research field situations where access to electricity (such as required for alternative sterilization procedures) is unavailable. This method therefore increases the accessibility of growing microorganisms by lowering a normally cost- and safety-prohibitive obstacle. This method includes risks and is not highly scalable due to the time and care it takes to create the portal, but we hope that sharing this method further fosters innovation in tools, equipment, and practices to make microbial cultivation more accessible. Ultimately, we believe the development of methods such as this will increase the safe use of microorganism-associated education initiatives.

## SUPPLEMENTAL MATERIALS

Appendix 1: Materials and detailed protocol for the Growsafe method

Appendix 2: Efficacy testing of the Growsafe method

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