

Antimicrobial Testing of Dry Surfaces through Large Droplet Inoculation

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Method Article

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Abstract

The Large Droplet Inoculation (LDI) protocol for testing antimicrobial coatings and treatments is simple, reproducible, and closely mimics real-world conditions at solid/air interfaces. This is an advancement of the current ISO 22196/JIS Z 2801 standard method, as it provides greater ease-of-use and more closely resembles the contamination of common surfaces. The protocol involves inoculating 100 μL droplets containing $\sim 10^7$ microbial cells of a test microorganism onto sets of antimicrobial-treated and untreated control sample material. The sets are protected from dust accumulation and allowed to air dry, which causes all cells within each droplet to encounter the sample surface. The surviving cells are then collected by vortexing in a collection liquid and enumerating to evaluate differences in survival on the antimicrobial-treated and untreated control samples. Overall, the LDI protocol requires 10 hours of basic microbiology over one week and provides a simple means of assessing the efficacy of various antimicrobial coatings and treatments.

Introduction

Prior to its use in determining the efficacy of antimicrobial-treated surfaces, the LDI protocol was developed to study the survival and persistence of desiccation-tolerant pathogenic and non-pathogenic bacteria.^{1–5} For example, in the work done by Ronan et al.,⁶ the method was used to accurately inoculate glass slides with predetermined loads of the opportunistic pathogen *Pseudomonas aeruginosa* (PA01-gfp) cells⁷ as well as six other isolates from indoor air. Of those six isolates, a notably desiccation-tolerant strain of *Arthrobacter* sp. (IAI-3) was subsequently used in a range of experiments to study the effect of interspecies interaction on bacterial survival during desiccation. Since the completion of that study, this desiccation-tolerant bacterial strain was reclassified as *Glutamicibacter soli* (IAI-3).^{8,9} It has been cryopreserved for use as a model organism in the LDI protocol, as it is representative of desiccation-tolerant bacteria that may persist for extended periods in air and on dry surfaces.

The LDI protocol was subsequently used by Porosa et al.¹⁰ as a method of testing the antimicrobial properties of a surface-bound treatment. These trials involved determining bacterial survival on benzophenone-anchored long-chain aliphatic quaternary ammonium compound (QAC) coatings, using the previously mentioned *G. soli* and *P. aeruginosa* strains, as well as the food pathogen *Listeria monocytogenes* (Scott A). The ability for the latter to persist on food preparation surfaces is a major cause of contamination in fresh produce and processed meats, and has led to many disease outbreaks and product recalls.¹¹ The LDI protocol was able to show a clear difference in bacterial survival on untreated control samples versus those treated with the QAC coatings; a biosafety cabinet-assisted drying step was found to impact the survival of desiccation-sensitive species.

The protocol has since been successfully employed to test the antimicrobial properties of sulfonamide QACs, as well as QAC polymeric systems and related phosphonium derivatives.^{12–14} These

studies allowed for optimization of the procedure which improved the reproducibility of the results. The drying process was adjusted to allow the droplet to air dry unassisted rather than under laminar airflow in a biosafety cabinet, which extended the drying time from 3 to 24 hours. This reduced desiccation stress on the cells and lead to improved survival on all untreated control surface samples regardless of the test microorganism. A variation of the protocol, named the Liquid Reservoir Inoculum (LRI) method, was also developed to examine the adhesion of microbial biofilms to antimicrobial-treated surfaces under aqueous conditions.¹²

The LDI protocol reliably demonstrates the antimicrobial effects of ionic coatings and has been developed with adaptability in mind.^{10,12-14} While the simplest application of the protocol examines the survival of pure cultures on antimicrobial-treated and control surfaces, co-cultures and microbial communities could also be used as inocula, as demonstrated in the development work that led to this method.⁶ The protocol could also incorporate swabbing to collect surviving cells from the surface samples, particularly when the surface material could be damaged by the prescribed saline vortex collection method. Any changes to the procedure, such as the microorganisms used, the concentration and volume of deposited cells, the cell collection method, the choice of control, and drying time must be reported.

Antimicrobial efficacy testing of treated surfaces has previously been carried out using established standard methods¹⁵⁻¹⁷ and published protocols^{18,19} which have been examined at length in critical reviews.^{20,21} A major deviation of the LDI protocol compared to the ISO 22196/JIS Z 2801 standard methods is the exclusion of a plastic cover film used to sandwich the inoculum droplet to the sample surface. Though omitting the cover film does increase desiccation stress on the test microorganism, it more closely mimics a natural contamination event by avoiding the entrapment of liquid inoculum on the surface while also reducing experimental error caused by the adhesion of cells to the film rather than the test material. This shares similarity to carrier-style disinfection standards, except the treatment is applied to the test surface before the inoculation step of the LDI procedure.²² As for some of the protocols that have been published, many use an aerosol chamber to deposit bacteria onto their test materials.^{23,24} During development of the LDI procedure, Ronan et al.⁶ also attempted to use an aerosol chamber to deposit *P. aeruginosa* and bacterial isolates from indoor air onto glass slides. The results obtained from aerosol deposition were highly variable in the quantity and survival of deposited cells, which negatively affected overall reproducibility.

The LDI method offers simplicity in its application at a low cost while providing reproducible results. A 100 µL inoculating droplet placed directly onto antimicrobial-treated and untreated control surfaces dries naturally without using a cover film. This allows all cells to encounter the sample surface due to the coffee ring phenomenon²⁵ and is a good approximation of how surfaces in the built environment are commonly contaminated. All the equipment and tools necessary for the protocol can be found in most basic microbiology laboratories, and the required consumables are generally inexpensive and readily available from lab suppliers.

This test is a comparison of bacterial survival on the surface of non-porous antimicrobial-treated and untreated materials. Therefore, the untreated control samples should be made from the same material as the antimicrobial-treated samples and absent of artifacts that may impact the survival of microorganisms during the trial period. If an untreated material is unavailable for use as a control sample, a non-degradable material such as polypropylene or stainless steel can be used as an alternative.

For textiles and other porous materials, it is recommended that the inoculum absorbs completely within the sample during the drying period. This is to prevent cells from being deposited elsewhere instead of the sample. To accomplish this with thin fabrics, a larger piece of material should be folded multiple times to increase its thickness, or multiple swatches of material should be stacked together as used in the AATCC 100 standard method.²⁶

One of the critical components of the LDI protocol is the choice of medium for the inoculum suspension. Use of a saline solution leads to the formation of salt crystals during drying, which can impact the survival of non-halotolerant organisms. It is therefore recommended that the inoculum be suspended in sterile, potable fresh water prior to inoculation.^{6,27} The purpose of this is to avoid the formation of solid materials on the sample surface while providing enough solutes to help withstand hypotonic stress. Alternatively, a soil load medium can be used to stabilize the inoculum and mimic dirty conditions.²² After the cells have been suspended, the concentration of cells in the inoculum is enumerated to determine the initial microbial load added to the antimicrobial-treated and untreated control surface samples. The inoculum can be diluted if necessary, based on optical density measurements, to achieve any desired concentration prior to inoculation.

Once the antimicrobial-treated and untreated control surface samples are inoculated, they are dried in a covered container to minimize external influence. An example of a simple, yet highly replicable environment is to keep the samples in empty Petri dishes on a workbench at room temperature for 24 hours to allow the inoculum droplets to completely dry. If the object the samples represent is expected to be used under unique conditions (e.g., at altitude) or if the test microorganism is incapable of surviving ambient temperatures and humidity, an environment-controlled incubator must be used. This change must be factored in when considering the effect on drying time as it could increase desiccation stress on the inoculum.

Collection of the surviving cells after drying should be performed by vortexing the air-dried samples at max speed for one minute within a conical tube containing 5 mL of phosphate buffered saline (PBS) solution. If the test microorganisms are not sensitive to changes in pH, simple isotonic saline could be used as a substitute for PBS. If there is a chance that antimicrobial leachate could be present, it is recommended that an appropriate neutralizing agent be used during the cell collection.

Due to the simplicity of the LDI protocol, most of the required equipment and expertise are available and taught in entry-level microbiology courses. A senior undergraduate biology student should be able to perform this method when given the appropriate materials and access to a containment level 1

facility. After appropriate instruction and safety training, the protocol was successfully employed in a national high-school science fair project (CWSF 2019).

There are a few limitations that can affect the utility of the results obtained with this protocol, though many of these can be easily overcome. The most likely source of error is the environmental contamination of sample materials. These natural contaminants can be vastly outnumbered by depositing high colony forming unit (CFU) counts within the 10^7 range, derived from the enumeration of undiluted overnight cultures of *G. soli* and *Escherichia coli*. The benefit of using a high microbial load is that it increases cell survival on control samples and it simplifies the inoculum preparation by removing a cell reduction step, although the starting inoculum can be adjusted using optical density measurements. There is also a risk of antimicrobial leachates during the cell collection step, which can be mitigated by exhaustively rinsing the samples with distilled water and testing the rinse solution prior to inoculation. If this is not possible, it is recommended that an appropriate neutralizing medium should be used during the cell collection. Conversely, the leachate could be retained and tested to determine if the leachate has antimicrobial properties.

Some of the limitations that are inherent with this protocol are also common in the real-world and need to be considered beforehand. Samples that are soiled or pre-contaminated prior to inoculation will likely prevent the test microorganisms from encountering the treated or control material surface, which can affect the reliability of the results. The same is also true if the samples are re-tested using the LDI protocol without cleaning the material since the surface will remain covered in extracellular polymeric substances deposited during the first trial. Incomplete drying would also affect the reliability of the results since the remaining liquid could act as a safe reservoir for the inoculated species. The test microorganism used in the LDI protocol also needs to be sufficiently desiccation-tolerant to allow for comparison of survival on antimicrobial-treated versus untreated control samples.

Reagents

-Agar A, powdered (BioBasic, cat. no. FB0100)

-*Glutamicibacter soli* (*G. soli*, IAI-3)

-*Escherichia coli* (*E. coli*, ATCC 11229)

-Distilled water

-Potable fresh water

-Phosphate buffered saline tablets (BioBasic, cat. no. PD0435)

-Tryptic soy broth, powdered (Sigma-Aldrich, cat. no. 1.05459)

Equipment

- Petri dishes, polystyrene, 92 x 16 mm (Sarstedt, cat. no. 82.1473.001)
- Conical tubes, polypropylene, 50 mL (Sarstedt, cat. no. 62.547.205)
- Micropipette tips, polypropylene, 200 μ L (Sarstedt, cat. no. 62.547.002)
- Micropipette tips, polypropylene, 1000 μ L (Sarstedt, cat. no. 70.762.100)
- Micropipette tips, polypropylene, 5 mL (Sarstedt, cat. no. 70.1183.001)
- Microcentrifuge tubes, 2 mL (Sarstedt, cat. no. 72.695.500)
- Micropipette, 2-20 μ L (Sartorius, cat. no. 725030)
- Micropipette, 20-200 μ L (Sartorius, cat. no. 725060)
- Micropipette, 100-1000 μ L (Sartorius, cat. no. 725070)
- Micropipette, 500-5000 μ L (Sartorius, cat. no. 725080)
- Microcentrifuge, (Eppendorf, cat. no. 022620321)
- Autoclave (Yamato Scientific, cat. no. RK-10771-10)
- Shaking incubator (Thermo Scientific, cat. no. SHKA4450CC)
- Inoculating loop
- Benchtop vortexer (VWR, cat. no. 58816-121)

Procedure

REAGENT SETUP

Tryptic soy broth Rehydrate 3 g of powdered tryptic soy broth in 1 L of distilled water. Autoclave at 121 °C for 20 minutes. Tryptic soy broth (TSB) solution is stored at 4 °C and remains usable until it appears turbid, indicating contamination.

Tryptic soy agar Rehydrate 3 g of powdered tryptic soy broth and 12 g of agar A powder in 1 L of distilled water. Autoclave at 121 °C for 20 minutes. Safely pour 25 mL of molten tryptic agar into 40 Petri dishes before agar solidifies. Tryptic soy agar (TSA) plates are stored in air-tight packaging and remain useable if uncontaminated.

Sterilized potable water Autoclave 100 mL of potable fresh water at 121 °C for 20 minutes. Sterilized fresh water remains usable for one month, or until it appears turbid, indicating contamination.

Phosphate buffered saline Rehydrate 5 phosphate buffered saline (PBS) tablets in 500 mL of distilled water. Autoclave at 121 °C for 20 minutes. PBS solution remains usable until it appears turbid, indicating contamination.

(Figure 1 here)

PROCEDURE

DAY 1 | Overnight Culturing of Inoculating Bacteria

1. Use an inoculating loop to transfer freshly streaked bacterial culture (*G. soli*) into a 50 mL conical tube containing 10 mL of tryptic soy broth.

Critical Step: Prepare an aseptic environment and use sterile consumables to avoid contamination of bacterial cultures and samples.

Pause Point: Incubate conical tube using optimal liquid growth parameters (200 r.p.m., 30 °C for *G. soli*).

Timing: 15 minutes.

Anticipated Results: Overnight culture should appear turbid.

(Figures 2, 3 and 4 here)

DAY 2 | Preparation of Inoculum and Inoculation of Samples

1. Pipette two 1 mL aliquots of overnight solution into separate microcentrifuge tube and spin at 9000 × g for 4 minutes.

Critical Step: Prepare an aseptic environment to avoid contamination of bacterial cultures and samples.

2. Remove supernatant fluid, resuspend each bacterial pellet in 1 mL sterilized potable water and repeat centrifugation to rinse cells.

3. Remove supernatant fluid and resuspend each bacterial pellet in 1 mL sterilized potable water. Combine resuspended bacterial pellets to form inoculum.
4. Using a dilution series consisting of 7 microcentrifuge tubes filled with 900 μL of PBS, successively dilute 100 μL of the inoculum by a factor of 10^0 - 10^7 .
5. Individually dispense ten 10 μL drops of each of the 10^5 , 10^6 , and 10^7 dilutions of the inoculum onto nutrient agar plates and incubate at optimal solid growth parameters (inverted when dried, 30 °C for *G. soli*).
6. Prepare triplicate samples of antimicrobial-treated and untreated control materials by cutting and placing 4 cm^2 coupons into labelled, empty Petri dishes.
7. Pipette 100 μL of the 10^0 inoculums onto each of the treated and control samples, cover and place on a benchtop to dry.

Pause Point: Allow inoculated samples to air dry. (Requires 24 hours at ambient humidity)

Timing: 3 hours

(Figures 5 and 6 here)

DAY 3 | Collection and Plating of Surviving Bacteria

1. Individually place each inoculated sample into its own 50 mL conical tube containing 5 mL of PBS.

Critical Step: Make sure samples are dry before starting step 1 to verify that all cells have encountered the sample surface.

2. Vortex each tube at max speed for 60 seconds to remove any adhered cells from the sample surface.
3. Using a dilution series consisting of 3 microcentrifuge tubes filled with 900 μL of PBS for each inoculated sample, successively dilute 100 μL of the sample by a factor of 10^0 - 10^3 .
4. For each sample, individually dispense ten 10 μL drops of each of the 10^0 , 10^1 , 10^2 , and 10^3 diluted sample rinse solution onto individually labelled nutrient agar plates and incubate at room temperature for 3-7 days.

Timing: 4 hours.

(Figure 7 here)

TABLE 1 | Survival of *G. soli* (IAI-3) against sulfonamide antimicrobial on polystyrene (\pm indicates standard deviation $n = 3$). Limit of detection is 50 CFU (1 colony in 5 mL undilute collection fluid).¹²

(Figure 8 here)

DAY 4 | Bacterial Enumeration and Data Analysis

1. Using the lowest dilution factor (DF) that can be reasonably counted (between 20 to 200 CFU), determine the number of bacterial colonies present per sample and for the inoculum.
2. Use the following equation to determine the number of viable bacterial cells per sample.

(Figure 9 Formula here)

3. Calculate the geometric mean and sample standard deviation from the geometric mean by averaging the log-transformed sample CFU values.

Timing: 1 hour.

Anticipated Results: The average geometric mean for the antimicrobial-treated sample should be lower than that for the untreated control samples. Higher consistency in the antimicrobial treatment of the sample will result in lower standard deviation values.

Troubleshooting

Problem: Overnight does not appear turbid after incubation.

Possible Reason: Overnight culture did not grow.

Solution: Repeat Day 1, Step 1 and adjust incubation temperature, shaking speed and time. Make sure that the bacteria used has been freshly streaked and try to transfer cells from multiple colonies.

Problem: Solution appears foamy when vortexing samples in the rinse solution.

Possible reason: Antimicrobial treatment is leaching into the solution and acting as a surfactant.

Solution: Test an aliquot of rinse solution for the presence of a leachate. Make note if the samples are leaching and repeat the experiment with non-leaching antimicrobials. If leaching is unavoidable, use an appropriate neutralizing media during collection (e.g., D/E broth, Sigma-Aldrich, cat. no. D3435).

Problem: There is uniform microbial growth on all plates between the different sample dilutions.

Possible reason: The saline solution is contaminated.

Solution: Repeat the experiment using freshly autoclaved saline.

Problem: There is no growth on the untreated control samples.

Possible reason: The microbial species used during the trial did not survive desiccation.

Solution: Repeat the experiment using a different microbial species or try to ease desiccation stress by lengthening the drying period using a humidified incubator. Note that *G. soli* has been successfully tested using a 3 h drying period.

Time Taken

Anticipated Results

References

1. Pettit, F. & Lowbury, E. J. L. Survival of wound pathogens under different environmental conditions. *J. Hyg. (Lond)*. **66**, 393–406 (1968).
2. Wendt, C., Dietze, B., Dietz, E. & Rüden, H. Survival of *Acinetobacter baumannii* on dry surfaces. *J. Clin. Microbiol.* **35**, 1394–7 (1997).
3. Rose, L. J., Donlan, R., Banerjee, S. N. & Arduino, M. J. Survival of *Yersinia pestis* on Environmental Surfaces. *Appl. Environ. Microbiol.* **69**, 2166–2171 (2003).
4. Makison, C. & Swan, J. The Effect of Humidity on the Survival of MRSA on Hard Surfaces. *Indoor Built Environ.* **15**, 85–91 (2006).
5. Stone, W., Kroukamp, O., Korber, D. R., McKelvie, J. & Wolfaardt, G. M. Microbes at Surface-Air Interfaces: The Metabolic Harnessing of Relative Humidity, Surface Hygroscopicity, and Oligotrophy for Resilience. *Front. Microbiol.* **7**, 1563 (2016).
6. Ronan, E., Yeung, C. W., Hausner, M. & Wolfaardt, G. M. Interspecies interaction extends bacterial survival at solid–air interfaces. *Biofouling* **29**, 1087–1096 (2013).

7. Ghadakpour, M. *et al.* Integration and Proliferation of *Pseudomonas aeruginosa* PA01 in Multispecies Biofilms. *Microb. Ecol.* **68**, 121–131 (2014).
8. Roh, S. W. *et al.* *Arthrobacter soli* sp. nov., a novel bacterium isolated from wastewater reservoir sediment. *J. Microbiol.* **46**, 40–44 (2008).
9. Busse, H.-J. Review of the taxonomy of the genus *Arthrobacter*, emendation of the genus *Arthrobacter* sensu lato, proposal to reclassify selected species of the genus *Arthrobacter* in the novel genera *Glutamicibacter* gen. nov., *Paeniglutamicibacter* gen. nov., *Pseudoglutamicibacter* gen. nov., *Paenarthrobacter* gen. nov. and *Pseudarthrobacter* gen. nov., and emended description of *Arthrobacter roseus*. *Int. J. Syst. Evol. Microbiol.* **66**, 9–37 (2016).
10. Porosa, L. *et al.* UV-Curable Contact Active Benzophenone Terminated Quaternary Ammonium Antimicrobials for Applications in Polymer Plastics and Related Devices. *ACS Appl. Mater. Interfaces* **9**, 27491–27503 (2017).
11. Ramaswamy, V. *et al.* Listeria—review of epidemiology and pathogenesis. *J. Microbiol. Immunol. Infect.* **40**, 4–13 (2007).
12. Caschera, A. *et al.* Surface-attached sulfonamide containing quaternary ammonium antimicrobials for textiles and plastics. *RSC Adv.* **9**, 3140–3150 (2019).
13. Shum, R. L., Liu, S. R., Caschera, A. & Foucher, D. A. UV-Curable Surface-Attached Antimicrobial Polymeric Onium Coatings: Designing Effective, Solvent-Resistant Coatings for Plastic Surfaces. *ACS Appl. Bio Mater.* **3**, 4302–4315 (2020).
14. Bedard, J., Caschera, A. & Foucher, D. A. Access to Thermally Robust and Abrasion Resistant Antimicrobial Plastics: Synthesis of UV-Curable Phosphonium Small Molecule Coatings and Extrudable Additives. *Materials (Basel)*. Submitted
15. ISO 22196:2007 - Plastics – Measurement of antibacterial activity on plastics surfaces. 1–16 (2007).
16. JIS Z 2801:2010 - Antibacterial products - Test for antibacterial activity and efficacy. 1–19 (2010).
17. *ASTM E2149-13a Standard Test Method for Determining the Antimicrobial Activity of Antimicrobial Agents Under Dynamic Contact Conditions*. (ASTM International, 2013). doi:doi.org/10.1520/E2149
18. Halder, J., Weight, A. K. & Klivanov, A. M. Preparation, application and testing of permanent antibacterial and antiviral coatings. *Nat. Protoc.* **2**, 2412–2417 (2007).
19. Rio, L. *et al.* Comparison of methods for evaluation of the bactericidal activity of copper-sputtered surfaces against methicillin-resistant *Staphylococcus aureus*. *Appl. Environ. Microbiol.* **78**, 8176–82 (2012).

20. Green, J. D., Fulghum, T. & Nordhaus, M. A. Review of immobilized antimicrobial agents and methods for testing. *Biointerphases* **6**, CL2-43 (2011).
21. Campos, M. D., Zucchi, P. C., Phung, A., Leonard, S. N. & Hirsch, E. B. The Activity of Antimicrobial Surfaces Varies by Testing Protocol Utilized. *PLoS One* **11**, e0160728 (2016).
22. ASTM E2197 - 17: Standard Quantitative Disk Carrier Test Method for Determining the Bactericidal , Virucidal , Fungicidal , Mycobactericidal and Sporocidal Activities of Liquid. 1–13 (2017).
doi:10.1520/E2197-17E01.2
23. Tiller, J. C., Lee, S. B., Lewis, K. & Klivanov, A. M. Polymer surfaces derivatized with poly(vinyl-N-hexylpyridinium) kill airborne and waterborne bacteria. *Biotechnol. Bioeng.* **79**, 465–471 (2002).
24. Gao, J. *et al.* Surface Grafted Antimicrobial Polymer Networks with High Abrasion Resistance. *ACS Biomater. Sci. Eng.* **2**, 1169–1179 (2016).
25. Sempels, W., De Dier, R., Mizuno, H., Hofkens, J. & Vermant, J. Auto-production of biosurfactants reverses the coffee ring effect in a bacterial system. *Nat. Commun.* **4**, 1757 (2013).
26. AATCC 100: Test Method for Antibacterial Finishes on Textile Materials: Assessment of. (2004).
27. Smith, E. J., Davison, W. & Hamilton-Taylor, J. Methods for preparing synthetic freshwaters. *Water Res.* **36**, 1286–1296 (2002).

Acknowledgements

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Figures

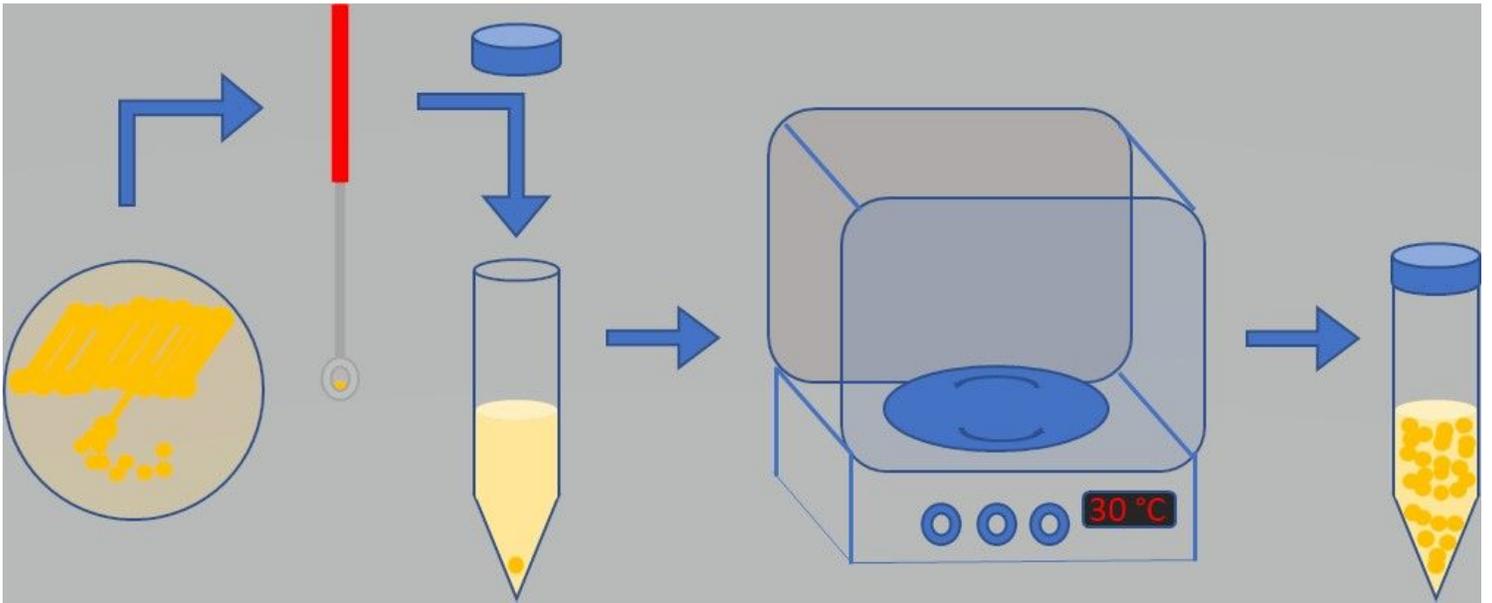


Figure 1

Day 1: Overnight Culturing of Desired Microorganism For Inoculating

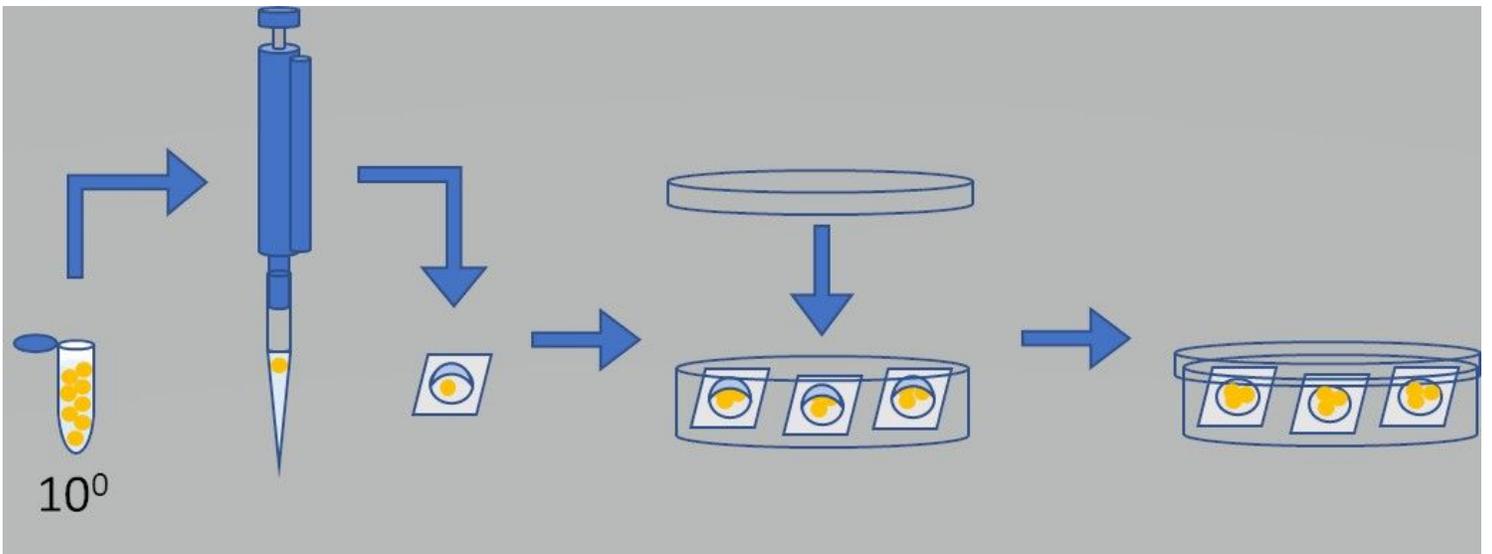


Figure 2

Day 2A: Inoculum Preparation Via Centrifugation of Overnight Culture

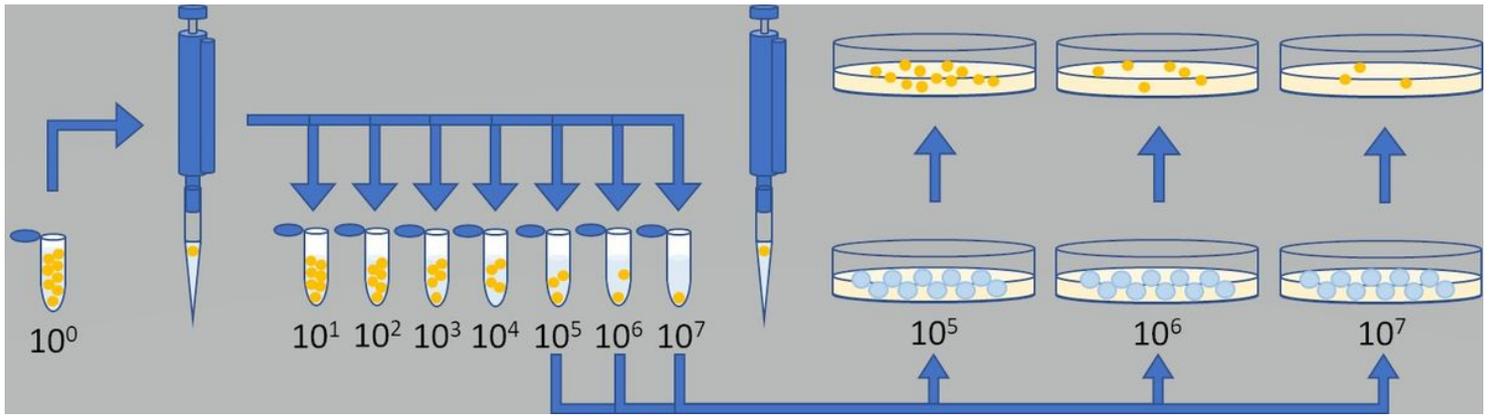


Figure 3

Day 2B: Dilution Series and Spot Plating of Inoculum for Determining Inoculum Load

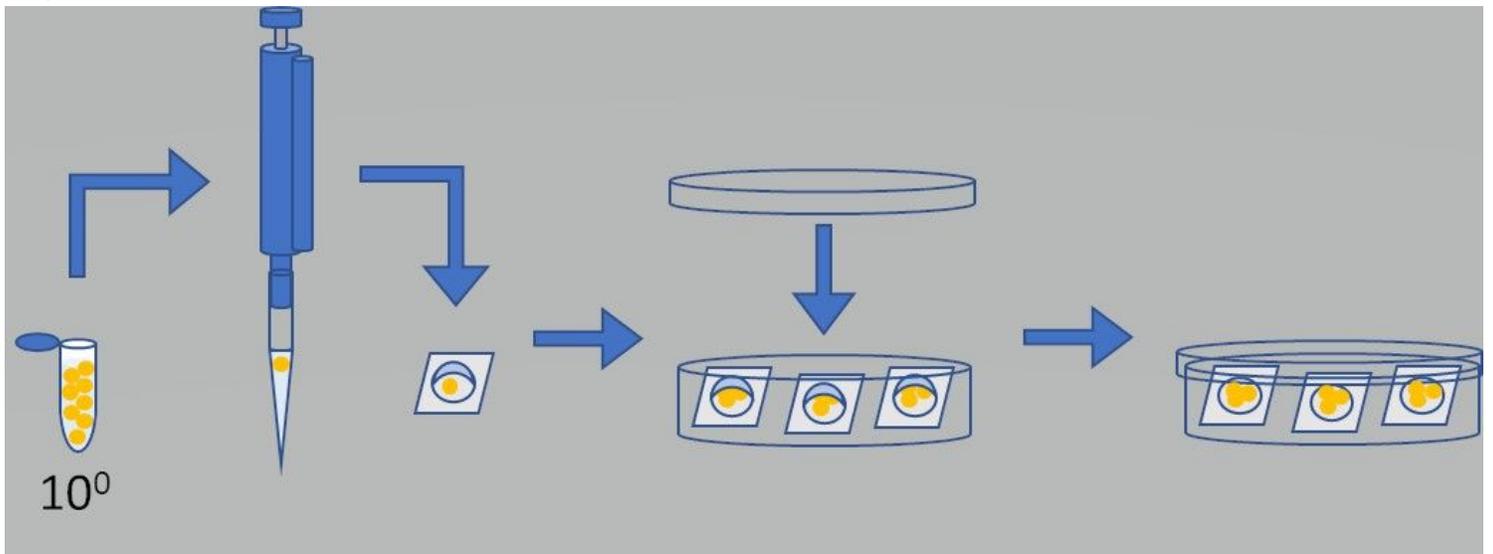


Figure 4

Day 2C: Inoculation of Desired Microorganism on to Sample Sets

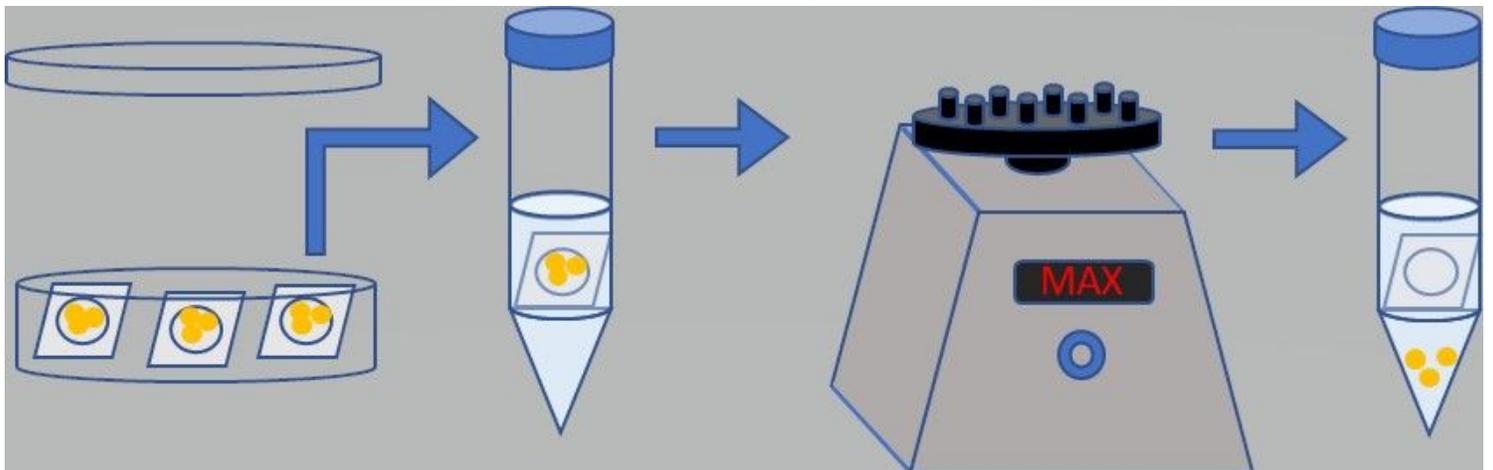


Figure 5

Day 3A: Sample Cell Collection via Vortexing Cells in Rinse Solutions

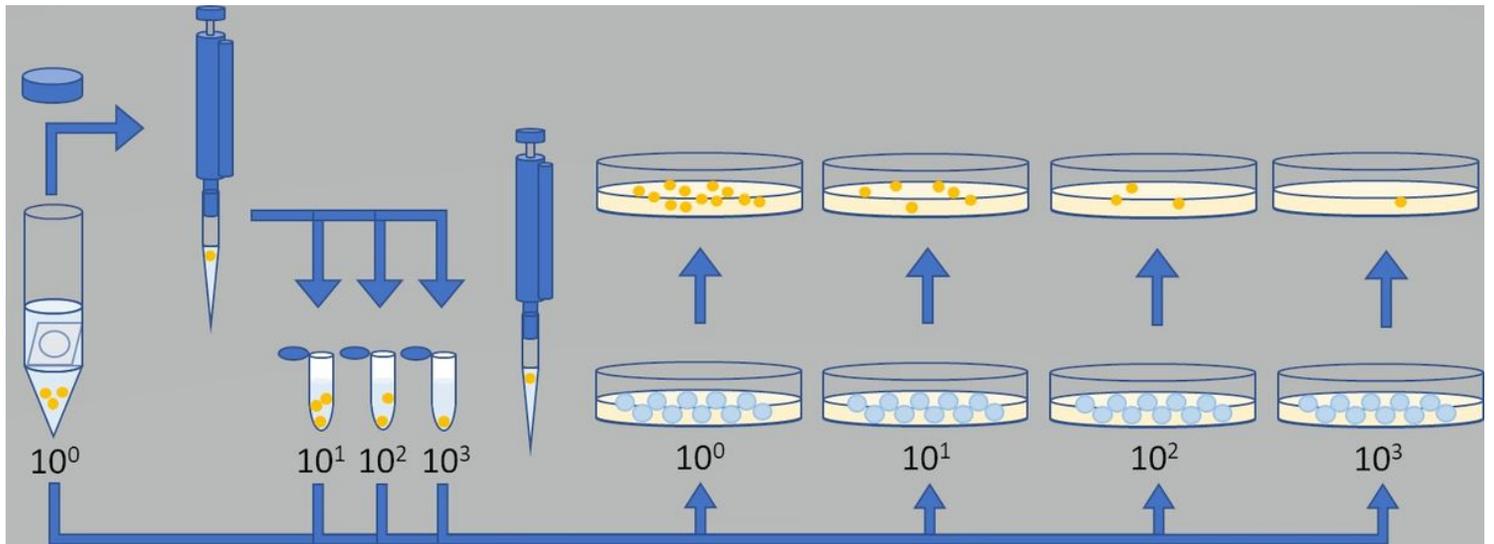


Figure 6

Day 3B: Dilution Series and Spot Plating of Collected Rinse Solution

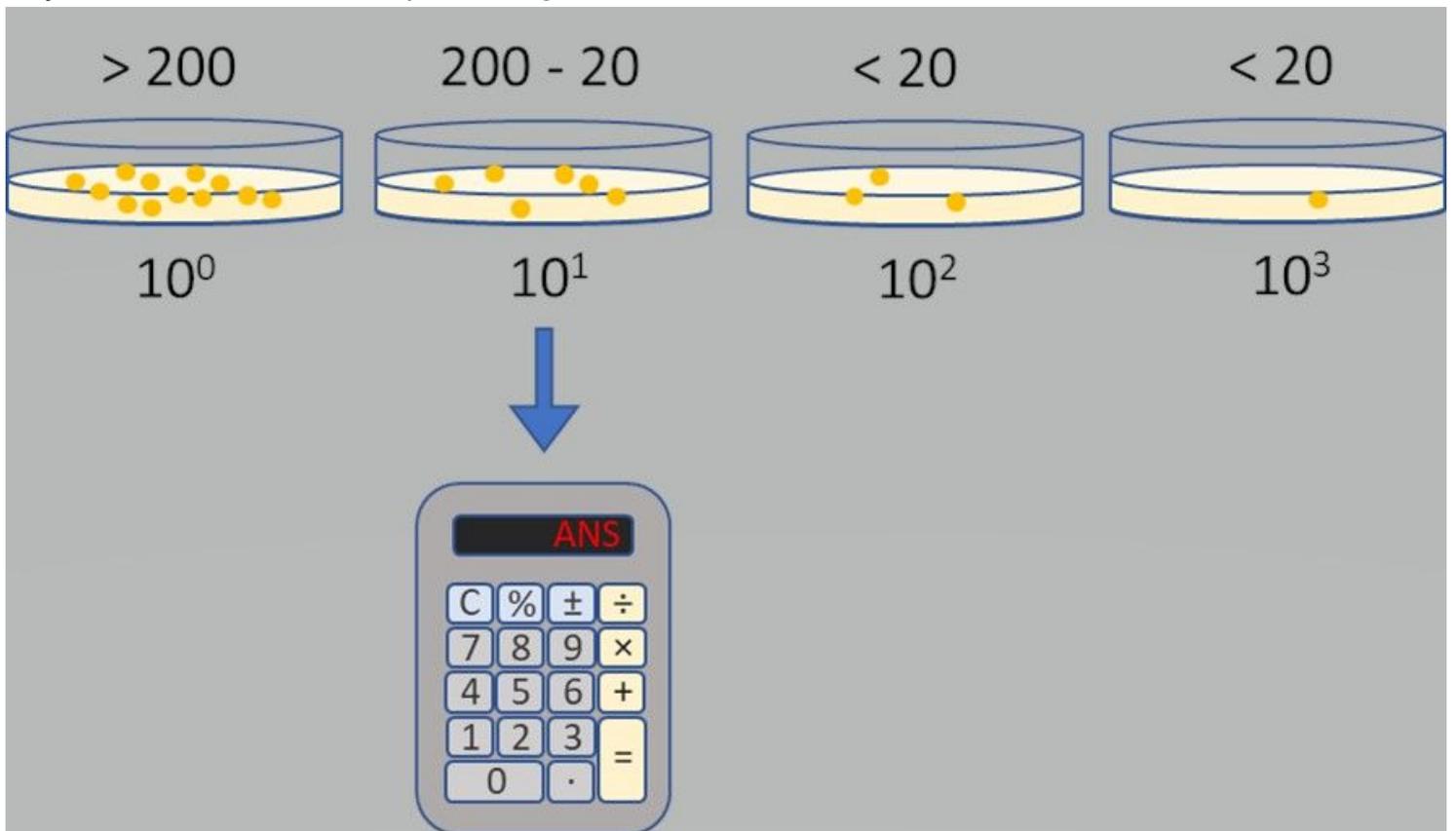
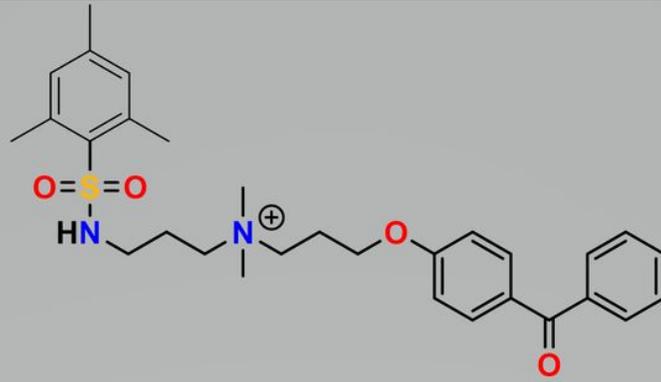


Figure 7

Day 4A: Data Enumeration and Results Processing of Spot Plating after Incubation



Bacteria	Conditions	Average survival log(CFU)		
		Inoculating load	Control sample	Treated sample
<i>G. soli</i> (IAI-3)	24 h, RT, on polystyrene	7.01	4.96 ± 0.08	< 1.70 ± 0.00

Figure 8

Day 4B: Sample Results from LDI Method

$$CFU_{sample} = \frac{CFU_{plate} * 0.1 \text{ mL} * DF}{5 \text{ mL}}$$

Figure 9

Day 4C: Equation for Determining Number of Cells on Samples after Spot Plating

Supplementary Files

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