Rapid Inactivation on Microscope Slides

A Platform for Improving Mass Spectrometry

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Abstract

Molecular imaging using mass spectrometry (MSI) enables identification of the changes in diseased versus neighboring cells within a tissue sample, including demonstrating delays in drug delivery to sites of need for effective treatment. Despite this, MSI for infectious disease research is hampered by the need to inactivate pathogens within tissue samples before analysis. Current inactivation techniques utilize a stepwise process of freezing excised tissues, inactivation via UV, X-ray, or gamma irradiation, then processing samples for MSI. This is a laborious and time-consuming process and is not efficient for studying dynamic cellular interactions during time of tissue harvest. **We hypothesize that alternate inactivation strategies can be conducted "on-slide" reducing the delay from tissue sample collection to MSI and improving the capture of dynamic interactions.** In this study, we used *Mycobacterium bovis* Bacillus Calmette-Guerin (BCG), an attenuated *Mycobacterium* that can be manipulated in standard laboratory conditions to test our hypothesis. We tested chemical and UV on-slide inactivated methods and found promising preliminary results using UV exposure on-slide. Our aim is to develop a protocol using BCG as our model organism, expand our protocols for use with virulent *M. tuberculosis*-infected tissue samples and eventually other high containment pathogens.

Background

- Mass Spectrometry Imaging (MSI) analyzes the location and type of molecules like proteins and lipids to understand biological processes[1].
- *Mycobacterium bovis* Bacillus Calmette-Guerin (BCG) is a part of the *Mycobacterium tuberculosis* complex[2].
- Inactivation requires maintaining cellular components while also ensuring the pathogenic ability of the organism is reduced[3].

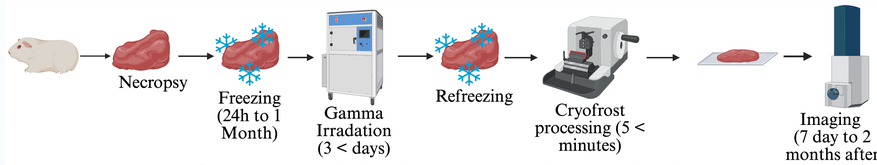

Figure1. Current method used to inactivate *Mycobacterium tuberculosis*

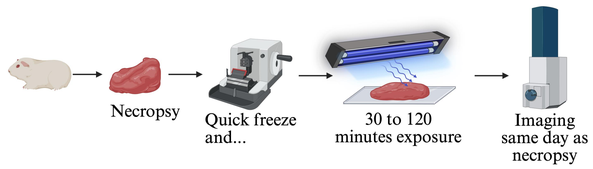

Figure2. Method we are trying to achieve. **analysis at the time of harvest*

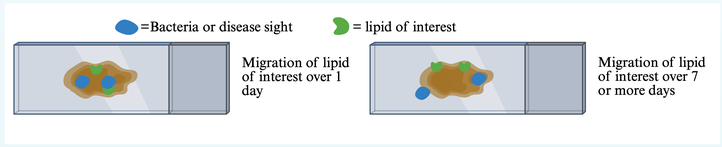

Figure3. De-localization of the lipid of interest over a period of time with current inactivation methods.

Methodology

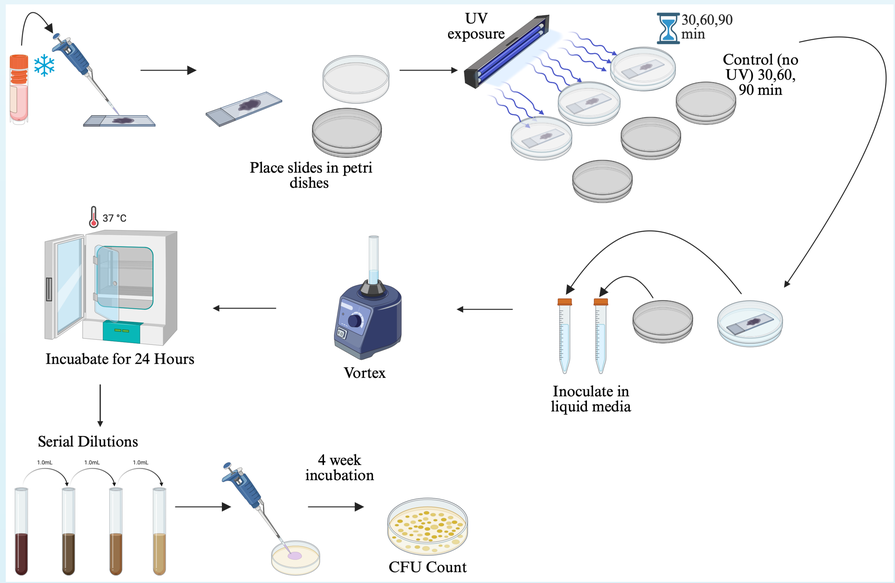

Figure4. Experimental design. Cryovial containing BCG was plated onto slides, placed in a petri dish, and then tested for inactivation at 30, 60, and 90 minutes UV exposure times. BCG on slides were transferred in liquid broth, and serial dilutions were made. Dilutions were plated for growth, and growth was counted as colony forming using (CFUs). The control group slides were placed in petri dishes covered in aluminum foil.

Results

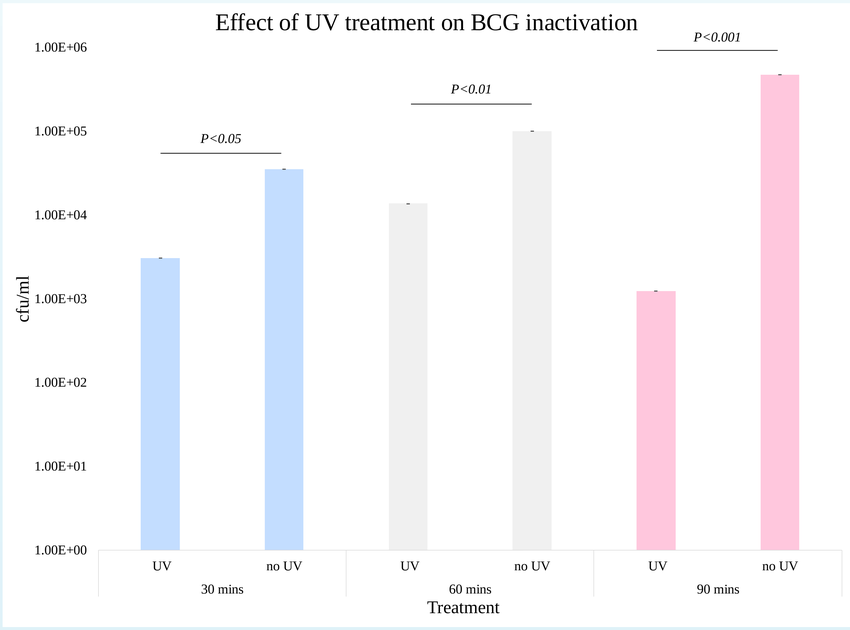

Figure5. Shows all three timepoints of UV treatment and No UV Treatment in comparison to each other with No UV exposure.

Conclusions and Future Directions

- A reduction in growth is observed after UV treatment.
- More than a 3-log fold reduction in viable BCG was observed with 90 minutes of UV exposure.
- We will test a combination of UV and hydrogen peroxide to achieve complete inactivation of BCG.
- Given the discrepancies in the 30- and 60-minute UV exposure, we would replicate the study to observe trends with an increase in UV exposure time.
- Identifying reduction in viable cells using UV treatment opens avenues for testing this protocol on other pathogens.

References

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