Determining TD50 of the Naja Naja Snake Venom Using Serial Dilution and Image Analysis with Error Report

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Introduction

Motivation: Snakes provoke a high number of human deaths due to envenoming characteristics. Venom lethality is expressed as median lethal dose (LD50) and toxic dose that kills 50% of the cells(TD50).

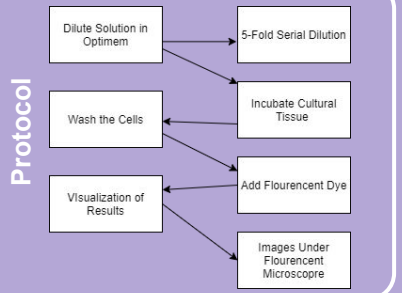
Purpose: Learn how to handle tissue culture, introduce fluorescent dyes to cells and record fluorescent images. Use the result to accurately evaluate the toxic activity of specific venom.

Research Question: Can we calculate the TD50 of the Naja Naja snake venom using fluorescence based imaging in mouse fibroblasts?

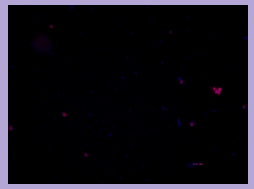
Materials

- Naja naja snake venom
- Hoechst dye
- Propidium Iodide
- Optimem
- Glass bottom 96 well plate
- 3T3 cells
- Fluorescence microscope
- 1.5mL tubes for venom dilutions

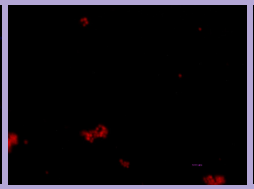
Protocol



Results



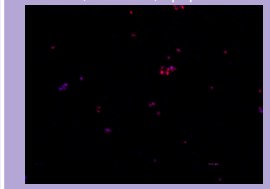
Well 1: 4% Snake Venom
Live: 11, dead: 10, apoptotic: 15



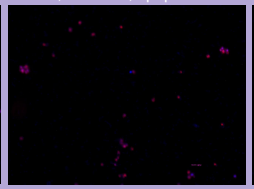
Well 2: 0.8% Snake Venom
Live: 0, dead: 30, apoptotic: 0

Well 3: 0.16% Snake Venom
Live: 0, dead: 1, apoptotic: 1

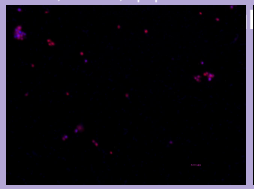
Well 4: Unable to open blue image



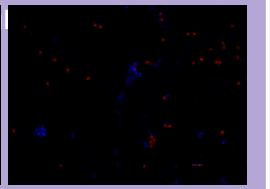
Well 5: 0.0064% Snake Venom
Live: 3, dead: 22, apoptotic: 24



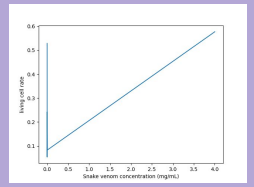
Well 6: 0.00128% Snake Venom
Live: 6, dead: 21, apoptotic: 33

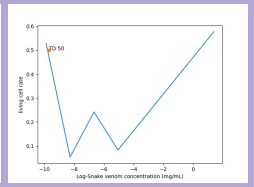


Well 7: 0.000256% Snake Venom
Live: 1, dead: 36, apoptotic: 24



Well 8: 0% Snake Venom
Live: 31, dead: 57, apoptotic: 0





Live Cell Rate vs. Snake Venom Concentration (with LD 50)

Demonstration

- Venom concentration design: 4e-2, 8e-3,16e-4,32e-5,64e-6,
- Aspirating liquid from wells might have removed some cells due to lack of experience
- The images were taken > 3 hrs after incubation with dye
- One image was found out to be broken (unreadable)

Error Report

1. Imaging errors:
   - Well 2: blue image did not seem to capture anything (light seems to be off)
   - Well 3: no cells in the vision field
   - Well 4: blue image faulty
2. Long time period between incubation and imaging probably led to death of more cells than expected
3. imperfect aspiration skill may cause more cells to be removed than expected. This leads to lower no. of samples and less convincing result
4. Difficulty in separating cells that stick very closely together in the image in counting

Analysis

- Picture 1 has similar numbers of live cells and dead cells when ignoring the apoptotic cells.
- Picture 5-8 show that most cells are killed by the venom. A lot of cells died by apoptosis.
- We plotted the living cell rate against snake venom concentration on both the standard scale and the logarithmic scale
- the log scale plot was used to determine the TD 50 of the snake venom
- The shape of the plot was not quite as expected - expect a monotonous plot
  - Probably due to long time of incubation - enough time for venom to kill cells regardless the concentration

Conclusion

We conclude that the TD 50 of the Naja Naja Snake Venom is approximately EXP(-9.7) = 6.13 * 10^{-5} mg/mL.

However, this result is only an approximation and could be faulty due to the errors appeared in the experimental process mentioned above.

If we were to do this experiment again in the future, we would make the following changes:

- We would again use a 5x dilution, but starting with a slightly lower initial concentration, since we found that the TD 50 was much closer to 0% snake venom than 4% snake venom.
- We would make sure that the images were analyzed immediately after the final incubation period.
- We would practice aspirating beforehand.

Reference

Xing, F., & Yang, L. (2016). Robust nucleus/cell detection and segmentation in digital pathology and microscopy images: A comprehensive review. IEEE Rev Biomed Eng. 2016; 9: 234–63.;
Lab 5: Determining Snake Venom Toxic Dose (TD50) in mouse fibroblasts, Available on Canvas
Parveen G, Khan MF, Ali H, Ibrahim T, Shah R (2017) Determination of Lethal Dose (LD50) of Venom of four Different Poisonous Snakes found in Pakistan. Biochem Mol Biol J. Vol.3 No.3:18. doi: 10.21767/2471-8084.100046