Purification of Fabs Under Financial Constraints

By Amanda Lams and Zoey Hua, Ph.D.

Introduction

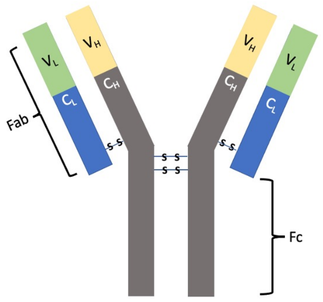


Figure 1: A structure of an antibody is shown. An antibody contains variable light and heavy (V_L, V_H) and constant light and heavy (C_L, C_H) chains on its Fabs. Each antibody contains two Fabs and one Fc, which are connected by disulfide bonds (S-S).

What is a Fab?

A Fab is a part of the antibody (see Figure 1), which helps the immune system recognize and appropriately respond to foreign particles such as viruses, allergens.

Why should we purify a Fab?

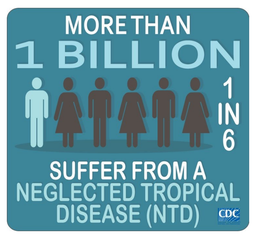
To conduct antibody research and accurately assess interactions between Fab and its binding partner, a pure sample is crucial.

What is the importance of antibody research?

Antibody research provides therapeutic treatment for diseases, such as sickle cell, HIV, and many more[1;4]. From the table shown on the right, antibody treatment has proven to be versatile in its ability to treat many different diseases[4]. While many diseases have been approved for antibody treatment, a large category of diseases, Neglected Tropical Diseases, has yet to undergo antibody research, due to a lack of funding[2].

List of FDA Approved Antibody Treatment for Diseases[4]

HIV infection	2018
Hairy cell leukemia	2018
Paroxysmal nocturnal hemoglobinuria	2018
Acquired thrombotic thrombocytopenic purpura	2019
Osteoporosis in postmenopausal women at increased risk of fracture	2019
Plaque psoriasis	2019
Diffuse large B-cell lymphoma	2019
Macular degeneration	2019
Sickle cell disease	2019



As a result, to provide treatments for diseases, such as Neglected Tropical Diseases, ample antibody research ought to be conducted, but due to the lack of funding, this has proven difficult[3]. Thus, the most likely option to conduct research to provide antibody therapeutics to individuals affected by NTD would be to lower cost of antibody research as a whole.

This research project aims to improve upon purification methodology in antibody research under financial constraints and with limited instrumentation by:

- Using chloroform as a nonmechanical, cost-efficient method to lyse bacterial cell walls
- Using simple affinity column chromatography to purify the Fab retrieved from aforementioned cell lysis method

References

1. Yan, L.; Ehrlich, P. J.; Gibson, R.; Pickett, C.; Beckman, R. A. How Can We Improve Antibody-Based Cancer Therapy? *mAbs* **2009**, *1* (1), 67– 70.
2. R&D funding for neglected diseases by disease. https://www.who.int/observatories/global-observatory-on-health-research-and-development/monitoring/r-d-funding-flows-for-neglected- diseases-by-disease-year-and-funding-category#data-sources (accessed Mar 10, 2022).
3. Global health - newsroom - neglected tropical diseases (ntds). https://www.cdc.gov/globalhealth/newsroom/topics/ntds/index.html (accessed Mar 10, 2022).
4. Lu, R.-M.; Hwang, Y.-C.; Liu, I.-J.; Lee, C.-C.; Tsai, H.-Z.; Li, H.-J.; Wu, H.-C. Development of Therapeutic Antibodies for the Treatment of Diseases. *Journal of Biomedical Science* **2020**, *27* (1).

Methodology

The purification methodology begins with a chemical treatment of bacterial pellet with chloroform to lyse cell wall.

The chemical lysing approach selected uses 5mL of chloroform as the cell lysing agent, due to its lower cost. Cell lysing refers to the separating of the lysate from the bacterial pellet. 10mL of PBS was then added to tube. The tube was centrifuged using Sorvall SH-3000. The resulting lysate layer was then extracted using a micropipette.

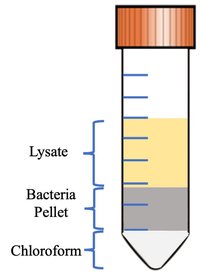


Figure 2: This figure displays the expected results from the nonmechanical cell lysing method used in this research .

Cell lysis was followed by affinity column chromatography, which utilized a commercial Protein L resin (Capto™ L by GE Healthcare Life Sciences). Affinity column chromatography enabled Fab purification through the strength of intermolecular interactions between the Fab and the resin of the column. The steps of affinity column chromatography are found below:

Step 1: Pour the lysate into the resin (twice) to ensure Fab is bound to resin and collect Flow Through fraction

Step 2: Pour 20mL of 1x high-salt phosphate buffered saline to separate nonspecific binding proteins from the resin and collect Wash fraction

Step 3: Pour 1mL of 10% acetic acid to remove 1x high salt phosphate buffered saline from column and collect Elute 1 fraction

Step 4: Pour 3mL of 10% acetic acid (twice) to break interaction between resin and Fab to collect purified Fab and collect Elute 2 and 3 fractions

Expected content of Eppendorf Tubes can be found in Figure 3. Gel electrophoresis was then conducted to confirm purification of Fab, and successfully purified Fabs were then concentrated through centrifugation and stored for later research use.

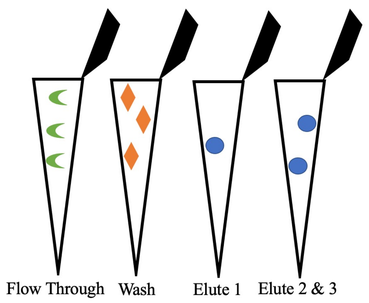


Figure 3: This figure shows the expected content of the Eppendorf tubes when conducting affinity column chromatography.

Acknowledgments

Special thank you to Dr. Zoey Hua for mentorship during this research project, and the Elmhurst University Department of Chemistry and Biochemistry for providing financial support, lab space, and reagents.

Results

Shown to the right is the successful separation of the cell using the chloroform cell lysing method

- Top layer contains lysate
- Middle layer contains bacterial pellet
- Bottom layer contains chloroform

The top layer, which contained the Fab, underwent the purification process using column chromatography

Figure 4: This displays the layers of the chemical cell destruction method utilizing chloroform, which can be seen in the bottom layer. Photo by Hatixhe Rojba and Melissa Ortega

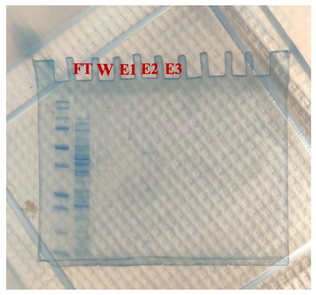


Figure 5: This gel displays results from the cell lysing and purification process from Trial 1.

Looking at results of Trial 1 in Figure 5, the lanes of the wash, elute 1, elute 2, and elute 3 are all empty. This causes a modification of protocol, due to the zero yield of a purified Fab, which can be found below

- Elongate the wash and elution processes by using 20mL of 1x high-salt phosphate buffer saline solution, rather than the original 10mL, and using 3mL of acetic acid per elute trial, rather than the original 2mL.

In Figure 6, the Fab yield improved visibly in the gel. However, the yield was not satisfactory. The modifications to protocol were made below to increase yield:

- Elongate the cell lysing process by incubating bacterial pellet with 5-10 mL of chloroform for 30 minutes
- Lyse a bacterial pellet that contains ample Fab initially

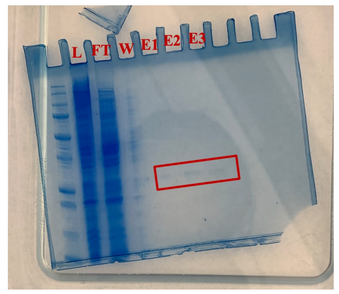


Figure 6: This gel displays results from the cell lysing and purification process from Trial 2.

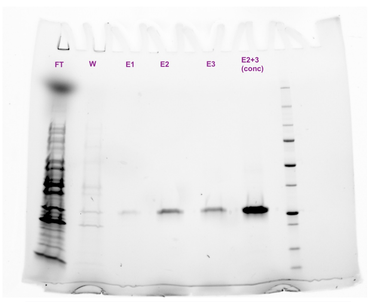


Figure 7: This gel displays results from the cell lysing and purification process from Trial 3.

All three modifications listed were followed in Trial 3, and the gel electrophoresis results from Trial 3 is shown in Figure 7, which is the final trial. Elute 1 was not concentrated due to the expected contamination of 1x high salt phosphate buffered saline solution.

Conclusion and Future Directions

From the results above, it was determined that the cell lysing method required roughly 5-10mL of chloroform to be added to sufficiently lyse the cell. In addition, the affinity column chromatography method, using Protein L column, improved in its Fab yield through the three modifications: using additional chloroform, using additional buffer, and a Fab-rich bacterial pellet, as confirmed by the gel in Figure 7. Thus, chloroform was determined visually to work as a sufficient, cost-efficient cell lysing agent, and the goal of lowering the cost of antibody research through successful lysing and purification was achieved in this project.

As a follow-up to the successes of this project, the following research topics below are suggested:

- Improve the 1x high-salt phosphate buffered saline removal from the column to avoid waste of Fab and increase Fab yield
- Prepare a quantitative efficiency determination for the chemical cell lysing technique using chloroform in comparison to a mechanical method.