Lectin Properties of Synthetically produced Glucoronate, Alginate, and Related Boronates

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Research Question

- Do synthetically produced compounds have lectin properties through agglutination with different red blood cell types?

Goals

- Test synthetically produced compounds with A positive, B positive, and O positive cells
- Determine lectin characteristics of compounds through visible agglutination
- Lectin applications in the medical field:
  - Blood grouping
  - Mitogenic activity
  - Stem Cell Transplantation

Background on Lectins

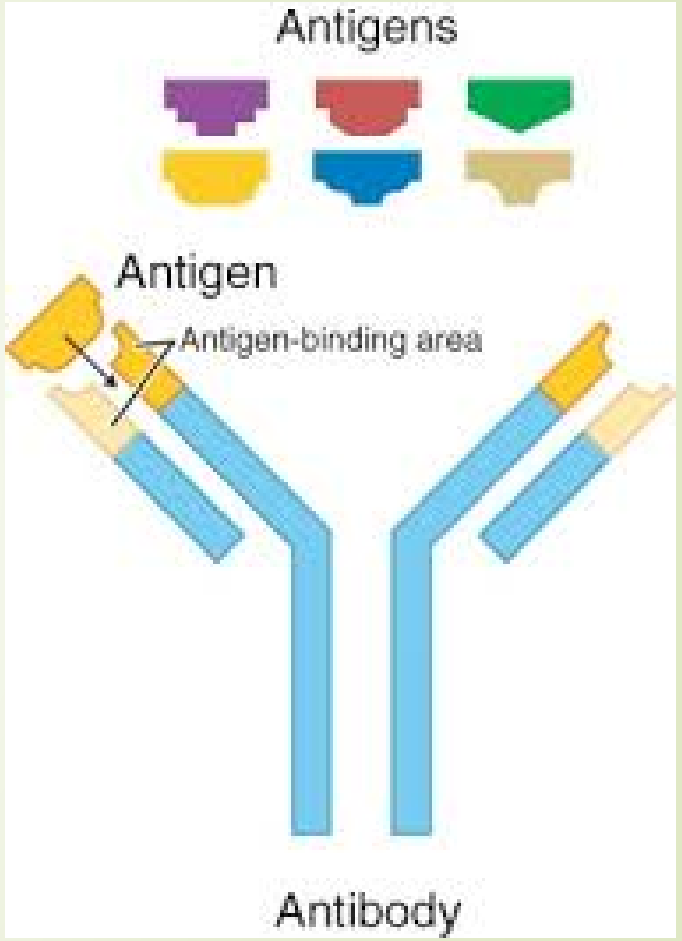
- Derived from the Latin word “legere,” meaning to choose or to “select”
- Lectin sources:
  - Seeds of leguminous plants
  - Fruiting bodies of fungi
  - animals
- 19th Century researchers discovered the ability of some proteins to agglutinate red blood cells
- Originally named, “phytohemagglutins” or “ hemagglutinins”
- Later, particular hemagglutinins were found to agglutinate red blood cells of a particular human blood group in the ABO blood group system

Major historical Lectin Landmarks in history

- Peter Stillmark (1888)
  - Isolated toxic extracts from seeds of castor tree (*Ricinus communis*)
  - Major Discovery: Hemagglutinating proteins agglutinated erythrocytes (Red blood cells) and named them "ricin"
- William Boyd and Karl Renkonen (1940)
  - Major discovery: Extracts of lima bean, *Phaseolus limensis* agglutinated blood type A, but not type B or type O
- W. G. Bird (1959)
  - Major discovery: Precipitants from *Dolichos biflorus* seeds reacted with part of the A-substance of human red blood cells, specifically an A-substance component from individuals of sub-groups A1 and A2

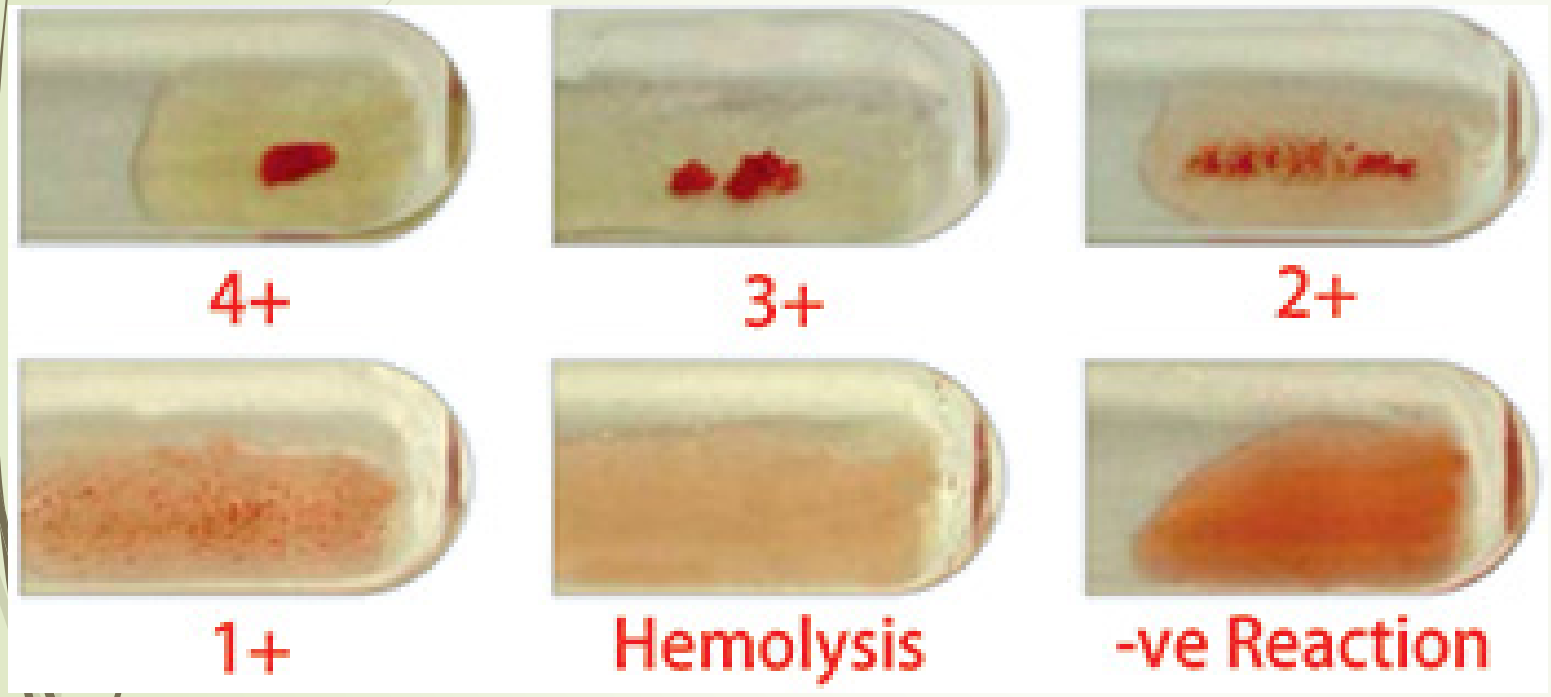
Intro to agglutination method

- Agglutination of human red blood cells will be tested for lectin activity
- Definition of agglutination: standard serological method in the clinical laboratory to detect antibody-antigen interactions through visible clumping and is graded on a scale
- Antibody: Produced in response of a foreign substance entering the body (Defense)
  - Comparison: Superhero
- Antigen: Foreign substance that induced the immune substance to produce antibodies
  - Comparison: Villain



https://www.ck12.org/book/ck-12-biology-concepts/section/13.48/

Agglutination reactions



https://www.remilabworld.com/serology-centrifuge/

https://www.indiamart.com/proddetail/test-tube-agglutination-viewer-21718451048.html

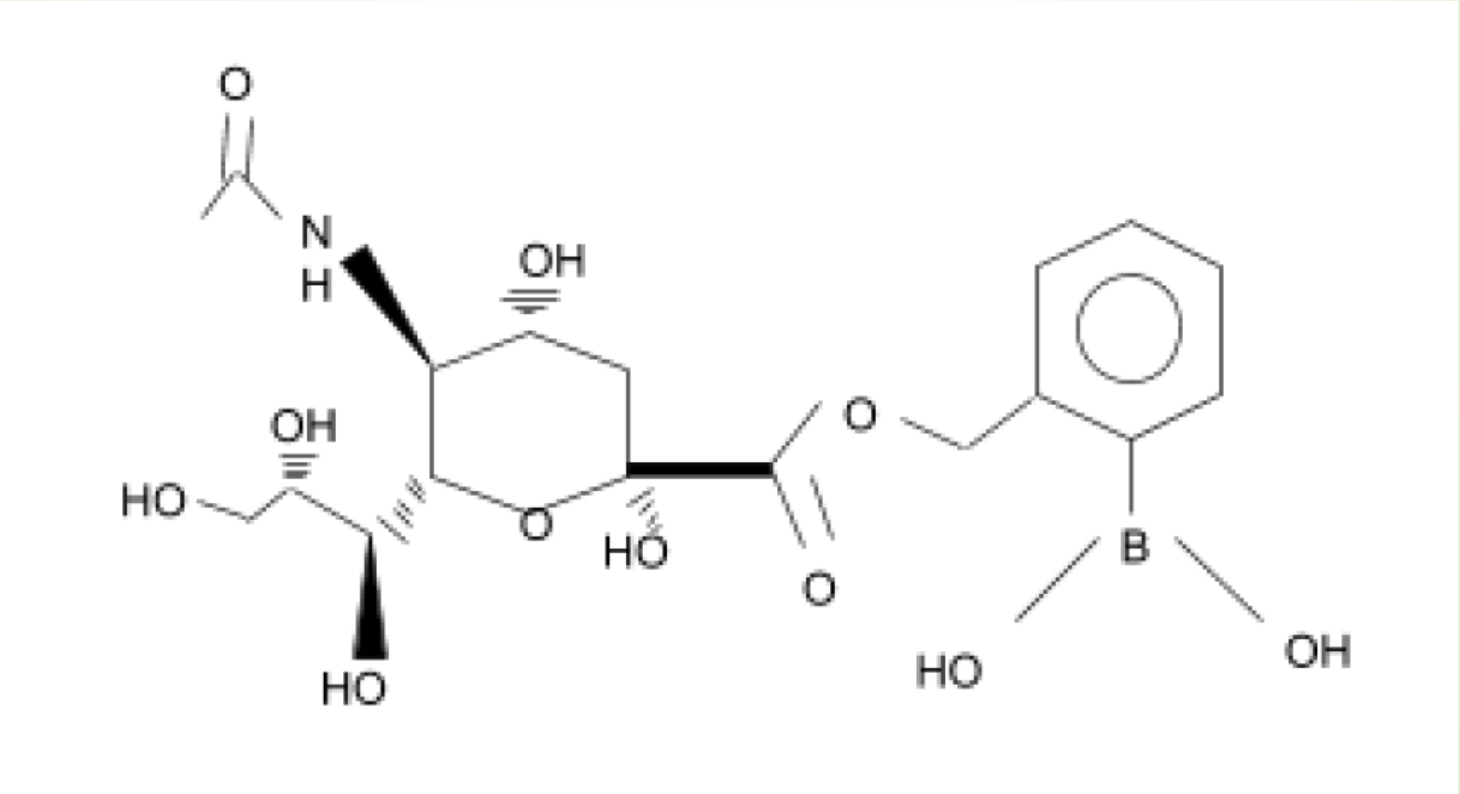
Method

- Prepare stock solution
  - 0.02 grams of compound with 0.5 grams of DMSO and 0.5 ml of normal saline (0.9 NaCl)
- Test Compounds with red blood with known ABO Rh type in 12 x 75 mm tube along with control with each run
  - Control: 0.5 ml of DMSO and 0.5 ml of normal saline
- Add one drop of stock solution with one drop of red blood cells
  - A positive
  - B positive
  - O positive

- Five compounds, two tested
  - 4-bromomethyl phenyl boronic acid and glucuronic acid (compound 1)
  - 2-bromomethyl phenyl boronic acid and acetylneuraminic acid (compound 2)

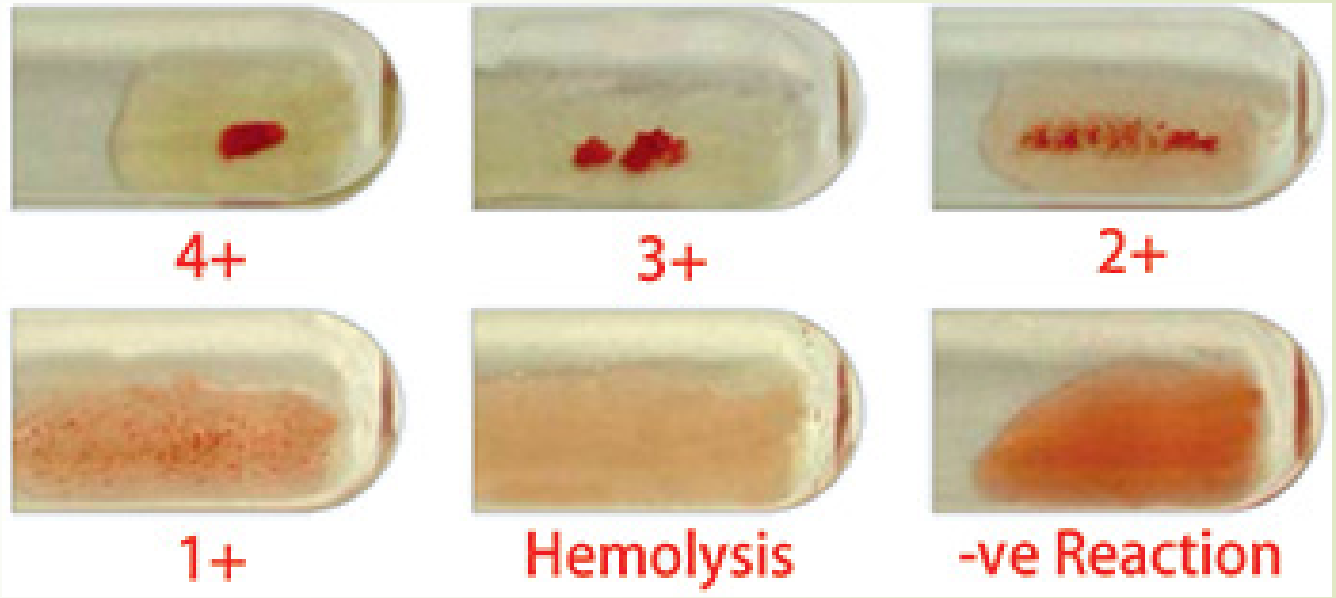
Typical Day in the Lab. . .

Structure for compound 2

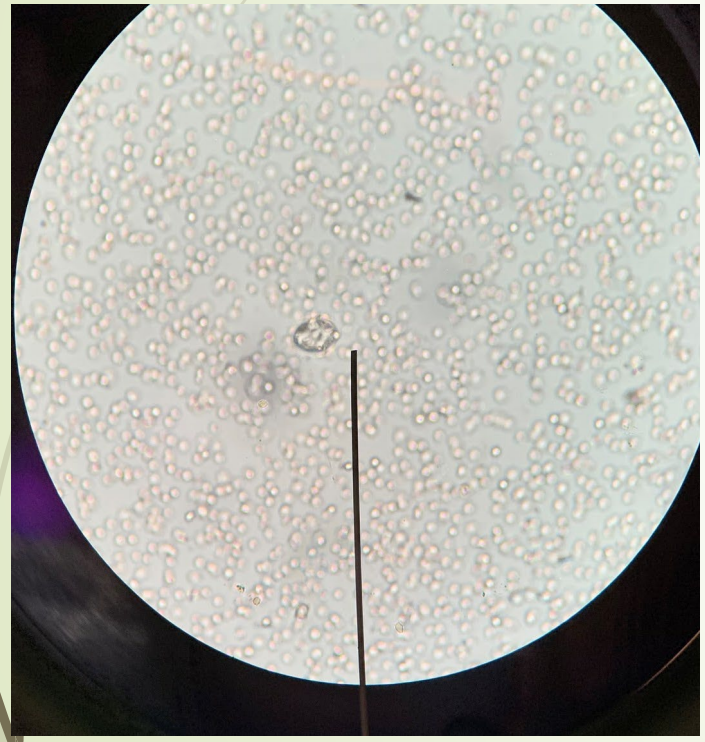


2-bromomethyl phenyl boronic acid and acetylneuraminic acid

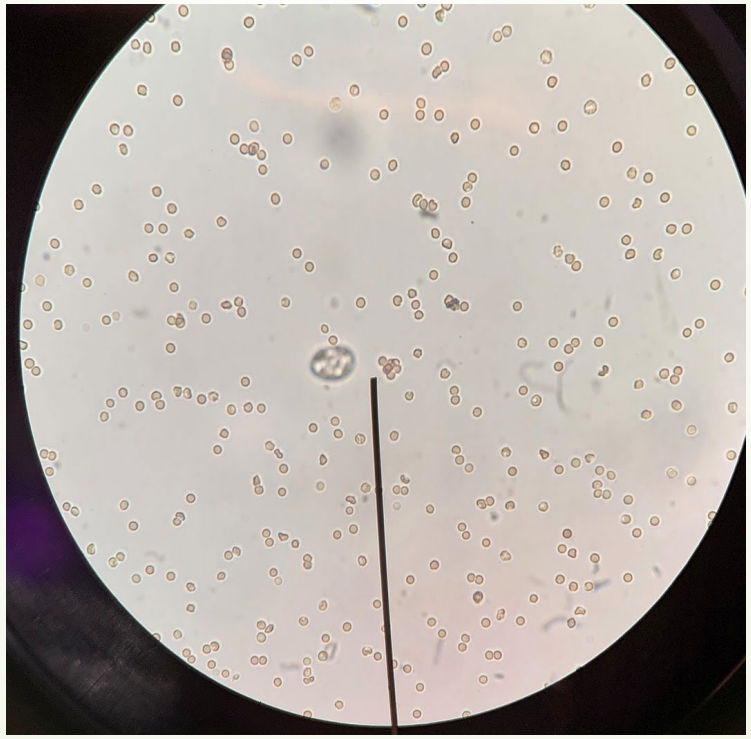
Results with the tube method



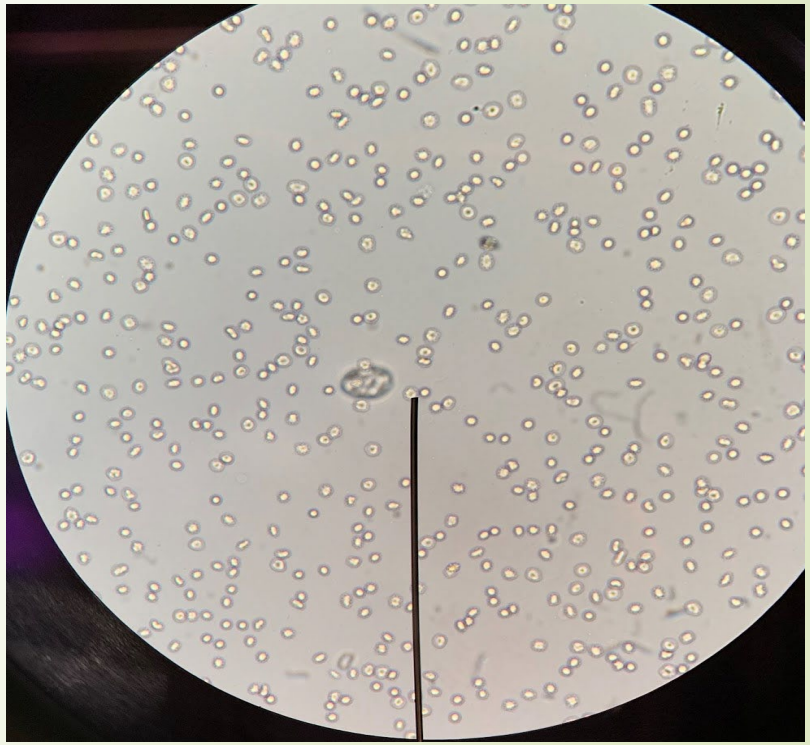
Results under the microscope



A Positive (Negative)



B Positive: Slight agglutination



O Positive: (Negative)

Conclusion, further Research, and Limitations

- 2-bromomethyl phenyl boronic acid and acetylneuraminic acid showed some slight agglutination with B positive cells
- These results suggest some selective hemagglutination or lectin activity for compound 2
- Further Research:
  - Test compound 2 lectin activity with Rh negative phenotypes
  - Explore different solvents since solubility was a problem
- Limitations
  - Solubility
  - Quality Control with DMSO

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Questions?