Polyagglutination: Lectin Isolation for T-Activated Red Cells

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Abstract

Polyagglutination is a rare condition that can occur during infections with certain microorganisms that produce enzymes capable of modifying carbohydrates on the erythrocyte membrane. These enzymes can expose cryptantigens that react with naturally stimulated antibodies found in nearly all adult sera. Cryptantigens can be detected in-vitro by the use of lectins. Lectins are proteins that bind specifically to certain carbohydrates, causing agglutination of targeted cells. United States manufacturers have discontinued the production of lectin kits; companies make no profit due to the rarity of this condition. This leaves reference laboratories without a commercially available way to test for polyagglutination, leading them to either adopt unapproved storage methods for expired lectin kits or create reagents in-house. This study produced a lectin kit that can be made in-house and used for accurate testing of T-activated polyagglutination for months if stored in proper conditions. To isolate the lectin, supernatants of ground *Arachis hypogaea* seeds suspended in phosphate buffered saline were collected by centrifugation. T-activated cells were produced by combining washed Group O erythrocytes with a neuraminidase enzyme via a *Streptococcus pneumoniae* filtrate. Traditional tube hemagglutination was used to test the lectin's potency with the T-activated polyagglutinable erythrocytes. We conjecture that our *A. hypogaea* lectin reagent and quality control red cells can be stored for up to three months while retaining the potency to positively identify T-activated cells. In addition, we disseminated a survey to assess the current status of lectin testing procedures in immunohematology reference labs.

Introduction

Various seeds have been identified to contain lectins with specificity to modified RBC antigens. Extracted lectin can be used to identify cases of polyagglutination and can be used in a panel to identify what antigen modification is causing the polyagglutination. RBC antigens can be modified *in vitro* using enzymes harvested from bacteria to create different types of cryptantigen activated cells. This study aims to determine how long this in-lab created kit will last at refrigerated temperatures and be used to positively identify cases of polyagglutination since reagent kits are not longer available for purchase.

Extracting Lectin

Seeds were placed in PBS (7.5mL for every 1.00g of seed) and soaked for 24 hours. Seeds were ground using a mortar and pestle, using the 7.5mL of PBS to create a paste. The liquid was separated by centrifuging the paste, supernatant was removed, and sodium azide was added in a 1:1 ratio to prevent microbial growth during storage.

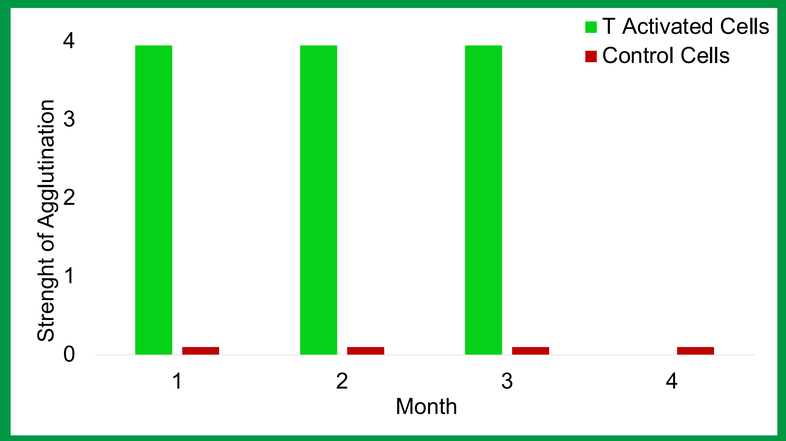
T-Activation Using Neuraminidase From *S. pneumoniae*

S. pneumoniae was cultured in a TSB tube for 24 hours. The tube was centrifuged, and supernatant was filtered though a 0.45-micron filter to separate the neuraminidase enzyme. The enzyme was added to group O RBC's in a 1:1 ratio and incubated at 37°C for 24 hours. Once T-activation was confirmed though testing, Alsever's solution was added to support longevity of RBC life span during refrigeration.

Lectin Panel	T	Th	Tk	Tx	Tn
Arachis hypogaea	+	+	+	+	-
Glycine max (soja)	+	-	-	-	+
Vicia cretica	+	+	-	-	-
Medicago disciformis	+	+	-	-	-
Salvia sclarea	-	-	-	-	+
Salvia horminum	-	-	-	-	+
Griffonia (Bandeiraea) simplicifolia	-	-	+	-	-
Vica hyrcanica	+	+	+		-

Figure 1. Known agglutination results of lectins found is specific seeds and their corresponding cryptantigen. Panel can be used to identify type of polyagglutination present in patient (Irish Blood Transfusion Service).

Results



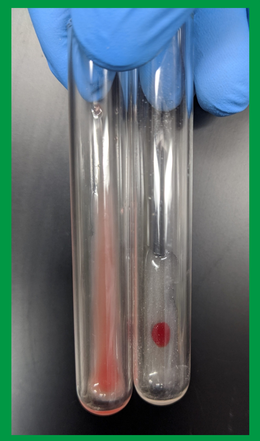


Figure 2. Agglutination results of lectin and T-activated cells. After removal from the fridge, cells were washed 3 times to remove hemolysis. Packed cells were made into a 3-5% cell suspension. One drop of the suspension was mixed with two drops of lectin. Tube was gently mixed and centrifuged for 15 seconds. Tube was then gently resuspended, and strength of agglutination was graded.

Methods

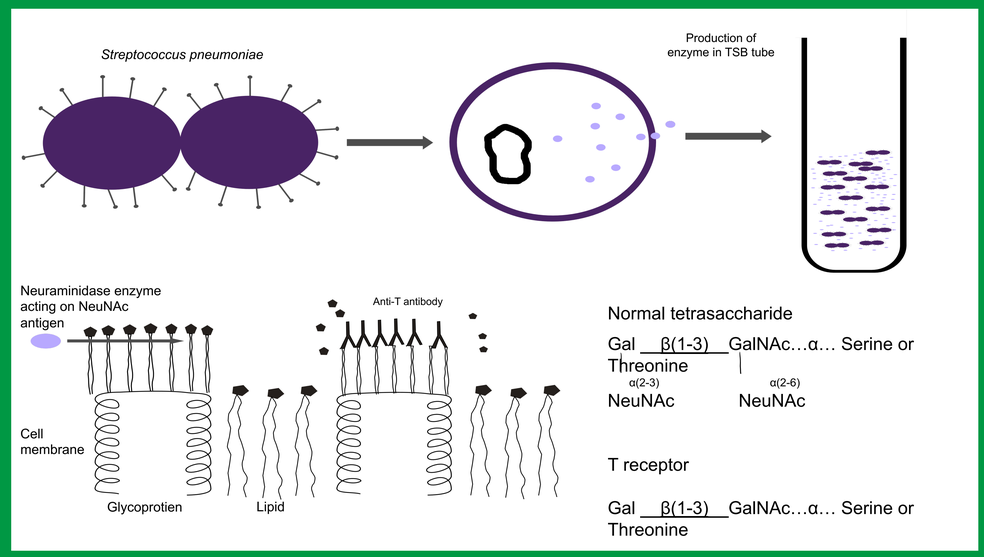


Figure 3. *S. Pneumoniae* grown in a TSB tube, producing neuraminidase enzyme. Enzyme is shown cleaving the N-acetyl neuraminic acid residues on MN antigens on portions of glycophorin A, making the antigen appear foreign and named the "T antigen". Antibodies are shown attaching to the modified antigen, which will cause agglutination of RBC's in proximity. Normal antigen is shown with linkage to the N-acetyl neuraminic acids compared to an antigen with N-acetyl neuraminic acids removed.

Discussion

- T-activation of RBC's *in vitro* was readily achieved with reproducible results.
- Preparation of lectin to detect T-activation readily producible.
- Kit was found to be stable for at least 3 months and still able to give a 4+ agglutination reaction.
- RBC's stored in Alsever's solution found to be stable for longer than 3 months, and lectin found to outlast treated RBC's, showing that the lectin would not need to be remade as frequently as control positive cells.

Formation of Lattice

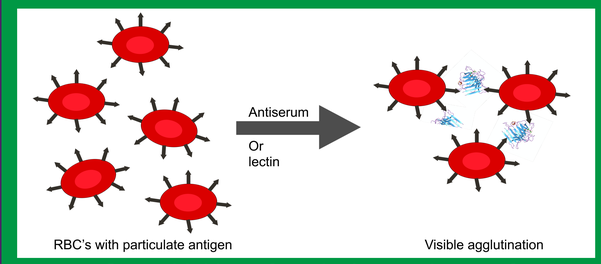


Figure 4. Cross-Linking of particulate antigen to form larger, also visible, complexes.

Limitations

- Tk antigen expression failed when attempted activation with enzyme filtered from *B. fragilis* as well as treatment with a powdered form of beta-Galactosidase
- T-activation required a 24-hour incubation when Judd's method only calls for a 1-hour incubation
- T-activated cells shelf life was only 3 months, testing of month 4 not possible due to hemolysis of RBC's.

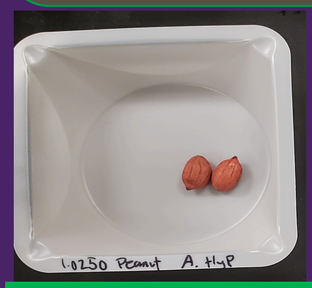


Figure 5. *Arachis hypogaea* (peanut) ground using a mortar and pestle to extract lectin.

Acknowledgements

Special thanks to the Office of Undergraduate Research and the Kem and Carolyn Gardner Foundation for funding our research, Dr. Harley Department Chair of the Botany department for being an outstanding resource in the knowledge of lectins, Dr Walker for his knowledge of enzyme function in biological systems, Nicholas Quesnell, Alyson Stanger for bacteria growth help and to the Medical Laboratory Science department for use of facilities.

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