

INTRODUCTION

- Lectins are carbohydrate-binding proteins or glycoproteins, mostly obtained from seeds of selected species of Leguminosae plants⁽¹⁾.
- Carbohydrates are universally present on the outer surface of the cell membranes⁽²⁾.
- Lectins can be used to detect specific antigens on cells.
- Lectins are non-immune in origin.
- They have special properties of binding to specific structures and ability to cause agglutination of RBCs.
- Lectins plays a pivotal role in blood typing- especially to detect A sub groups and H antigen⁽³⁾.
- Anti-A¹ lectin used to identify A¹ subgroup and are important for solving grouping discrepancies.
- Blood centres rely on commercially available lectins for this purpose.
- In-house preparation of lectins can act as an alternative to obtain the Anti-A¹ lectin, which offers advantages like reduced costs and greater flexibility in titration of lectins⁽⁴⁾.
- Anti-A¹ lectin are prepared from seeds of *Dolichos biflorus*⁽³⁾
 - herbs/shrubs
 - leaves have single blades or compound (divided into three leaflets)
 - flowers—purple or white
 - fruit -flattened legume pod with upto 10 seeds
 - Seed – reddish brown



OBJECTIVES

- Comparison between In-house prepared lectin with commercial available Anti-A1 lectins.
- Comparison will be in terms cost-effectiveness, reliability, specificity, and ease of implementation.

MATERIALS

- Seeds – *Dolichos biflorus* - obtained from the local stores
- Normal Saline
- Glass beakers
- Mortar & Pestle
- Test tubes
- Eppendorf tubes

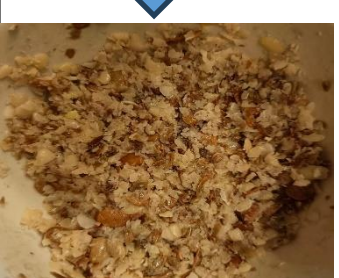
METHODS



Dolichos biflorus seeds - obtained locally



Seeds are soaked in Saline for 12 hours



Seeds crushed with Mortar and pestle till coarse particles were obtained



Grounded seeds were next soaked in saline (saline thrice volume of seeds) overnight at room temperature, with occasional stirring



Supernatant was transferred to test tubes and centrifuged to get clear supernatant



Clear supernatant was filtered, following which it was adjusted for reactivity against titres of A¹ cell at 32 and A¹B at 16 respectively

After the titres were confirmed the Anti A¹ lectins were transferred to Eppendorf tubes and stored

RESULTS

- The in house prepared lectin was assessed for appearance, specificity, avidity, reactivity and potency as per guidelines⁽²⁾, prior to applying for clinical use.
- Comparison of the prepared lectin with the commercially prepared lectin was in terms of the quality requirements of Anti A¹ lectin as per guidelines, and in terms of the cost and ease of preparation.
- The titres were adjusted to be reactive to A¹ cells at titres 32 and A¹B cells at titres 16.
- The in house prepared lectin were found to have comparable quality parameters with the commercially available Anti-A¹ lectins table 1.
- They were transferred to Eppendorf tubes and stored at 2⁰ to 8⁰ Celsius.
- The prepared lectins were stored for upto seven days maintaining the reactivity and potency.
- When comparing costs, the store-bought *Dolichos biflorus* seeds were priced at approximately 55 rupees for 500 grams.
- For the preparation of the lectin, 100 grams of these seeds were used, along with 500ml of normal saline costing 600 rupees.
- This meant that for 250 ml of the in house prepared lectins the cost had come to 611 rupees (about 12.25Rs for 5ml).
- The cost of commercially available Anti-A¹ lectin was 400 Rupees for 5 ml, with which we can do the test for about 20 samples.

Table 1: Comparison of quality parameters⁽⁵⁾ of In house prepared lectin & Commercial lectin

Parameter	Required Quality	Commercial Anti A1 Lectin	In House Prepared Anti A1 Lectin
Appearance	No turbidity, precipitation, particles or gel formation	+	+
Specificity	Clear reaction with A ¹ & A ¹ B cells. No reaction with A ² , A ² B, B, O cells (and other subgroups of A antigen)	+	+
Avidity	Visible agglutination with 40-50% A ¹ red cells suspension - slide test in 5-20 seconds	+	+
Reactivity	No immune haemolysis or prozone phenomenon, and rouleaux formation	+	+
Potency	Titre	A ¹ cells: 1:32 A ¹ B cells: 1:16	A ¹ cells: 1:32 A ¹ B cells: 1:16

CONCLUSION

- The In-house prepared Anti-A¹ lectin was found to be as effective as commercially available lectin, with substantial lower cost to prepare and store.
- It can be readily prepared and use as per the requirements of the immunohematology laboratory.
- It has an advantage of being cost effective, reliable and with relative easy mode of preparation.
- This can be adopted and used in resource constrained immunohematology laboratories to resolve blood grouping discrepancies.
- The disadvantage observed was the limited time of storage and the time taken to prepare the lectin (at least 24 hours).
- Lectins have tremendous potential in the field of transfusion medicine and stem cell research.
- Lectins play an important role in the diagnosis and differentiation of polyagglutination and immunohematological management of the patient⁽⁶⁾.
- In this era of molecular biology and biotechnology, it is possible obtain available lectins for various biological procedures in immunohematology and stem cell biology⁽⁶⁾.

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