# An Interdisciplinary Enrichment Laboratory on Lectin Production

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## ABSTRACT

In order to provide students with an appreciation for how lectin reagents have historically been obtained, the medical laboratory science (MLS) immunohematology instructor arranged with a botany instructor to provide an enrichment laboratory. MLS students observed lectin extraction and processing of extract from *Pisum sativum* seeds to purify *Pisum* lectin by affinity chromatography. Students were provided the opportunity to use a NanoDrop Lite Spectrophotometer to monitor the presence of the lectin during chromatography. This activity provided students the opportunity to see how various analytical methods are used outside the MLSs. The results of an anonymous postactivity survey indicated the respondents would recommend the activity for future students and were interested in seeing applications for analytic methods outside the MLS profession. The quiz results indicated good retention of the material learned approximately 6 months after the activity was conducted.

**ABBREVIATIONS:** MLS - medical laboratory science, PBS - phosphate buffered saline, PSA - *Pisum sativum* agglutinin, Tris - Tris(hydroxymethyl)aminomethane.

**INDEX TERMS:** hemagglutination, blood banking, lectins, antigens.

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## INTRODUCTION

Lectins are proteins that bind to carbohydrates and can be isolated from diverse plant and animal sources.[1–4] Seed lectins can be bivalent or tetravalent, allowing a single lectin to bind to carbohydrates on the surfaces of several different cells, causing agglutination. If the target carbohydrate is on a red blood cell (RBC) membrane, the lectin mixed with the RBCs will demonstrate hemagglutination.[5–7]

In 1888, Stillmark obtained the first lectin to demonstrate hemagglutinating properties from the seeds of the castor oil plant *Ricinus cummunis*.[1] Plant-derived lectins, or phytohemagglutinins,[8–9] have been used to detect various RBC antigens in the immunohematology laboratory for many years. For example, *Dolichos biflorus* is a commonly used lectin to differentiate $A_1$ from non-$A_1$ RBCs, and *Ulex europaeus* lectin has specificity for the H antigen and can differentiate group O RBCs from the rare Bombay phenotype.[5–7] A panel of lectins can be used to presumptively identify polyagglutinable RBCs, and often includes lectins from seeds of *Glycine max* (soy), *Arachis hypogaea* (peanut), *D biflorus* (horse gram), *Salvia sclarea* (clary sage), *Salvia horminum* (blue clary sage), and *Griffonia* (*Bandeiraea*) *simplicifolia*, an African woody shrub.[7]

Students enrolled in our medical laboratory science (MLS) program's Principles of Immunohematology course are provided *U europaeus* and *D biflorus* seeds to grind and prepare their own lectins for use in the student immunohematology laboratory. In conjunction with this laboratory activity, the MLS students attended an enrichment laboratory in the university's botany department to learn how lectins are isolated and purified. The demonstration illustrated how lectins are purified from *Pisum sativum* (pea) seeds by affinity chromatography. Pisum lectin *P sativum* agglutinin (PSA) binds to glucose residues. Sephadex, a beaded glucose polymer used for separating proteins by size, can be used as an affinity matrix for glucose-binding lectins like those from *P sativum* and *Lens culinaris* (lentil).

For this activity, a crude extract of supermarket split peas was passed through a Sephadex column. Following washing of the column to remove nonbinding proteins, the column was connected to a fraction collector, and PSA was eluted with a glucose solution. Students took turns reading the $A_{280}$ of the fractions to detect protein and thus find the fractions containing the eluted lectin.

## MATERIALS AND METHODS

To prepare the crude extract, 10 g of dry pea seeds (*P sativum*) were imbibed by soaking in water at 4 °C overnight. The imbibed seeds were homogenized in 50 mL of 50 mM phosphate buffer, pH 7.2, with a blender for 30–60 seconds until the puree was smooth. The puree was centrifuged at 10 000 × *g* for 10 minutes. After centrifugation, the still-cloudy supernatant solution was decanted through 2–4 layers of Kimwipes into a beaker with a stirring bar. The pH of the solution was lowered to 5 by slowly adding

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2 M citric acid dropwise while the supernatant was being stirred on a stir plate, monitoring the acidity with a pH meter. The solution was again centrifuged at 10 000 × *g* for 10 minutes. The now-clear supernatant was decanted into a beaker with a stirring bar, returned to the pH meter, and 2 M tris(hydroxymethyl)aminomethane (Tris) was added dropwise while stirring until the pH increased to 7.

The neutralized supernatant was loaded onto a 50-mL (2.5 × 10.5 cm) Sephadex G-100 column. Once all of the sample had entered the column, the column was washed with 4 column volumes (200 mL) of phosphate-buffered saline (PBS) containing 10 mM sodium phosphate, 150 mM NaCl, and a pH of 7.4 to flush out the proteins that did not bind to the column. The column outlet was then connected to a fraction collector. PBS with 0.1 M glucose was then passed through the column to elute *Pisum* lectin while collecting 7-mL fractions.

The students in the class then took turns reading the $A_{280}$ of the fractions, using a NanoDrop Lite Spectrophotometer to determine which fractions contained lectin. The absorbance readings were recorded on the classroom whiteboard, enabling students to see the rise and fall in the readings as the lectin eluted from the column.

In a follow-up to the enrichment activity and after approval from the university's institutional review board, the students were surveyed anonymously approximately 6 months later. The survey instrument collected feedback on the activity (Table 1) and assessed the students' retention of their knowledge of how lectins are extracted, purified, and used in the immunohematology laboratory (Table 2).

**Table 1. Postactivity survey results**

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|---|
| Question 1. Was it interesting for you to see where lectins come from? |
| Yes, it was interesting to see all of the steps that it actually took to isolate lectins. |
| Yes. |
| Yes, very. |
| Yes. I had imagined that they were created artificially at first. When I arrived at the lab, I hadn't realized the process of extracting lectins from seeds would be as simple as it actually is. |
| Yes! |
| Yes. |
| Yes. |
| Yes, I learned that lectins were something some reagents were made of and that was it so I wanted to know a bit more. |
| Yes. |
| Yes, it was. We talked about lectins a lot in class, and getting to see how they were made was very interesting. |
| Yes, it is always helpful to make what we're learning more real. |
| Very much, yes. |
| Yes! Very interesting and neat to learn about. |
| Question 2. What did you enjoy most about this activity? |
| How we could participate and check the concentrations of the lectins. |
| I liked that we were able to see real applications of how reagents like lectins come from. |
| Going to the greenhouses. I thought it was very all very informative and quite interesting. |
| The botany professor was engaging. She was enthusiastic about her field and did an excellent job at involving individual students in the process. |
| How hands-on it was and exploring a different area of the university. |
| It was neat to see the actual process of where the lectins originated from. |
| It was interesting to see that lectins could be made from other substances like peas. |
| Going to another department to see how they do things. |
| Getting to see the intersection of very different sciences: botany and immunohematology. |
| I enjoyed the process of purification. The mush of peas went from looking gross to a nice, pristine, clear solution. |
| I enjoyed how hands-on and how involved the instructor was with the audience. |
| Listening to the presenter and visiting the greenhouse. |
| Knowing what lectins are and what they can be used for. |
| Question 3. Would you recommend future classes do this? |
| Yes, I would. |
| Absolutely. |
| Yes. |
| Absolutely. It's valuable to understand where our reagents are coming from. |
| Yes. |
| Yes. |
| Yes. |
| Yes. |
| Yes, absolutely. |
| Yes, I would highly recommend this at least once per semester. |
| Yes. |
| Yes. |
| Yes. |

**Table 1.** (*Continued*).

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| Question 4. During this activity, a nanodrop machine was used to measure the concentration of the extracted lectin. Was it interesting for you to see applications for analytical methods (eg, spectrophotometry, and so forth) outside of the medical laboratory sciences? |
| Yes. |
| Yes. I had no idea of the practices outside of MLS. |
| Yes. It was cool to see the whole set up and then participate in it as well. |
| It is interesting that spectrophotometry is so widely employed. |
| Yes. |
| Yes. |
| Yes. |
| Yes. I got to see the same machines we have here but smaller and more high tech. |
| Yes, and also to see how advanced spectrophotometers can be. |
| It was interesting to see "clinical lab methods" being used outside of the clinical lab. This field is very broad, and has lots of opportunities, so it was good to see that. |
| Yes, I loved how much effort was put into the presentation. |
| Yes. |
| Yes. To know the same machines can be used for so many different things. |
| Question 5. What would you have like to have seen, in addition to what was done? |
| I think everything I saw was great. |
| See how the lectins are processed later. |
| N/A |
| I was satisfied with what I saw and did. |
| Maybe have a class swap? We can show botany majors what lectins can be used for in our field. |
| No. |
| Nothing. |
| An activity where we make some lectins for blood bank lab. |
| I thought it was good the way it was. |
| It would be nice to see a couple different kinds of lectin being made. |
| I can't think of anything. |
| N/A |
| How it related to blood banking activities. |
| Question 6. Do you have any recommendations for improvements to this activity? |
| No recommendations. It was great. |
| Use the lectin to test a patient sample. |
| No. |
| It would be valuable to have more hands-on activities for all the students (though probably difficult to prepare). |
| Nope. |
| No. |
| Have the students study how lectins are made before coming to the activity. |
| Provide some food? |
| It would be cool to do a Ulex europaeus or a Dolichos biflorus extraction to see exactly what is done with our reagents. |
| Do a couple different lectin types. |
| A room where everyone can face front. |
| No. |
| N/A |

Abbreviation: MLS, medical laboratory science; N/A, not applicable.

## RESULTS

Responses to the survey questions (Table 1) indicated that the students were interested to see where lectins come from, that they enjoyed the activity, that they would recommend the activity for future students, and that they were interested in seeing applications for analytic methods outside the MLS profession. One respondent recommended inviting botany students into the immunohematology laboratory to experience how lectins are used in blood typing, and one respondent recommended hosting the botany students in the immunohematology laboratory. The quiz average was 83% (Table 2), indicating good retention of the material learned approximately 6 months after the survey was conducted.

## DISCUSSION

It is important to provide students the opportunity to learn the background of the techniques they will use in their professions. The following techniques were employed in this enrichment activity: affinity chromatography/column

**Table 2. Postactivity quiz results**

Quiz Respondents ($n = 11$)

Question 1. Lectins are:

A. Proteins that bind to carbohydrates (9)*
B. Carbohydrates that bind to proteins (1)
C. Antibodies that bind to carbohydrates (1)
D. Antibodies that bind to proteins

Question 2. The Ulex europaeus lectin has specificity for:

A. A1 antigens
B. B antigens
C. Rh antigens (1)
D. H antigens (10)*

Question 3. The Dolichos biflorus lectin has specificity for:

A. A1 antigens (11)*
B. B antigens
C. Rh antigens
D. H antigens

Question 4. In the lectin extraction laboratory facilitated by the botany laboratory, which of the following methods of analysis was employed to measure the concentration of the extracted lectin?

A. Affinity chromatography
B. Gel electrophoresis
C. Spectrophotometry (11)*
D. Phytohemagglutination

Question 5. In the lectin extraction laboratory facilitated by the botany laboratory, which of the following methods is employed to purify the extracted lectin?

A. Affinity chromatography (6)*
B. Gel electrophoresis (2)
C. Spectrophotometry
D. Phytohemagglutination (3)

Question 6. How could you determine if a newly discovered lectin might be useful as a reagent for blood typing?

A. Affinity chromatography (3)
B. Gel electrophoresis
C. Spectrophotometry
D. Phytohemagglutination (8)*

*Note*: The asterisks denote the correct answer for the question.

chromatography, micropipetting, use of the fraction collector, centrifugation, pH meter, and spectrophotometry (NanoDrop). An interdisciplinary approach was applied because of its ability to enrich the students' overall educational experience.[10]

Interdisciplinary education refers to the collaboration of two or more disciplines in relation to research or instruction.[10–12] This type of learning allows students to apply the knowledge they have gained within their own discipline to another field of study, to form connections between concepts across disciplinary boundaries, and to develop transferrable skills.[10] In addition, interdisciplinary collaborations are increasingly used in both research and medicine because they are recognized for their ability to find solutions to complex questions and to determine the optimal treatment regimens for individual patients.[12]

We concluded that this is an excellent opportunity to emphasize to students that discoveries are ongoing, that new lectins continue to be found in diverse plant and animal sources, and that their use is not restricted to blood typing in the immunohematology laboratory. In future planned activities, an electrophoresis demonstration will be added to show the purity of the lectin compared with the mixture of proteins in the crude extract.

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