# *LECTINS AND THEIR USES AS BIOTECHNOLOGICAL TOOLS*

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## Introduction

On February 1, 1888, a dissertation presented by Stillmark[1] entitled 'Ricin, a toxic enzyme from seeds of *Ricinus communis* L and some other Euphorbiaceae' at the Medical School of Dorpat (now Tartu) in Estonia, began the era of lectins. In this paper Stillmark described "interesting effects of ricinus (extraction) on the blood", ie their ability to agglutinate erythrocytes, without realizing that ricin had binding sites for carbohydrate groups. There have since been many significant historical landmarks in the studies of lectins and a few are shown in Table 1.

Lectins are polyvalent carbohydrate-binding proteins or glycoproteins of non-immune origin and seem to be present in all organisms. They have many biologically significant activities, such as the ability to agglutinate cells and precipitate polysaccharides and glycoproteins.[2–4] Although our understanding of the biological functions of lectins in plants is scant, lectins are now widely used as biochemical tools in many types of research. A large number of lectins have been purified, characterized and their carbohydrate-binding properties described (Table 2).

Earlier studies of inhibition of agglutination by lectins suggested that they bound to monosaccharides; however, several investigators, such as Kornfeld and Ferris,[5] demonstrated that the binding constant of a lectin for a specific free monosaccharide may be orders of magnitude lower than the binding constant of the lectin for a glycoconjugate containing that monosaccharide residue. Many other such studies indicate that the binding sites of lectins are large and accommodate structurally complex carbohydrate determinants. The monosaccharide constituents are now considered to be only part of the overall determinant.

*Table 1* *Lectins: An Historical Overview*

| *Year* | *Investigator(s)* | *Discovery* |
|---|---|---|
| 1888 | H Stillmark | *Ricinus communis* plant extract has hemagglutinating properties |
| 1890 | P Erlich | Lectins as antigens in immunology |
| 1908 | K Lansteiner and H Raubitsheck | Different hemagglutinating properties in various seeds extracts |
| 1919 | J B Sumner | Crystallization of Concanavalin A |
| 1936 | J B Sumner | Lectins demonstrated to bind sugar |
| | S F Howell | Concanavalin A precipitates glycogen from solution |
| 1940 | W C Boyd, R M Reguera and K O Renkonen | Specificity of some lectins for some human blood group antigens |
| 1954 | W C Boyd and E Shyleigh | The name lectin proposed instead of hemagglutinin |
| 1960 | P C Nowell | Lectin from *Phaseolus vulgaris* found to be mitogenic to resting lymphocytes |
| 1960 | J C Aub | Lectins preferentially agglutinate malignant cells |
| 1976 | Y Reisner | Peanut agglutinin discriminates cortical from medullary cells in mice |
| 1974 | G Ashwell and A G Morell | First mammalian lectin identified: hepatocyte asialoglycoprotein receptor specific for terminal galactose in serum glycoproteins |

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*Table 2 Some Activities of Plant Lectins*

| |
|---|
| Agglutinate Cells |
| Act as mitogens to peripheral blood lymphocytes and other resting cells |
| Stimulate human leukocytes to produce gamma-interferon |
| Cause death of many types of cells |
| Mediate cell adhesion to substrates |
| Stimulate histamine release from mast cells and basophils |
| Inhibit growth of fungi |

The topic of lectins is a fertile one for teaching students. Not only are the proteins themselves intrinsically interesting as are speculations on their possible functions, but also the area provides an opportunity for an extensive review of the structures of carbohydrates. In addition, there is the general question of how to determine binding constants and illustrations of the very wide use of lectins as biochemical and biotechnological tools. Practical laboratory experiments are very easily designed, based on such commonly available immobilized plant lectins as Concanavalin A-Sepharose, for example.

## The distribution of lectins

*Microorganisms* Many enterobacteriaceae have lectins in their pili that are essential for their successful adhesion to the intestinal epithelium leading to infections in the urinary and gastrointestinal tracts.[6,7] For example, both *Escherichia coli* and *Salmonellae* spp make several lectins with different carbohydrate-binding specificities.[8,9] Because of their apparent association with other proteins in the pili, these lectins have proved difficult to purify and characterize.

*Invertebrates* Almost a century ago it was observed that hemolymph from *Limulus*, the horseshoe crab, had agglutinating properties that might be important in resisting infections. Since then a large number of lectins have been found in all invertebrate phyla and several lectins have been isolated from the hemolymph and sexual organs of mollusca and arthropoda such as: *Helix pomatia*, *Limulus polyphemus*, *Homarus americanus*, *Limax flavus*, *Cancer antennarius*, and *Tridacna maxima*. Soluble lectins in invertebrates may play a role 'analogous' to that of immunoglobulins in vertebrates; this hypothesis, however, is far from being firmly established, since except for the lectin from *H pomatia*, which recognizes *N*-acetylgalactosamine, the majority of these hemolymph lectins appear to recognize sialic acid residues.

*'Higher' animals* Lectins are produced by animals both in a soluble and in a membrane-bound form. Many of the membrane-bound lectins are receptors for physiological ligands, such as the mannose 6-phosphate receptor,[10] which binds lysosomal enzymes, and the asialoglycoprotein receptor, which binds serum asialoglycoproteins.[11] Many other important animal lectins have recently been discovered, such as the 'selectin' family of cell adhesion molecules expressed by platelets, endothelial cells and lymphocytes.[12–14] The biological functions of many other animal lectins, however, particularly those that are soluble, are less clear. At least three general models have been put forward for the functions of membrane-bound and soluble lectins in animals: (a) cell-surface lectins can mediate pinocytosis of modified glycoproteins[11] which may be a preliminary step to their catabolism; (*b*) lectins can play a significant role in the intercellular interactions during fertilization, vertebrate development and inflammatory responses; and (*c*) circulating or soluble lectins may act as protective agents against infections by binding to and/or agglutinating pathogens.

*Algae* Molecules with agglutinating activity have been found in a large number of marine algae, but few have been studied in detail. A few of the representative species which have been investigated are *Pilota plumosa*, *Agardhelia tenera*, *Cystoclonium purpureum*, *Gracilaria verrucosa*, *Palmaria palmata*, *Fucus vesciculosum*, and *Codium fragile*.[15–16]

*'Higher' plants* The majority of lectins studied so far and those that are commercially available have been extracted and purified from seeds and plant tissues.[4] It has been suggested that the plant lectins, and perhaps even the bacterial lectins, play a role in the symbiotic relationships between bacteria and leguminous plants.[17] There is also some evidence that lectins act in preventing plant infections.[18] However, the physiological role of lectins in plants is still a matter of much research and discussion.

**Carbohydrate-binding properties**

Watkins and Morgan[19] demonstrated that monosaccharides constitute the determinants of blood group specificity. Lectins are now used routinely in blood banks to facilitate blood typing and they can be used for the identification of blood group substances secreted in saliva and other biological fluids (Table 3). The modern perspective on the applications and use of lectins was opened by the demonstrations that some lectins, such as that from *Phaseolus vulgaris*, can stimulate mitogenesis of lymphocytes by binding to cell surface carbohydrates[20] and by the findings that lectins react differently with malignant cells compared to their non-malignant counterparts. These studies focused attention on the potential importance of protein-carbohydrate interactions in the normal growth and metabolism of cells.

Despite a wide diversity in three dimensional structures and in the probable binding sites among lectins, there are some features of the interaction of protein with carbohydrate that appears to be common to all lectins. Both hydrogen bonds and van der Waals interactions are involved in stabilizing these interactions.[21] Lectins often display hydrophobic interactions with derivatives of glycosides that are stronger than with the glycoside alone. For example, *N*-dansylgalactosamine binds 60 times more strongly than *N*-acetylgalactosamine to *Erythrina crystagalli* lectin.[22] Lectins which show similar specificity for monosaccharides can differ in their recognition of the fine structure of oligosaccharides and glycoproteins.

The great majority of lectins studied so far contain metal ions, normally $Ca^{2+}$ and $Mn^{2+}$ (Table 4). They often require metal ions for maintenance of conformation and their ability

*Table 3* *Human Blood Groups and Recognition by Plant Lectins*

| *Blood type* | *Oligosaccharide structure* |
|---|---|
| A | Fuc<br>↓ α1,2<br>GalNAcα,1,3Galβ1,3(or 4)GlcNAcβ-R |
| B | Fuc<br>↓ α1,2<br>Galα1,3Galβ1,3(or 4)GlcNAcβ-R |
| H(O) | Fuc<br>↓ α1,2<br>Galβ1,3(or 4)GlcNAcβ-R |

| *Lectin from* (common name) | *Blood type* | *Monosaccharide recognized* |
|---|---|---|
| *Griffonia simplicifolia* (GS-I) | A, B | α-D-Gal, α-D-GalNAc |
| *Griffonia simplicifolia* (GS-$IB_4$) | B | α-D-Gal |
| *Griffonia simplicifolia* (GS-$IA_4$) | A | α-D-GalNAc |
| *Helix pomatia* (HPA) | A | α-D-GalNAc |
| *Ptilota plumosa* | B | α-D-Gal |
| *Anguilla anguilla* | H | α-L-Fuc |
| *Ulex europaeus* (UEA-I) | H | α-L-Fuc |
| *Laburnum alpinum* (LA-I) | H | $(GlcNAc)_{2-3}$ |
| *Laburnum alpinum* (LA-II) | H | Gal |

*Table 4* *Metal Content of Lectins* (Adapted from Goldstein and Hayes, 1978; ref 45)

| *Lectin from* | *Metal ions (atom/mol)* $Mn^{2+}$ | $Ca^{2+}$ | $Zn^{2+}$ |
|---|---|---|---|
| *Canavalia ensiformis* | 4 | 4 | — |
| *Dolichos biflorus* | 0.7 | 3.5 | 0.5 |
| *Ulex europaeus* (UEA-I) | 0.42 | 2.0 | — |
| *Griffonia simplicifolia* (GS-I) | 1.2 | 2.0 | — |
| *Erythrina cristagalli* | 1.0 | 1.9 | — |
| *Euonymus europeaus* | — | 8.0 | 0.7 |

to bind carbohydrates. Most metal ions are strongly bound and are not easily removed from their site in the lectin even after long treatment with chelating agents such as EDTA.

The mechanism(s) by which lectins interact with monosaccharides is somewhat similar to enzyme-substrate interactions with a low rate association constant equal to $10^4\ M^{-1}\ s^{-1}$, two orders of magnitude less than a $K_A$ for many enzymes.[23] The affinity $K_A$ of many lectins for an appropriate monosaccharide is approximately $10^3\ M^{-1}$ (see ref 24). It has been shown, however, that lectins with low affinity for monosaccharides can bind with much higher affinity ($K_A$ greater than $10^6\ M^{-1}$) to an oligosaccharide containing two or more specific monosaccharide residues in an appropriate linkage. In addition, because lectins are multivalent, they can bind simultaneously to more than one oligosaccharide, expressing an 'avidity' which can result in an increased affinity (Table 5).

*Table 5 Oligosaccharide Binding Specificities of Some Lectins*

| *Lectin* | *Oligosaccharide specificity* | *Mono/disaccharide inhibitors* |
|---|---|---|
| *Ricinus communis* (RCA) | Biantennary oligosaccharides containing:<br>Gal β1,4GlcNAc- linked at nonreducing end<br>Gal β1,3GalNAc1-Ser or Thr | β Gal/Lactose |
| *Sambucus nigra* (SNA) | NeuAcα2,6Gal > NeuAcα2,6GalNAc | Sialic acid |
| *Canavalia ensiformis* (ConA) | Man α1- linked > Glc α 1-linked ><br>GlcNAc α 1-linked<br>High affinity for trimannosidic core structure | αD-Man>α-D-Glc><br>α-D-GlcNAc |
| *Lens culinaris* | [R-GlcNAc] ↘ β1,6<br>[R′-GlcNAcβ1,2] Man<br>↓ α1,6<br>Manβ1,4GlcNAcβ1,4GlcNAcβ1-Asn<br>↑ α1,3 ↗<br>[R″-GlcNAcβ1,2]Man α1,6Fuc | α-D-Man > α-D-Glc> LCA<br>α-D-GlcNAc |
| *Helix pomatia* (HPA) | GalNAc α1,3GalNAcβ1,4Galα1,4Galβ1,4Glc | αGalNac>αGlcNAc |
| *Bandeiraea simplicifolia* (BS-1) | GalNAcα1,3Gal | αGal>αGalNAc |
| *Phaseolus vulgaris* (PHA-E) | Galβ1,4 GlcNAc β1,2 Man<br>↓ α1,6<br>GlcNAcβ1,4Manβ1,4GlcNAcβ1,4-R<br>↑ α1,3<br>GlcNAcβ1,2Man | GalNAc |
| *Datura stramonium* (DSA) | Galβ1,4GlcNAcβ1,6<br>Galβ1,4GlcNAcβ1,2ManαR<br>$[3Gal\beta1,4GlcNAc\beta1]_{n>2}$ | (D-GlcNAc)$_2$ |
| *Maakia amurensis* Leukoagglutinin (MAL) | NeuAcα2,3Galβ1,4GlcNAc-R | ? |

Students could be asked to devise a suitable experiment to determine the binding constant of a given lectin for a carbohydrate. Alternatively, they could be given some data and asked to make calculation regarding the binding constant. In addition, the students could be asked to consider the experimental differences between determining the binding constant of a lectin for a monosaccharide *versus* an intact cell, such as an erythrocyte.

Surolia *et al*[24] reported that the $K_A$ for RCA-1, a major lectin in seeds from the plant *Ricinus communis*, in its binding to glycolipids in membranes is three orders of magnitude higher than that seen for lectin-binding to monosaccharide inhibitors. The binding of a lectin is sensitive to host matrix composition and the nature of the receptor and in general the lectin binding is a direct function of the number of 'receptors' in membranes.

**Applications**

A great amount of interest in lectins was generated in the 1960s and 1970s when numerous studies were published demonstrating that lectins preferentially agglutinate malignant cells and that certain lectins, when bound to cell surface of lymphocytes, can stimulate mitosis in these cells. The preferential agglutination of malignant cells seems to result from a combination of both altered glycosylation in the malignant cells and an increased receptor mobility in transformed cells which permits a lectin-induced clustering.[20,22] Several recent studies have now identified specific glycosylation differences between malignant and non-malignant cells (reviewed in ref 25).

Students can hardly fail to be interested in this aspect of lectins with relation to all biology and malignancy. It might be a valuable class exercise to set the reading of two or three papers in this area with the purpose of preparing a short essay on this aspect with an assessment of its potentialities and actual use clinically.

Interest in lectins as biotechnological tools was also generated by the demonstrations that lectins can be used for separating and analyzing glycoproteins and glycolipids and for isolating specific oligosaccharides. There is now a large, and continuously increasing, number of lectins that are commercially available and used in biochemical studies and there is an expanding emphasis on the identification, purification, and characterization of new lectins.

Lectins are attractive biotechnological tools because they are highly stable, relatively easy to purify, readily available, highly specific for carbohydrate determinants, and amenable to chemical modification and conjugation. Lectins have applications in many fields, including cancer research,[20,26] immunology,[27] basic biochemical studies on membrane structures,[28] glycoprotein purification,[29] cell identification,[30] histology, and cytochemistry and others (see ref 4).

Students might at this point be set the problem of devising a method for purifiying Concanavalin A from jack beans. As a help, they could be told what carbohydrates the protein binds.

Because of the "practical" analogy of saccharide-lectin interaction to antigen-antibody interactions, lectins are increasingly used in the identification and purification of glycoconjugates. Techniques derived conceptually from immunology and applied to lectins include:

(1) *Polyacrylamide gel electrophoresis* In this procedure, glycoproteins are identified on gels stained by radioactively labeled or fluorescent lectins;

(2) *Affinityimmunoelectrophoresis* In this procedure glycoproteins are separated by a two-dimensional electrophoresis, using a lectin in the first dimension and an antibody in the second;

(3) *Affinity chromatography of glycoproteins* A lectin is immobilized in a solid matrix such as agarose. Cell or tissue derived glycoproteins are passed through the column and only specific carbohydrate structures are recognized and captured by the lectin. Glycoproteins can be released from these immobilized lectins by addition of high concentrations of competing monosaccharides in one step or using a concentration gradient. Using this type of procedure many glycoproteins have now been purified, such as carcinoembryonic antigen from human colon adenocarcinoma,[31] band 3 from human erythrocytes,[32] glycophorin from human erythrocytes,[33] and human histocompatibility antigen from lymphoblastoma cells.[34]

(4) *Serial Lectin Affinity Chromatography* In recent years immobilized lectins have been used extensively for purifying oligosaccharides from animal cell glycoproteins and

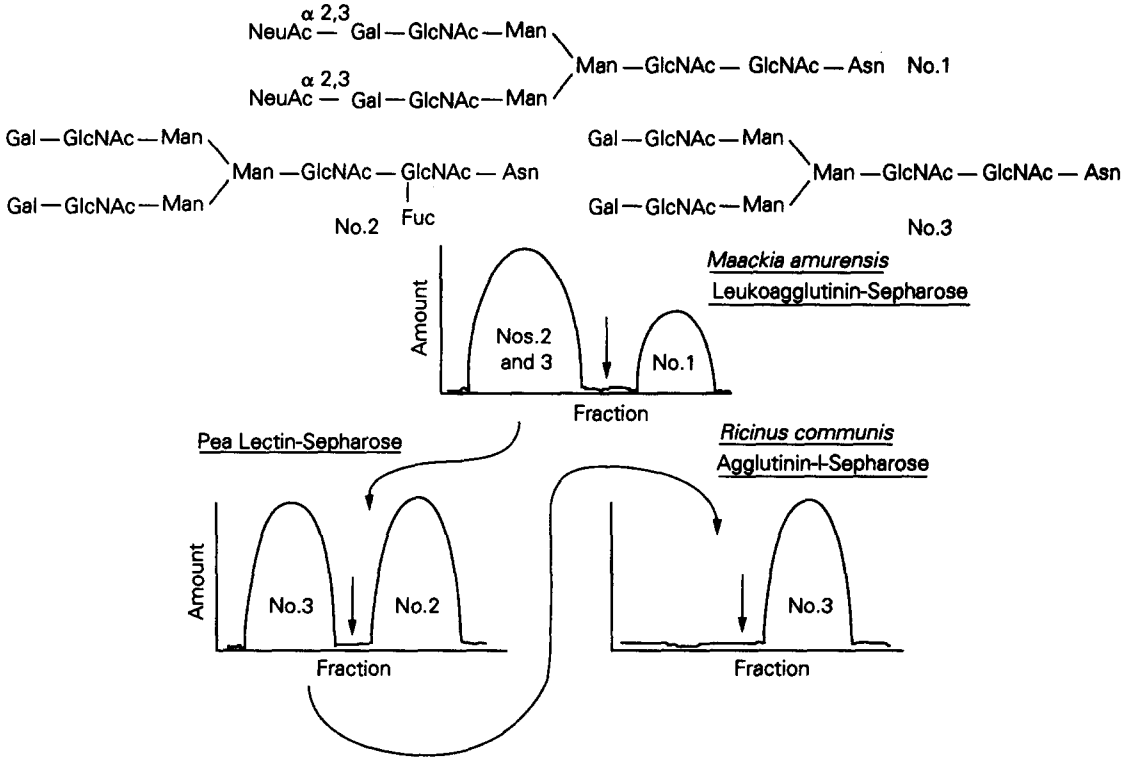


*Figure 1 Principle of serial lectin affinity chromatography*

glycolipids. The combined use of many different lectins, in a procedure called serial lectin affinity chromatography (SLAC), can greatly facilitate the isolation and purification of oligosaccharides from a complex mixture[28,35] (Fig 1). The SLAC analysis permits the isolation and the identification of a minimal structure of *N*-linked oligosaccharides. Usually, radiolabelled material produced from a metabolically-radiolabeled cell culture is solubilized, and the oligosaccharides enzymatically cleaved from the protein. The mixture is passed through different immobilized lectins in a 'logical' sequence using a very small amount of material. The method has been used to analyze the glycosylation of murine antigen a,B chains[36] and for the fractionation of oligosaccharides from mouse lymphoma cells.[37] For a detailed description see Cummings *et al.*[38]

(5) *Solid Phase Assay* An important recent advance in the application of lectins is their use for identifying the products of glycosyltransferase assays in solid phase techniques, similar to the type shown in Fig 2. Glycosyltransferases are a group of enzymes, normally present in the membranes of endoplasmic reticulum and Golgi apparatus, that catalyze the synthesis of oligosaccharides by transferring monosaccharides from a glycosyl nucleotide donor substrate to an acceptor substrate.[39] Glycosyltransferases have been studied for understanding the metabolic deviation in neoplastic modifications with the aim of identifying a clinically relevant tumor marker. Many of the glycosyltransferases, whose expression is cell and tissue specific and altered by malignant transformation, are present in body fluids, such as blood and milk. There have been several reports suggesting the possibility that alterations in glycosyltransferase activities in serum may be indicative of cancer.[40–42] There is considerable interest in developing rapid and specific glycosyltransferase assays to allow a comparison of the activities of the enzymes in sera from patients with different tumors and other diseases for both diagnostic and prognostic purposes.

In our laboratory a simple, reliable and fast assay has been prepared using a lectin as an important tool[43] (Fig 2). The neoglycoprotein acceptor, GlcNAc-bovine serum albumin, was prepared synthetically for use as an acceptor substrate for a specific β1,4 galactosyltransferase in serum. The GlcNAc-BSA is immobilized on a microtiter well plate and the enzymatic reaction is carried out using serum, UDP-Gal as donor substrate, $Mn^{2+}$, and buffer in each well. After incubating for one hour at 37°C the reaction is stopped and the wells are thoroughly washed. The product formed, Galβ1,4GlcNAc-

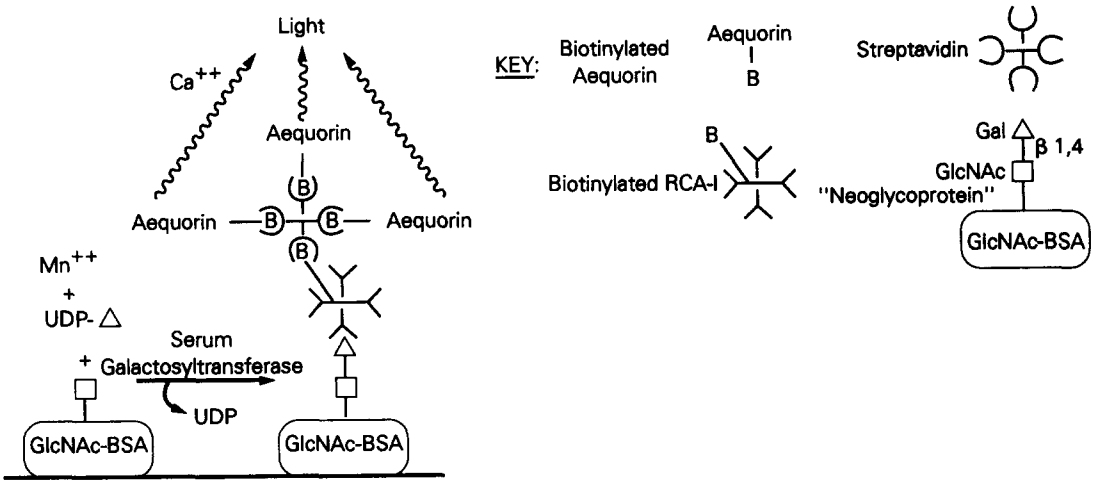


*Figure 2* *Assay configuration for β1,4-galactosyltransferase*

BSA, is detected using biotinylated-*Ricinus communis* agglutinin, which specifically interacts with galactose in β1,4 linkage. The unbound lectin is washed away, streptavidin is added to form a bridge between the biotinylated-lectin and the biotinylated photoprotein, aequorin. The unbound streptavidin and biotinylated aequorin are washed away. Aequorin is a newly available recombinant bioluminescent protein which emits light in the presence of $Ca^{2+}$. The light emitted after automatic injection of $Ca^{2+}$ in each well is measured using a commercially available luminometer giving a rapid and sensitive method for determining the activity of the galactosyltransferase in the serum of patients. It is also likely, as shown in our preliminary studies, that other lectins may be generally usefully used in this manner for assaying other glycosyltransferases and for identifying the altered glycosylation of serum proteins which occurs in patients with cancer and other diseases.[44]

**Summary** This short overview illustrates the great interest scientists have in lectins and their wide applications in biotechnology and biochemical research. In the hundred years since Stillmark's announcement, there has been a continuous and growing regard for the characterization and exploitation of lectins in biochemical research. It is likely that the future will find lectins, not only from plant but also from animals and microorganisms, playing increasing important roles in the research and clinical laboratory.

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