

D P C T e c h n i c a l R e p o r t

AlaBLOT™ Allergen Immunoblotting

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The measurement of allergen-specific IgE has become a standard method for use in the diagnosis of allergic disease. First developed in 1967 by Wide et al.,¹ the method has been improved by many companies, incorporating new technology. The most recent technological advancement, developed by DPC, incorporates the allergen in a liquid-phase reagent rather than in the historical solid-phase matrix.

It is well known that one of the most problematic characteristics of allergy diagnostic tools is the variability of the allergen used for testing. Researchers have devised many methods for evaluating allergen sources which are used by many manufacturers for raw material evaluation and quality control. Analysis of allergen extracts used for diagnostic testing, both for *in vitro* and *in vivo* testing, indicates that they are complex mixtures of proteins, carbohydrates, and nucleic acids. Many allergen sources have been extracted, studied, and characterized biochemically and immunochemically. The IgE-binding components of these mixtures are the allergens.

As an example, allergen extracts from grass pollen are known to contain several hundred different components. One hundred or fewer, however, have

been shown to bind to IgE from grass-allergic individuals corresponding to approximately 10 groups of allergens, each of them containing a few to up to 25 closely related isoforms.² In fact, the evidence indicates that relatively few components of allergenic mixtures are commonly bound by IgE from allergic individuals. When more than 50 percent of allergic individuals have antibodies to an allergen component, it is called a major allergen. Identification and characterization of major and clinically important minor allergens has been a focus of study for many decades.

Individual allergens are named using a convention based on the genus and species of the allergen source, and the order in which the allergens were discovered and characterized. For example, the botanical name for timothy grass, for example, is *Phleum pratense*, and allergens characterized from timothy grass are named Phl p #, where # is the order in which the allergen was characterized. Several clinically important allergens – found among hundreds of others that are not – have been characterized from timothy grass and are known as Phl p 1, Phl p 2, Phl p 5, and Phl p 6.

The essential characteristics of an allergen are that it be capable of coming in contact with the reactive mucosa in allergic individuals, generally the airways, eyes, mouth, skin or gut; and that it bind IgE from patients who are known to be allergic to the material. The analysis of allergens has improved with technological advances. As more allergens are isolated and sequenced, genetic database analysis offers the promise of identifying common structural features of allergens. Since the number of raw allergen sources is so high, and the components in each source so complex, only selected allergens can be thoroughly analyzed. For the vast majority of allergens, the ability to bind specific IgE remains the defining test.

Immunoblotting has become one of the most useful tools for evaluating allergens because it uses IgE from allergic individuals to visualize allergens in complex mixtures that can be separated by several biochemical

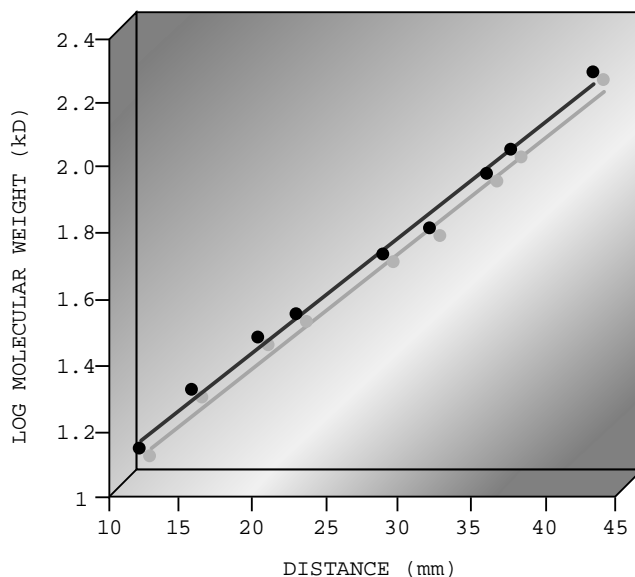


Figure 1. Calibration curve of protein standards from molecular weight marker strips used to develop the molecular weight estimation formula.

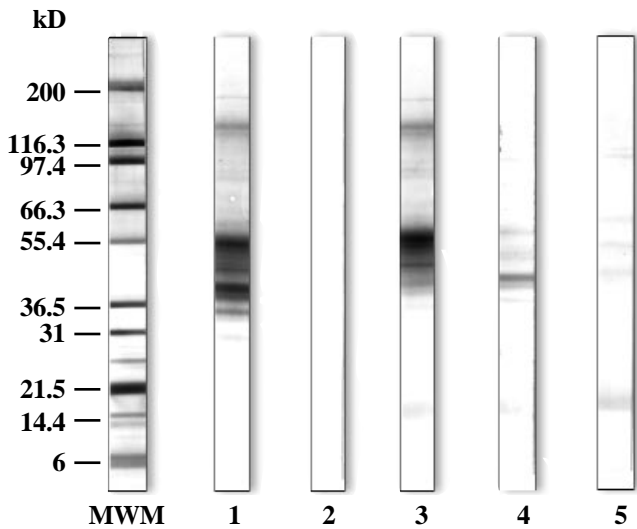


Figure 2.

parameters. The two most common biochemical methods used to characterize allergens are molecular mass and isoelectric point determinations. Each parameter is independent of the other, and together they are used in two dimensional electrophoresis. Immunoblotting can be carried out with either or both biochemical procedures, but it is most commonly used with molecular mass separation. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is a method for separating proteins and glycoproteins by their molecular weights. By transferring the separated proteins and glycoproteins to a matrix like nitrocellulose, the separated mixture can be probed with IgE from allergic patients. Each IgE-reactive component of the allergen mixture appears as a band corresponding to a molecular mass that can be analytically determined. Immunoblotting of allergens has been an important research tool,³ and recently it has become a useful tool for allergen analysis.

AlaSTAT® AlaBLOT™ provides all the reagents necessary to perform immunoblotting using any of 20 different allergen sources. Each allergen strip permits analysis of allergenic components found in complex mixtures. Characterized allergen extracts have been carefully electrophoresed and transferred to nitrocellulose under well-controlled conditions, and each is marked with a dye front for estimation of

molecular mass. Each reagent has been quality tested and is provided ready for use. Following the kit protocol, it is possible to visualize the reactive allergen components in less than 5 hours. AlaBLOT enhances availability of information to clinicians, researchers and patients. It is intended for research use only, and not for use in diagnostic procedures.

With AlaBLOT, molecular mass estimation of reactive allergen components is possible. The laboratory can measure, in millimeters, the distance between the center of the reactive band and the bottom edge of the dye front. The molecular mass can then be estimated

using the formula provided with allergen strips.

The formula has been determined by DPC using molecular mass markers (MWM) ranging from 14 to 200 kilodaltons (kD). The estimation of molecular mass less than 14 kD is not accurate, because the mobility of the smaller proteins is not in the linear range of the plot. (See Figure 1.)

Allergen inhibition can be a potentially powerful use of AlaBLOT. By modifying the AlaBLOT procedure slightly, one can test extracts of allergens, or potential sources of allergens to evaluate any allergic individual's sera. The fine definition of the allergen components by electrophoresis allows better differentiation of the individual components that may be found in immunotherapy mixtures or materials to which allergic individuals may be exposed.

In addition, AlaBLOT could be potentially useful for patients who have been chosen to undergo immunotherapy. Patient sera could be tested with AlaBLOT to identify allergenic components to which the patient had specific IgE. The immunotherapy allergen could then be mixed with the patient sera and tested to determine whether the allergenic components are present in the immunotherapy extract. Their presence would be demonstrated by the inhibition of patient IgE binding to the specific components to which

it had previously bound. Such use has not been correlated to the success of immunotherapy, but the inhibition procedures can be useful for such evaluations. (See Figure 2.)

Several possible uses of AlaBLOT have been described, and certainly many others will develop with the availability of this product. Diagnosed allergic individuals can be characterized, using AlaBLOT to identify the specific components contained in diagnostic methods such as DPC's AlaSTAT® Specific IgE. Research in the area of immunotherapy may also benefit from AlaBLOT testing, by allowing better definition of components in immunotherapy mixtures. Studies of crossreactive allergens may reduce the number of allergens necessary for immunotherapy. A consistently manufactured product such as AlaBLOT could be a useful tool in the hands of many qualified researchers. The potential applications of AlaBLOT are intriguing.

References

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In the US, for research use only; not for diagnostic procedures.

