

Standardization of Allergenic Extracts: The New Allergy Unit

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ABSTRACT

In recent years, several organizations have made concentrated efforts toward standardization of allergenic extract materials. A variety of standard extracts are now available for use by commercial manufacturers who, upon preparing new extracts, can calibrate their product to the standard of reference extract. There are several advantages to the use of standardized extracts, such as more accurate administration of extracts in treatment by immunotherapy and the ability for physicians to compare different individual extracts. Standardized extracts will most likely be more expensive, but they will also be more cost effective because of consistent potency. It appears that this is a small price to pay for quality control, which, until the onset of standardized extracts, has been lacking in the manufacture and use of allergenic extracts.

ALLERGENIC EXTRACTS, in terms of their relative potency, currently are labeled according to the method of manufacture (weight/volume) or according to the protein content of the final extract product (protein nitrogen unit, PNU). Unfortunately, these latter forms of labeling do not accurately reflect the potency of the extract. Consequently, physicians using allergenic extracts are forced to maintain consistency in extract source, and to use the same materials for skin testing as for immunotherapy. The current situation also means that diagnostic skin tests performed in one allergist's office are not easily interpretable in another allergist's office.

In an effort to reduce this rather chaotic state, the International Union of Immunologic Societies (IUIS), as well as the United States Food and Drug Administration (USFDA) Office of Biologics, have made available reference extract materials. Commercial manufacturers who prepare new extracts can calibrate their product to the standard of the reference extract.

There are several characteristics of allergenic extracts that create difficulty in assigning specific allergen units. The active principals found in crude extracts are mixed with variable amounts of inert materials and, as a result, isolation of the active material for measurement is often very difficult. Crude allergenic extracts contain multiple active principals. All of the active principals important to the disease should be present in materials prepared for diagnostic testing or immunotherapy. Finally, patients vary greatly in their response to specific allergens. Patients

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appear to react to unique patterns of components of allergenic extracts; some to only a few allergens, and others, to many.⁽¹⁾

There are several obvious advantages to the use of standardized extracts.⁽²⁾ Standardized extracts will allow the physician to describe accurately the dose of an extract that a patient has received. The allergist will better be able to characterize the sensitivity of a patient. This would be particularly useful in corresponding with referring physicians and in establishing first treatment doses. Standardized extracts will provide a framework for allergen research. Investigators in different research centers would be able to compare the sensitivity of patients under study with the sensitivity of study patients in different centers.

Standardized extracts will most likely be more expensive, but they will also be more cost effective because of consistent potency. This is a small price to pay for quality control, which, up to now, has been lacking in the manufacture and use of allergenic extracts.

There are *in vitro* and *in vivo* methods currently employed for characterization of allergenic extracts.

IN VITRO METHODS OF STANDARDIZATION

The *in vitro* methods include measurement of specific antigenic components, total protein analysis, the radioallergosorbent inhibition assay, and isoelectric focusing.⁽³⁾

The potency of an extract is known to be related to the quality of the source of material. The potency of allergenic extracts available today may vary 1000-fold.⁽⁴⁾ Among other things, differences in potency may result from differences in source material, extraction methods, and deterioration over time.

The radioallergosorbent inhibition assay is one of the more widely used *in vitro* tests for measuring total allergenic activity.⁽⁵⁾ The potency of an extract is tested relative to a reference preparation. The method measures the activity of a mixture of allergens, but is very dependent upon the reagents used to perform the test. The method is dependent upon a high-quality extract bound to a solid support, the reference material, the allergic serum pool, and the quality of the radiolabeled anti-IgE used in the final step of the procedure.

The isoelectric focusing procedure is a qualitative procedure for detecting stainable protein bands using pH gradient. It is invaluable for identifying extracts, since it functions as a "fingerprint." A loss in protein bands or change in pattern or intensity of staining of bands as seen in this test may be either the result of other contaminating allergenic material in the extract or deterioration of the extract product itself.⁽⁶⁾

IN VIVO METHODS OF STANDARDIZATION

The new allergy unit concept developed in the United States is based on an *in vivo* assay method. After an extract reference material has been assigned allergy unit potency by means of *in vivo* assay, other allergenic extracts can be compared to this reference material by *in vitro* tests, such as the radioallergosorbent inhibition assay.

The *in vivo* method currently recommended in the United States is the quantitative intradermal test procedure for relative potency of allergenic extracts using parallel-line bioassay.⁽⁷⁾ The parallel-line method is similar to that used for estimating relative potency by RAST inhibition. On each assay, approximately three allergic subjects are used. These subjects should have a history of allergic disease related to exposure to the test allergen, and they should be sufficiently sensitive so that injection of high concentrations of allergenic extract can be avoided. Patients who are not receiving and have not received immunotherapy are preferred. Serial threefold

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dilutions from the undiluted new extract product are labeled 1–17. An occasional highly sensitive patient may require dilutions up to #21. The skin test is carried out by intracutaneous injection so that low-potency extracts can be studied. Skin tests are read in 15 minutes and the wheal and erythema margins are outlined using a skin-marking pen. The sum of the longest orthogonal erythema diameters constitutes the skin response at that site. A randomization scheme is used for selecting the skin test sites, and each allergen dilution may be tested singly. A line using linear regression computation is calculated. Appropriate statistical requirements of correlation and linearity are applied.

In addition to relative potency, an Allergen Unit can also be assigned to the extract based upon the threefold dilution number producing a sum of erythema diameter of 50 mm (D_{50}) in each patient whose puncture test (bifurcated needle) was positive to the allergen concentrate. A scheme for assigning potency in terms of allergen units for these extracts has been developed based on a large number of extracts tested.⁽⁸⁾ The most potent extracts including short ragweed and grass pollen extracts usually have a nadol D_{50} of 14. That is, the 14th threefold dilution of the extract concentrate in a large number of highly allergic patients gave a sum of erythema measurement on skin testing of 50 mm. This D_{50} has arbitrarily been assigned 100,000 allergen units. Once an *in vivo* assay has been used to assign allergen units to a reference, then appropriate assays of *in vitro* relative potency, such as the radioallergosorbent inhibition assay, can be used to assign the new allergen unit to newly compounded extracts.

A major advantage of the new allergy unit derived from *in vivo* methods for standardization of allergenic extracts is that two different extracts of the same material can safely be switched at bioequivalent doses. This will permit development of a safe and effective dose regimen using allergenic extracts for extracts from diverse sources and in different allergist's offices. As of this writing, the new allergy unit has been assigned to extracts of the house dust mites *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*, and is considered being assigned to extracts of the pollens of timothy, rye, orchard, june and red top grasses, ragweed, and extracts of cat.

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