

potency sequences established by RAST-inhibition and C-assay displayed some parallelism (Table 1). This finding is in concert with other data from C-assay, skin tests and direct RAST (5) which suggest that, although IgE- and C-mediated mechanisms in house dust allergic patients operate independently, there may yet be some association between these phenomena.

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Standardization of Radioallergosorbent Test (RAST) and Allergen Extracts in Terms of the WHO Standard for IgE

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Allergen extracts have been reported to vary considerably with respect to their potency as reflected by their ability to react in human skin (1,6). At the present time, there are no potency standards for such extracts and commercial preparations are listed in terms of either protein nitrogen per volume (PNU) or as weight percent of allergen extracted (being related to Noon units). Standardization is of course desirable, but progress towards this goal has been held back because of the lack of accepted criteria and the technology for measurement.

The use of skin testing for the measurement of potency of allergen extracts has been proposed (7), often linked with a histamine standard (2). In addition to the ethical and legal barriers to this approach, the limited number of suitable human volunteers makes it impracticable for implementation by a control authority

(3). Such *in vivo* techniques have only been practised in isolated clinics.

The development of the radioallergosorbent test (RAST) for the detection of specific IgE antibodies in the sera of atopic patients (8) has led to its adaptation for the measurement of allergens. The inhibition of the RAST by soluble allergen has now emerged as the method of choice for the standardization of allergen extracts.

The major problem of this approach is that RAST itself is not a standardized technique. In particular, it is not possible to directly compare two assays performed at different times because of variations in the binding of the labelled anti IgE. This problem has been approached by ranking atopic sera in the direct RAST (5) or by comparison with a reference allergen as inhibitors of the RAST (4).

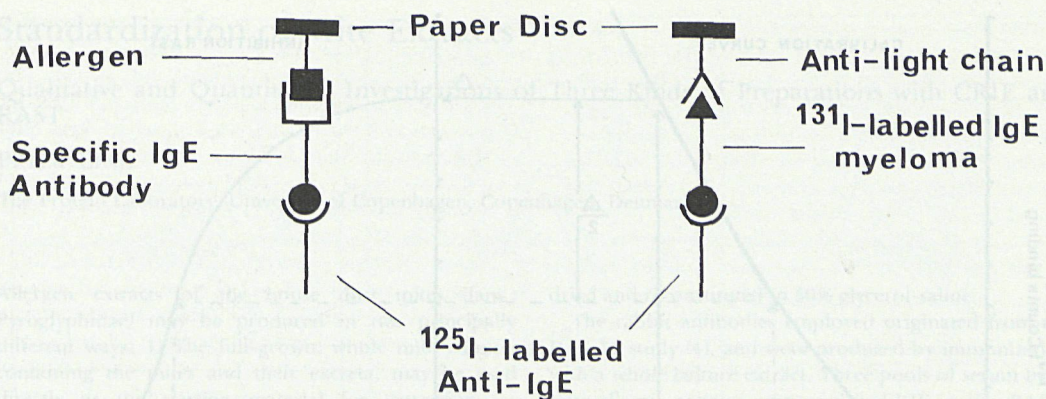


Fig. 1. Principle of method for standardizing the RAST

The application of the RAST to allergen standardization requires the collection of vastly differing reference materials. Man is exposed to a large number of antigens in his everyday life coming from plant, animal, chemical and micro-organism sources. Any of these may stimulate the IgE response and act as allergens, making the task of collecting reference materials daunting.

We report the standardization of the RAST by reference to the internationally accepted WHO standard serum for IgE. The actual weight of specific IgE attached to the allergen in the direct RAST is calculated. When soluble allergen is used in the RAST inhibition, exact quantification of the inhibition of specific IgE units bound can be calculated and standardized with reference to equivalent WHO IgE units.

Thus, using this procedure, reference allergen standards are rendered unnecessary as all allergens can be measured in terms of the same ultimate standard, i.e. WHO IgE units.

PROCEDURE

A purified IgE myeloma protein is radiolabelled with ^{131}I . The number of IgE Units contained in this radio-labelled preparation (IgE_m) is determined by comparison with the WHO Standard Serum for IgE by double antibody radio-immuno assay. This allows the specific radioactivity of IgE_m to be calculated (as c.p.m. per mU IgE).

The weight of the allergen specific IgE antibody reacting with the allergen disc is determined by comparison of the anti-IgE binding to it and that binding to a parallel disc bearing anti-light chain antibodies to which a known weight of IgE_m is bound (Fig. 1). The weight (expressed in WHO units) of IgE_m binding to this disc is calculated directly from the ^{131}I counts.

The ability to measure the actual weight of IgE antibody binding to an allergen disc allows the precise

conditions for the inhibition RAST to be set. The working concentration of atopic serum is determined, by titration, such that after incubation with an allergen disc it facilitates the binding of exactly 200 mU of IgE antibody to the allergen disc in the absence of the inhibiting allergen. Inhibition of this standardized RAST is in essence a competition between solid and fluid phase allergen for IgE antibodies in an atopic serum. Therefore, the weight of IgE molecules bound decreases with increasing allergen concentrations. At a given concentration of allergen, the weight of IgE antibodies prevented from binding will equal 100 mU IgE (Fig. 2). The concentration of soluble allergen preventing these 100 mU IgE from binding to the allergen disc is taken as the basis for the allocation of a potency unit to the extract.

Standardization of allergen extracts in terms of their ability to bind to defined weights of IgE antibodies has universal application being independent of allergen, reference allergen preparations and atopic sera, and dependent only upon the WHO standard serum for IgE for the quantification of IgE protein contained in a labelled myeloma preparation.

We believe the above procedure offers the following advantages.

- Represents a universal unit
- Independent of allergen reference preparations
- Independent of allergen and applicable to mixtures
- Independent of a patient panel and inherent variation of *in vivo* testing
- Few legal and ethical restrictions
- Independent of geographical area
- A measured parameter — not an arbitrary unit
- Suitable candidate as an international standard
- A relevant standard for RAST measurement.

The unitage derived from such a standard procedure makes it the most applicable for acceptance to form the basis of an International Reference for allergen standardization.

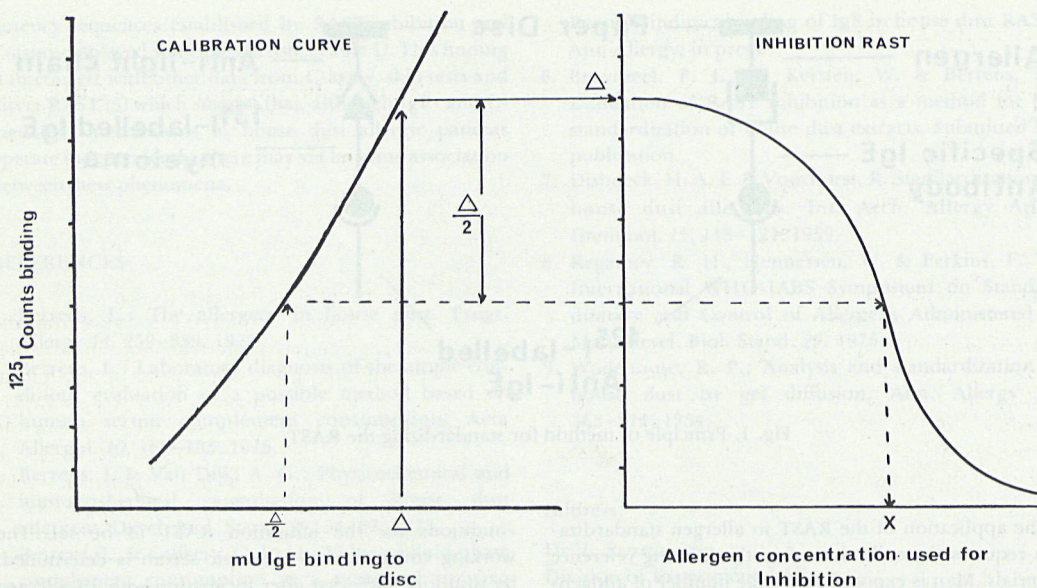


Fig. 2. Assay of allergens by inhibition of standard RAST

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