

Monoclonal antibody assays for allergens: Pick your antibodies with care!

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Allergen assays have two main fields of applications: the determination of the potency of allergen preparations intended for diagnostic or therapeutic use and the assessment of allergen exposure from environmental samples. These will be referred to as “extract analysis” and “dust analysis,” respectively. As with most analytic assays, the primary outcome is a dimensionless potency ratio: the potency of the analyte (ie, the unknown sample) relative to a reference. The three main issues to be evaluated are precision, reproducibility (eg, Do we get the same potency ratio when we use different reagents or different procedures?) and relevance (Does the assay outcome predict what we want to know?).

In assays in which the active substance in the unknown sample is qualitatively identical to that in the reference, the interpretation of the assay result is straightforward, the only issue being the analytic precision (ie, the 95% confidence interval of the potency ratio). In the case of allergen assays, the situation is almost always more complicated because of qualitative differences between the analyte and the reference. In the premonoclonal era, the multiplicity of the allergens within a single extract was a point of concern: for a valid assay with polyspecific reagents (eg, radioallergosorbent test inhibition) the relative concentrations of the major allergens within the unknown extract and the reference had to be similar. The obvious and sensible choice was to move to monospecific assays. This move has been greatly facilitated by the increasing availability of monoclonal antibodies to major allergens. The major advantages are well-definable specificity and inexhaustibility of reagent supply. Thus analytically robust and reproducible assays can and should be used.

We have had these problems all the time, but before the monoclonal era, we could blame the reagents for spurious results. The existence of well-defined reagents makes the real problems much more obvious. The main source of trouble is the variability of the allergen: biosynthetic variability (isoforms, glycosylation, etc) and postsynthetic

variability (fragments, aggregates, allergens modified chemically by UV or oxygen, [partial] denaturation, and others). These modifications may increase reactivity (gain variants) or decrease reactivity (loss variants). Problems arise if the effect of these modifications on immune reactivity is different from the effect on biological activity.

Three situations will be considered in which a modified allergen (modified in a broad sense: isotypic variation, denaturation on storage, etc) is compared with a reference allergen:

1. *Biological reactivity is lower; immune reactivity remains unchanged.* This is typically a problem for allergens that tend to lose biological activity during storage. Antibodies may or may not discriminate between active and inactive allergens. For extract assays, the latter is an undesirable situation because the potency is overestimated. On the other hand, for dust assays, it may be a desirable situation because the assay performs well even after storage of the samples.
2. *Immune reactivity is lower; biological reactivity remains unchanged.* For example, the antibody fails to detect an isoform of the allergen. Unless the nonreactive isoform is prominent (ie, represents more than 50% of the total allergen), the effect will be relatively small (a less than twofold underestimation). For many allergens, we do not know the relative abundance of the isoforms in dust or in the source materials used for extracts so there is some reason for concern. The avoidance of antibodies with marked isoform preference or the use of mixtures of antibodies (both the coating antibody and the detecting antibody) is prudent. Moreover, the use of reagent excess also will help to avoid major discrepancies (see below).
3. *Immune reactivity is higher; biological reactivity remains unchanged.* This situation may present itself in the case of isoform variability (see above) but has a potentially more dramatic impact in the case of neoepitopes: immune reactivity that is generated during processing or storage. Monoclonal antibodies with specificity for neoepitopes can be obtained accidentally (see the article by Batard et al [p 669]) or intentionally by immunization with (partially) unfolded allergen.¹ In these two cases, the antibodies show very little if any reactivity with the native allergen, but more subtle effects also are likely to occur: antibodies that show some reactivity with the native allergen but a much higher reactivity with the modified allergen. In this latter case, the difference in immune reactivity may be diminished by using a sufficiently large reagent excess.

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The debate on the clinical relevance of neoepitopes stems primarily from the field of food allergy in which an increase in biological activity has been reported in selected cases. The most prominent example is the selective sensitivity for cooked fish in relation to the original Prausnitz-Küstner reaction, but other examples of enhanced reactivity toward processed or partially digested food allergens have been described. From the point of view of allergen standardization, these inducible biologically active neoepitopes represent a challenge that most of us would not like to meet yet. Fortunately, it seems to be exceptional that such epitopes are overwhelmingly important. Antibodies reactive with neoepitopes that do not contribute to clinical symptoms should not be used for routine allergen quantitation in relation to either extract analysis or dust analysis. However, they may be useful to study deterioration of allergens.

The problems mentioned above indicate that monoclonal antibodies need to be used with care: they have a very well-defined specificity, but this specificity may be too high.^{2,3} Some persons might argue that we should return to human IgE and radioallergosorbent test inhibition. As discussed before, there are many reasons why this is not the best solution, one of which is that even human IgE antibodies do not necessarily correlate with biological activity.⁴ The next generation of assays is likely to be based on oligoclonal antibodies (ie, mixtures of monoclonal antibodies). Such assays are more robust than strictly monoclonal assays.

The selection of suitable antibodies is one concern. The other concern is how to use these antibodies. As mentioned above, some of the more subtle differences will become apparent only if the antibodies are used below critical con-

centrations. Differences in affinity among allergen variants will disappear when the antibodies are used in high enough excess. From an economical point of view, investigators might be tempted to use their antibodies at the lowest concentrations that give acceptable dose-response curves with the reference material. However, if the unknown samples (the extracts or the dust samples) contain allergen variants for which the reagents have a higher or lower avidity than for the reference allergen, the use of the highest acceptable reagent concentration might be indicated. Economic considerations may have to be balanced against reliability in the face of epitope variability.

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