Introduction:
In this article we will discuss issues pertaining to allergen extract endogenous enzymes and how they may deleteriously affect the allergen and immunotherapy half-life. If one allergen extract’s endogenous protease activity diminishes the immunogenicity of other allergens in the same solution, then the effective dose and benefit of these affected allergens is lowered. This is particularly applicable to proteolytic enzymes found in some fungal and whole-body insect allergenic extracts. Proteolytic degradation can lead to ineffective immunotherapy. Furthermore, if such immunotherapy is administered over a period of time potency will diminish, then injections from new vials (i.e. not initially degraded by endogenous enzymes) will be more potent and may lead to an increased risk of adverse reactions and possible anaphylaxis (1, 2).

1. Which Allergens can be Mixed Safely:
Several allergens such as molds and insects (cockroach) contain enzymes (proteases) which degrade other allergens mixed in the immunotherapy (IT) solution. For example, if grass pollen extracts are mixed with molds such as Aspergillus, Alternaria, or penicillium, studies have shown that after 6 months of storage, grass pollen potency is diminished by 85 percent (1). Therefore, we need to be careful and knowledgeable about which allergens can be safely mixed together in order to minimize the premature “allergen degradation” and subsequent loss of immunogenicity of the IT solutions.

Table 1 below illustrated compatible and incompatible mixed combinations of allergen extracts based upon their endogenous protease content (1, 2).

<table>
<thead>
<tr>
<th>Allergen-Extract</th>
<th>PROTEASE CONTAINING</th>
<th>EXTRACTS</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>INSECTS</td>
<td>MOLDS</td>
</tr>
<tr>
<td>Insects</td>
<td>M</td>
<td>C</td>
</tr>
<tr>
<td>Molds</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>Dust Mites</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Pollens</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>Cat hair/epithelia</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>Dog hair/epithelia</td>
<td>C</td>
<td>M</td>
</tr>
</tbody>
</table>

C = Compatible; M = Moderately Compatible; I = Incompatible
As demonstrated in table I above (where insects means cockroach), all combinations with a “C” are compatible and result in “safe” mixtures. Therefore for example, it is fine to mix a dog hair extract with extracts of insects (cockroach) and/or dust mites.

By examination of table #1, one can develop a scheme of compatible allergen solutions for immunotherapy injections. Such a 2 vial (or injection) scheme is presented in table 2 below.

<table>
<thead>
<tr>
<th>TABLE 2: IT VIAL CONSTITUENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vial 1</td>
</tr>
<tr>
<td>Pollens*</td>
</tr>
<tr>
<td>Dog Hair/Epith.</td>
</tr>
<tr>
<td>Dust Mites</td>
</tr>
</tbody>
</table>

*with minimal cross-reactivity (see previous article)  
* *can be in either vial 1 or vial 2

2. **Maximum Number of Extracts per IT Vial:**

Studies have demonstrated that the effective number of allergens per IT vial ranges from 7 to 15 (1). Thus, we recommend that the maximum number of allergens may be 10 per vial. We will utilize a maximum of 2 vials (see table 2 above). In the event there are more than 20 positive allergy scratch tests, then still a maximum of 20 only will be utilized. The selection of these 20 will depend upon the patient’s environment and the strongest positive allergen reactions as determined by the largest scratch test wheal with associated erythema (3). For example, if a patient is allergic to dogs and cats but only has a dog at home, then the IT vial will contain the dog extract, but not the cat extract. In addition after consideration of cross-reactivity (see previous article), if 17 positive scratch test wheal diameters with associated erythema are greater than 15 mm while 3 other positive tests are 10 mm wheals with associated erythema and 5 more positive test wheals with associated erythema measure 5 to 8 mm, then the IT vials will contain the 17 extracts whose reactions were greater than 15 mm and the 3 measuring 10 mm. However, the 5 allergen tests of 5 to 8 mm will not be included in the vials since 20 allergens have already been selected and are the maximum to be utilized (a maximum of 10 extracts per vial x 2 vials).
As discussed above, due to enzyme induced degradation (see table I above), only some extracts can be mixed together in the same vial. The specifics and regimen of compatible mixing are illustrated in Table 2 above.

4. **A Practice Example**

Suppose that Mr. Smith is found on allergy scratch prick testing to have the following allergen wheal with associated erythema diameter results:

Histamine -25 mm, Saline- 1 mm, Timothy grass- 20 mm, Orchard grass- 18 mm, Johnson grass- 19 mm, Bahia grass-16 mm, pigweed- 19 mm, lambs quarter 14 mm, red root-12 mm, Alder-18 mm, Oak-20 mm, Birch -15 mm, Hazel tree- 16 mm, ragweed mix- 7 mm, Kentucky blue grass- 8 mm, redtop grass -6mm, Dust mite mix- 20 mm, Cockroach mix-17 mm, Aspergillus fumigates- 9 mm, Cladosporium- 19 mm, Alternaria- 18 mm, Fusarium-8 mm, Helminthosporium-14 mm, dog dander-8 mm, and cat dander-9 mm.

Analysis:

1. Since Mr. Smith owns a dog but not a cat, the IT vials will contain dog dander only and not cat dander.
2. We have cross-reactive grasses consisting of Timothy, Orchard, red top and Kentucky blue (fig. 1, previous article). Also, Bahia and Johnson grasses are cross-reactive (fig. 1, previous article). Therefore, we can utilized Timothy and Johnson grass extracts only to be mixed in an IT vial in lieu of all 6 grasses.
3. The cross-reactive weeds: pigweed, lambs quarter, and red root can all be represented by the addition of pigweed only to an IT vial (table 1, previous article).
4. The cross-reactive trees: Alder, Oak, Birch and Hazel, can be represented by the addition of Alder tree extract only to an IT vial (table 1, previous article).
5. Based upon mold cross-reactivity principles (fig. 2, previous article), Alternaria can be used to represent itself and Cladosporium and Helminosporium. In a similar manner, Aspergillus fumigates can represent itself and Fusarium.
6. If we follow the scheme of table 2 (above), then the pollens (Timothy grass, Johnson grass, pigweed, and Alder tree) should be mixed with Dog Dander and the Dust Mite mix in IT vial # 1.
7. In contrast, the Molds (Aspergillus fumigates & Alternaria ) should be mixed with the Cockroach mix in IT vial #2.
8. Note that both vial # 1 (6 allergens-including mixes) and vial #2 (3 allergens-including mixes) contain less than 10 allergens each (maximum recommended). Also, by
following the scheme of Table 2 (above), we avoided problems with extracts containing proteases that might degrade other allergens in the mixture.

5. SUMMARY

The following steps are a review of the procedures for making IT vials:

1. Review the allergy scratch test results and eliminate the irrelevant positive allergen test results based upon the patient’s responses to questions about environmental-allergen exposure (e.g. dog and/or cat at home?).
2. Next, select the 20 largest scratch test results.
3. List the pollens (weeds, grasses, and trees), molds, cockroach, animal danders, and dust mites separately.
4. Use these lists (#3 above) in conjunction with the cross-reactivity information of the previous article to minimize the extracts needed to be added to each vial.
5. Then use table 2 to determine which extracts need to be added to vial 1 and vial 2.

References: