Title: Improved In-vitro Allergy Diagnostics using a Molecular Blend of Purified Peanut Allergens.

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Rationale: Peanut extracts and/or purified allergens are routinely used for allergy diagnostics. Allergen extracts contain mixtures of allergenic and non-allergenic substances with variable allergen composition which may complicate clinical interpretation. The aim was to produce a standardized molecular peanut blend (MPB) with high concentrations of major peanut allergens for diagnostic purposes.

Methods: Peanut allergens Arah1, Arah2, Arah3, and Arah6 were purified from defatted peanut. Equal amounts of the four purified allergens were combined and analyzed by SDS-PAGE. Total protein yield was measured by BCA and allergen composition was analyzed using allergen-specific ELISA and mass spectrometry. IgE binding was validated using a chimeric ELISA.

Results: SDS-PAGE showed pronounced bands of the expected molecular weights of 64kD (Arah1), 17-19kD (Arah2), 14-45kD (Arah3), and 15kD (Arah6). Mass spectrometry (LC-MS/MS) analysis confirmed the presence of all four allergens with matching abundances of ~25% (17-34%). Total protein concentration was 1.6 mg/ml, and each allergen was quantified at ~0.4mg/ml (0.3-0.45 mg/ml) using allergen-specific ELISA. All 16 sera from peanut-allergic individuals reacted to MPB and to a peanut-flour extract: 9/16 sera had ~66% higher reactivity to MPB, while two sera had moderately higher (~27%) reactivity to the peanut-flour extract.

Conclusions: Optimized, ISO-9001 compliant, bioprocessing pathways have been established to yield a standardized molecular peanut allergen blend with a defined allergen profile. The MPB should improve the diagnostic efficacy of *in-vitro* IgE tests and could, in future, provide an alternative to allergen extracts for allergy diagnosis.