Stability of Allergenic Extracts in Multi-Test II® and Duotip-Test® II Skin Testing Wells

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Abstract

Rationale: Skin testing routinely uses percutaneous devices that fill into plastic wells that contain the testing allergen extract. The allergen solution in the wells can be used many times (~100 to 300 times) with new devices for various lengths of time and practice conditions. The purpose of this study was to determine the stability of several allergenic proteins that contribute to the activity of the extracts in these wells.

Methods: Multi-Test II® and Duotip-Test® II devices from Lincoln Diagnostics were placed in their corresponding allergen Dipwell trays loaded with allergen diagnostic strength extracts (50% glycerin concentrates, ALK-Abelló, Inc.). The amount of major allergen protein was determined using microtiter ELSAs methods from ALK-Abelló or in the case of short ragweed. FDA-polymer Antib 1 serum. The wells were tested after storing 1 month at room temperature, 3–6 months refrigerated, or 3–14 days at 40°C.

Results: Timothy, Bermuda, English plantain, short ragweed, cat hair, olive tree pollen, mugwort, white birch, and mixed mite all showed very good stability even at room temperature for 1 month. Slight evaporation from the Multi-Test II wells was detected resulting in <10% increases in major allergen concentration. Non-glycerinated birch extract completely evaporated after 1 month at room temperature. The non-standardized extracts were 1:20w/v in 50% glycerin. Histatrol® positive histamine control was also placed in wells and measured using HPLC. Wells were tested at 1 month room temp, 3 and 6 months refrigerated and 3-14 days at 40°C.

Conclusions: Importantly allergens related to the potency of diagnostic extracts are stable in Multi-Test II and Duotip-Test II Dipwell trays for up to 3 months. Occasional room temperature exposure during this time would not affect the activity of these allergens.

Background: Diagnostic Allergen Stability

Allergen extracts are conveniently stored in skin test device trays, in some cases for several months, yet information about the stability of the allergen protein active ingredients is lacking. Stability of the standardized extracts; cat hair/pet, short ragweed, mite, and some grass pollens, at diagnostic concentrations in 50% glycerin, is known and reflected on the vial label, however, expiration dating is determined with these extracts stored in a sealed vial. Extracts used for skin testing using the tray wells are exposed to air, plastic devices, plastic wells and are at room temperature for various lengths of time.

Methods: Extract Characterization

Complexity

Protein profile by SDS-PAGE. 10-20% tris-glycine gels stained with Coomassie Blue or Silver Stain for cat hair.

Potency

Potency or amount of active ingredients.

Major allergen proteins determined by direct binding ELISAs developed and validated by ALK-Abelló. Timothy pollen Phl p 5, Mite Der 1 and Der 2, Cat hair Fel d 1, Birch Bet v 1, Bermuda Cyn d 1, Olive pollen Ole e 1, English plantain Pla l, and Mugwort Art v 1 were measured in the respective diagnostic strength extracts. (references available). Amb a 1 in Short Ragweed was determined using FDA rafael immunofluorescence test.

IgE binding ability determined with specific atopic sera by competition ELISA methods using FDA references and sera for standardized house dust mite, Timothy grass, and Bermuda.

Conclusions

* This study determined the stability of several standardized and non-standardized allergenic extracts in Multi-Test II and Duotip-Test II wells using both protein profile and potency methods.

* Extracts are stable for 3 months refrigerated and 1 month at room temp. Protein profiles were unchanged. Some decreases in potency and changes in profile occur by 6 months.

* Some extracts show significant loss of activity at elevated temperature (40°C) suggesting that care should be taken to note the ambient room temperature.

* Histatrol® positive histamine control was also stable to be able for 1 month room temp and 6 months refrigerated.