# New dot-blot methods for evaluating the effect of inactivators on mite and Japanese cedar pollen allergens

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**Background:** Sandwich ELISA has been used to evaluate potential allergen inactivators. However, inactivators included in the samples affect the assay's ability to measure allergen levels. Allergen levels measured by the ELISA may be underestimated due to the effect of the inactivator and, thus leading to overestimate the inactivation potential. In this study, to evaluate inactivators while avoiding interference with the assay's ability, we used the dot-blot method.

**Methods:** Each amount (4, 20, 100 ng) of mite (Der f 1) allergen was immobilized on PVDF membranes, which were treated with each inactivator (5 ppm sodium hypochlorite, 6 M guanidine, 3.5 mM sodium lauryl sulfate (SDS)) against the immobilized allergens and washed afterwards. Next, membranes were divided into two groups, then ones were immunostained for the allergens and the others were gold stained for protein detection. For immunostaining, the allergens on the membrane were detected with anti- Der f 1 IgG monoclonal antibodies against the allergens. For protein detection, gold staining was performed. The same operation was carried out for Japanese cedar pollen allergen (Cry j 1).

**Results:** Anti-Der f 1 IgG reactivity to allergens on the membrane was studied by using the immunostaining method. Anti-Der f 1 IgG was reactive under the following conditions: 4 to 100 ng of Der f 1 with PBS treatment (no activator), 20 and 100 ng of Der f 1 with guanidine treatment, and 100 ng of Der f 1 with sodium hypochlorite and SDS treatment. Next, protein staining was performed to confirm whether Der f 1 was detached from the membrane by the inactivator. All spots (4 to 100 ng) of Der f 1 in all membranes were detected using protein staining and confirmed that the allergen proteins were not detached from the membranes. The dot blot analysis of Cry j 1 allergen was almost the same as that of Der f 1.

**Conclusion:** We made it possible to accurately evaluate inactivators for allergens using the dot-blot method, which combines immunostaining and protein detection methods.