Test report

Hoshi University commissioned research No. 418-004-1

7 July, 2004

Applicant Mitsubishi Paper Mills, Ltd.

Specimen Aller Sweep

Title Tick allergen inactivation test

The result of the test to the specimen above is as follows on 7 June, 2004.

Hoshi University applied microbiology laboratory
2-4-41, Ebara Shinagawa-ku, Tokyo 142-8501 Japan

Tick allergen inactivation test

1. Applicant
   Mitsubishi Paper Mills, Ltd.

2. Specimen
   Aller Sweep

3. The purpose of the test
   To investigate the efficiency of the inactivation against the tick allergen.

4. Test outline (sandwich ELISA method)
   (1) Put 50µl of coating solution (1µg/ml of anti-purified mite antigen monoclonal antibody 15E11) into 96-well ELISA plate and leave them at 4℃ overnight.
   (2) After removing the coating solution, wash the well three times with 300µl of phosphate buffer (PBS-T, phosphate buffered saline with 0.1% Tween 20).
   (3) Add the specimen (Aller sweep, 25mm^2 of sample area) and 50µl out of 300µl mite antigen rDerf2 solution (125ng/ml) to the well and leave them for two hours at room temperature.
   (4) Wash each wells three times with 300µl of PBS-T, add 50µl of peroxidase stained anti-purified mite antigen monoclonal antibody (13A4PO, 100ng/ml) to them and leave them for two hours at room temperature.
   (5) Wash each wells three times with PBS-T, add 100µl of substrate solution to them and leave them for twenty minutes at room temperature.
   (6) Add 100µl of 1%-SDS solution to stop reaction. Measure the absorbance of 405nm solution to calculate the concentration of mite antigen rDerf2 solution.

[Reagent]

・PBS-T (Sodium chloride: 8g, Potassium, chloride: 0.2g, Sodium hydrogen phosphate 12 saturated solution: 2.9g, Potassium dihydrogen phosphate: 0.2g, Tween 20 1g/l)
・Mite antigen rDerf2 (made by Asahi Breweries Ltd.)
・Anti-purified mite antigen monoclonal antibody (made by Asahi Breweries Ltd.)
・Peroxidase stained anti-purified mite antigen monoclonal antibody 13A4PO (made by Asahi Breweries Ltd.)
・1%-SDS solution (1% Sodium dodecyl sulfate solution, made by Wako Pure Chemical

Corporation)

・Substrate solution (2.2'- Azino-bis (3-ethylbenzothiazoline 6-sulfonic acid) solution, made by Wako Pure Chemical Corporation)

5. The result of the test

The decrease of the amount of mite antigen rDerf2 in the solution can be confirmed by mixing specimen and mite antigen rDerf2.

The concentration of mite antigen rDerf2 in the solution at the beginning of the test	125ng/ml
The concentration of mite antigen rDerf2 in the solution three hours after mixing the specimen	42.8±3.0ng/ml (n=5)

Test report

Hoshi University commissioned research No. 418-003-1

7 July, 2004

Applicant Mitsubishi Paper Mills, Ltd.

Specimen Aller Sweep

Title Cedar allergen inactivation test

The result of the test to the specimen above is as follows on 7 June, 2004.

Hoshi University applied microbiology laboratory

2-4-41, Ebara Shinagawa-ku, Tokyo 142-8501 Japan

Cedar allergen inactivation test

1. Applicant
   Mitsubishi Paper Mills, Ltd.

2. Specimen
   Aller Sweep

3. The purpose of the test
   To investigate the efficiency of the inactivation against the cedar allergen.

4. Test outline (sandwich ELISA method)
   (1) Put 50µl of coating solution (1µg/ml of anti-purified cedar antigen monoclonal antibody mAb 013) into 96-well ELISA plate and leave them at 4℃ overnight.
   (2) After removing the coating solution, wash the well three times with 300µl of phosphate buffer (PBS-T, phosphate buffered saline with 0.1% Tween 20).
   (3) Add the specimen (Aller sweep, 25mm^2 of sample area) and 50µl out of 300µl purified cedar pollen antigen Cryj1 solution (125ng/ml) to the well and leave them for two hours at room temperature.
   (4) Wash each wells three times with 300µl of PBS-T, add 50µl of peroxidase stained anti-purified cedar antigen monoclonal antibody (mAb053, 50ng/ml) to them and leave them for two hours at room temperature.
   (5) Wash each wells three times with PBS-T, add 100µl of substrate solution to them and leave them for twenty minutes at 25℃
   (6) Add 100µl of 1%-SDS solution to stop reaction. Measure the absorbance of 405nm solution to calculate the concentration of purified cedar pollen antigen Cryj1 solution.

[Reagent]

・PBS-T (Sodium chloride: 8g, Potassium, chloride: 0.2g, Sodium hydrogen phosphate 12 saturated solution: 2.9g, Potassium dihydrogen phosphate: 0.2g, Tween 20 1g/l)
・Cedar antigen Cryj1 (made by Hayashibara Biochemical Laboratories)
・Anti-purified cedar antigen monoclonal antibody (made by Hayashibara Biochemical Laboratories)

・Peroxidase stained anti-purified cedar antigen monoclonal antibody mAb053 (made by Hayashibara Biochemical Laboratories)
・1%-SDS solution (1% Sodium dodecyl sulfate solution, made by Wako Pure Chemical Corporation)
・Substrate solution (2.2'- Azino-bis (3-ethylbenzothiazoline 6-sulfonic acid) solution, made by Wako Pure Chemical Corporation)

(7) The result of the test

The decrease of the amount of Cedar antigen Cryj1 in the solution can be confirmed by mixing specimen and Cedar antigen Cryj1.

The concentration of Cedar antigen Cryj1 in the solution at the beginning of the test	125ng/ml
The concentration of Cedar antigen Cryj1 in the solution three hours after mixing the specimen	46.6±3.8ng/ml (n=5)