GROUPE PSA

Materials test method

D19 5004

ASSESMENT OF A CAR'S FILTER EFFICIENCY TOWARDS ALLERGENS

This standard REPLACES standard B53 3240

This standard is published by the PSA Standard Department (DQI/DAPF/RHN).
For any question, contact us at the next address: normespsa@mpsa.com.
This standard is available in the internet: NORMES and on the portail B2B.
In the event of litigation, only the English version of this standard is taken.

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RECORDS

Index	Date	Type of modifications
OR	17/10/2018	CREATION OF THE METHOD.

PARTICIPANTS

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CONTENTS

1.PURPOSE AND FIELD OF APPLICATION

The purpose of this method is to describe a test for measuring the efficiency of filters towards allergens. An allergen is a substance capable of triggering an allergic reaction.

The purpose of this document is to describe the quantitative analysis of allergens on a surface. To reach this objective, a method in a controlled atmosphere (volume, T°, HR) is described.

2.REFERENCE DOCUMENT

2.1.NORMS

2.1.1.PSA INTERNAL NORMS

A10 0156 TEST REPORTS - WRITTING

2.1.2.EXTERNAL STANDARDS

Not applicable.

2.2.REGULATIONS

Not applicable.

2.3.OTHER DOCUMENTS

Not applicable.

3.TERMINOLOGY

A dictionary (glossary) with main definitions used at PSA R&D is available via DESP or directly at the address: SCPO_MGPJ07_0116.

3.1.DEFINITION

Filter: Device to be tested, micro-organisms trapping.

Security device: Guaranty the non-contamination of the media used and of the persons.

Test Bed: Device in Plexiglas with defined volume with controlled introduction of micro-organisms.

3.2.ACRONYMS

BSC **B**iological **S**avety **C**abinet
ELISA **E**nzyme-**L**inked **I**mmuno**S**orbent **A**ssay
HE **H**igh **E**fficiency

4.TEST DESCRIPTION

The principle is to quantify the amount of several allergens (pollen, mite, cat...) trapped by a filter in a hermetic and specific test bed. The filter to be tested is introduced in a Plexiglas specific device (developed by PSA, ref: FR1660043).

An air flow is produced by a system equipped with a fan in the device. A specific solution of powder of several allergens is introduced into the device.

After diffusion, the filter is collected and crushed in the aim to collect allergens trapped into the filter.

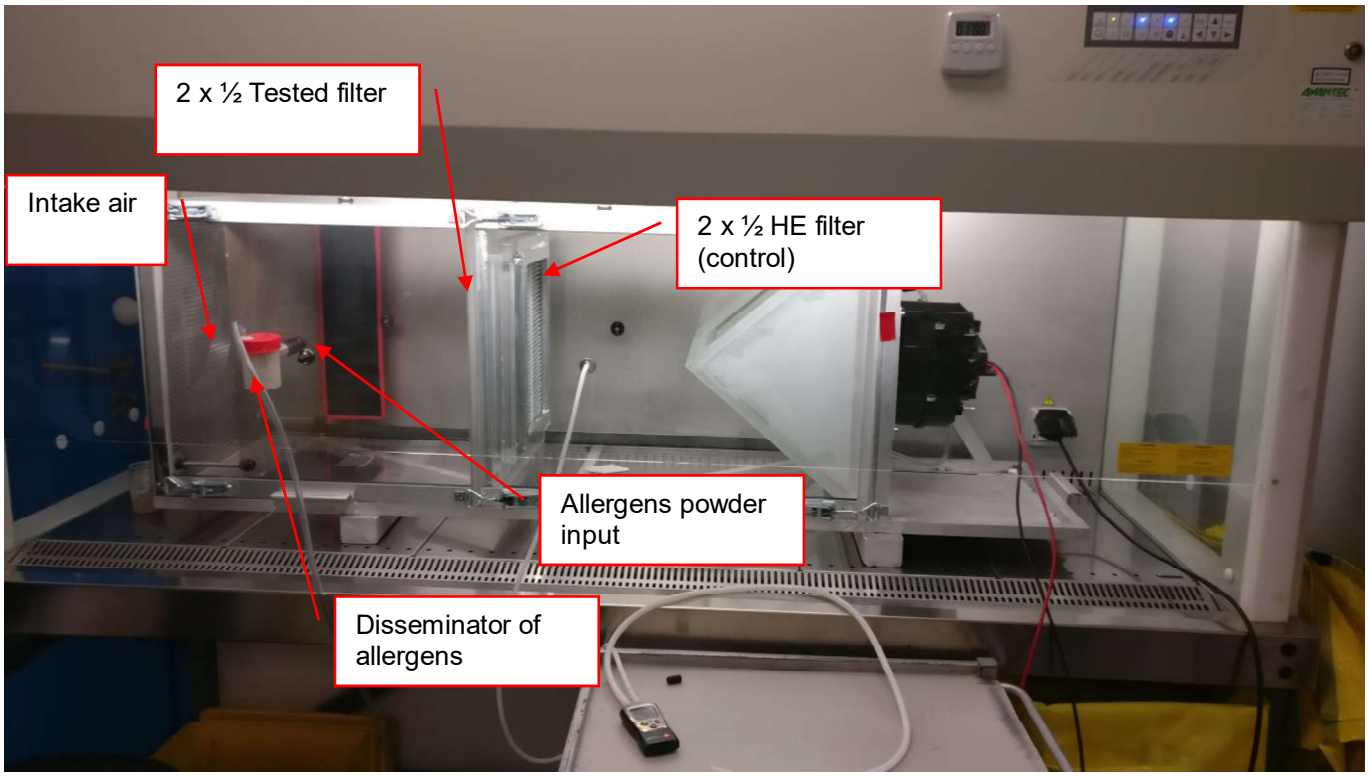
The device is equipped with a HE filter (High Efficiency) to collect the allergens not stopped by the tested filter. After the test, the amount of allergens trapped by the HE filter is measured following the same procedure than for the tested filter.

The efficiency of the filter is calculated by the difference between the two filters (tested filter and HE filter).

5.EQUIPMENT

5.1.TEST BED

The Test bed is placed under a Biological Safety Cabinet (type 2).



5.2.SPECIFIC EQUIPMENT FOR ALLERGEN TESTS (SEE CHAPTER 4)

- Biological Savety Cabinet,
- Agitator (Vortex),
- Mixer,
- Precision balance 0,001g,
- Microscope,
- Microplate Spectrophotometer with OD at 405nm,
- Micropipette (1000µl, 500µl, 100 µl),
- HE filter (reference: Peugeot B78HE 16 169 591 80).

6.ALLERGENS REPRESENTATIVENESS

Allergens are chosen by PSA with assistance of Pasteur Institute.

The allergens can be bought at Stallergene-Greer Laboratory.

- Birch (Betula pendula) - Greer ref.: RM527,
- Ragwwed (Ambrosia artemisiifolia) - Greer ref.: RM56,
- Timothy (Phleum pratense) - Greer ref.: RM28,
- Dander cat (Felis domesticus) - Greer ref.: RME63P,
- Mite (D. pteronyssinus) - Greer ref.: RMB84M,
- Mite (D. farinae) - Greer ref.: RMB83M,
- Alternaria alternata - Greer ref.: MY1.

The selection criteria must be mentioned into the test report A10 0156.

In any doubt, contact PSA Groupe [*DRIA/ACBI/THQA*]

6.1.PREPARATION OF ALLERGEN MIX

6.1.1.MINIMUM OF DETECTION FOR EACH ALLERGEN

The minimum of detection is on HE filter control. It is based on the minimum concentration (standard curve) and the volume of control filter washed:

Calculation method for minimum detection:

$$L = C_{mini} \times V_{mini} \times \frac{1}{1000}$$

L = Minimum of quantification (U or µg),
C_{mini} = Minimum Concentration on standard curve (U/ml ou ng/ml),
V_{mini} = Volume of wash liquid used for control filter (e.g. 250 ml),
1/1000 = dilution factor ng -> µg (used for ng only).

6.1.2.MINIMUM AMOUNT TO BE DISSIMINATED FOR EACH ALLERGEN

The amount of disseminate powder is based on the minimum of quantification and the efficiency required. For 99% efficiency, it's necessary to have a minimum of 100 times more quantity of allergens on the essay filter compared to the filter control (For 99,9% it's needed to collect 1000 times more).

An ELISA test is realized with a liquid solution of each allergen: 100mg of allergen in 100 ml of sterile water for example.
The result of ELISA reaction indicate an amount of antigen in 100 mg of allergen. The amount of allergen is calculated to obtain the minimum of allergen powder.

The equation is:

$$P_{min} = L \times 1000 \times \frac{Q}{p_{manual}}$$

P_{min} = Minimum of allergen powder (mg),
L = Minimum of quantification (U or µg), obtain with standard curve,
Q = Antigen amount on solution (100mg of allergen),
$P_{solution}$ = Amount of powder = 100mg.

The minimum of amount must be calculated for each batch. A test (3 essays) need to use a same batch.

6.1.3.PREPARATION OF MIX ALLERGEN

The mass of each allergen is define by the ELISA test sensitivity.
The amount below are defined with an efficiency of 99,9%, for example:

- Birch (Betula pendula): 250 mg,
- Ragwwed (Ambrosia artemisiifolia): 300 mg,
- Timothy (Phleum pratense): 400 mg,
- Dander cat (Felis domesticus): 100 mg,
- Mite (D. pteronyssinus): 1000 mg,
- Mite (D. farinae): 300 mg,
- Alternaria alternata: 2700 mg,
- Arizona dust: 5 g.

6.1.LABORATORY SAFETY EQUIPEMENT

Personal Protection Equipment:

- Safety glasses,
- Safety mask,
- Lab Coat,
- Chemistry gloves.

7.TEST PROCEDURE

7.1.CLEANING OF THE TESTBED

Before every test, the surfaces of the test bed must be cleaned with a alcohol solution (single use tissue). The disseminator is cleaning after essay.

7.2.PREPARATION OF DISSEMINATION

7.2.1. ALLERGENS PREPARATION

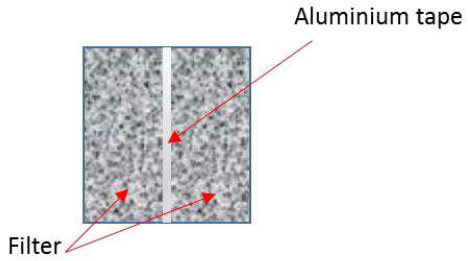
See 6.1.

7.2.2.FILTER PREPARATION

No specific treatment of the filter.

7.2.3.TEST INTO THE TESTBED

The filters are introduced into the specific area of the test bed. The filters are maintained with aluminum tape allaround the support.
To avoid the linkage, a double-sided tape is applied between the same filters (set of test filter and set of filter control).



7.3.DISSEMINATION

7.3.1.ALLERGENS DISSEMINATION

Dissemination of allergens procedure:

- Add mix (allergen + dust) in the nebulizer,
- Turn on the airflow of bed test with a debit of 500 $kg.h^{-1}$,
- Turn on the air compressor with a pressure between 0,5 and 1,5 bars: this variation create a variable air flow and accelerate the dissemination,
- Disseminate all the amount of mix in the nebulizer. A visual control of nebulizer permit to evaluate the end of experiment. Turn off the compressor. (when all the mix allergens is sprayed),
- Disassemble the filters.

7.3.2.ALLERGENS QUANTIFICATION

Collect of allergens:

- Cut each filter into 2 pieces,
- Put the pieces one at a time into a sterile box with 1000 ml of sterile water (volume to be adapted if needed),
- Wash during (by manual washing by steeping and kneading, friction of the filter to detach the maximum of biological particles) 5 -10 minutes each piece in the same water,
- Measuring of volume of washing liquid for each filter (pilot and controls). Volumes are measured for each test.

Note: *for control filter, the collect volume is 250 ml approx.*

- A dilution range (evaluated by experiment) of liquid is analysed with ELISA method (see appendix 1 for specific protocol provided by manufacturer).

8.RESULTS

8.1.STANDARD CURVE

The formula of standard equation used data of exponential curve:

$$y = a\ln(x) + b$$

a: slope
b: intercept

8.2.ANTIGEN AMOUNT ON FILTER

$$Q = \frac{C}{d} \times V_{total} \times \frac{1}{1000}$$

Q = quantity of antigen (µg),
C = concentration of allergen in well (x) (U/ml or ng/ml),
d = Dilution range,
V_{total}: Volume of wash solution (e.g. 600 ml for test filter / 75 ml for control filter),
1/1000 = dilution factor ng -> µg (used for ng only).

The results are expressed µg (or unit U) of allergen/filter.

8.3.TEST CONTROL

If one essay is too different of the other (more than 1log), this essay is not used for the calculation of efficiency.

8.4.EFFICIENCY CALCULATION

The ratio of the allergens on the tested filter/HE filter defines the rate expressed in % unit.

E = [Qtested/(Qcollecting + Qtested)] x 100

E: efficiency - %,
Qcollecting: Quantity of allergen on the collecting filter with the tested filter upstream,
Qtested: Quantity of allergen on the tested filter with the tested filter upstream.

The efficiency is calculated for each allergen.

9.TEST REPORT

See A10 0156 Standard.

The test report must include:

- The amount results,
- The standard used,
- The name and the address of the laboratory,
- The name of the technician,
- The device reference,
- The test date.

The incidents or unforeseen events must be reported.

APPENDIX 1:
ELISA METHOD WITH INDOOR BIOTECHNOLOGIES KIT

1. Coat polystyrene microliter plates (NUNC Maxisorp Cert NUNC catalog # 439454) With 100µl mAb 4B10 at 10µl/10ml, i.e. 1/1000 dilution of stock, in 50mM carbonate-bicarbonate buffer, pH 9.6, incubate overnight at 4°C.

2. Wash wells 3x With PBS-0.05% Tween 20, pH 7.4 (PBS-T). Incubate for 30 min. at room temperature with 100 µl of 1% BSA, PBS-T. Wash 3x with PBS-T.

3. Use doubling dilutions of the rBet v I standard to make a control curve ranging from 250 - 0.5ng/ml Bet v 1: Pipette 20 µl Bet v I standard into 180µl 1% BSA, PBS-T into wells A1 and B1 on the ELISA plate. Mix well and transfer 100 µl across the plate into 100 µl 1% BSA, PBS-T diluent to make 10 serial doubling dilutions. Wells A11, B11 and A12, B12 must contain only 1% BSA. PBS-T as blanks.

4. Add 100µl of diluted allergen samples and incubate for 1 hour at room temperature. House dust extracts for Bet v 1 analysis are routinely diluted tow-fold from 1/10-1/80. Other sample types, like air filter extracts and allergen extracts, may require different dilutions.

5. Wash Wells 3x with PBS.T and add 100µl diluted biotinylated anti-Bet v 1 mAb 2E10 The antibody solution contains 50% glycerol and must be diluted 1/1000 in 1%BSA, PBS-T. Incubate for 1 hour at room temperature.

6. Wash Wells 3x with PBS-T and add 100µl diluted Streptavidin- Peroxidase (Sigma S5512, 0.25 mg reconstituted in 1 ml distilled water). The reconstituted Streptavidin must be diluted 1/1000 in 1% BSA, PBS.T. Incubate for 30 minutes at room temperature.

7. Wash wells 3x and develop the assays by adding 100 µl_1mM ABTS in 70mM citrate phosphate buffer, pH 4.2 and 1/1000 dilution of H202. Read the plate when the absorbance at 405 nm reaches 2.0-2.4.

Notes: *The Bet v1 standard is recommended for immunoassay calibration purposes only. Not recommended for in-vitro antibody measurement, T cell studies, immunization purposes, or other uses.*
Buffer recipes, storage conditions and a list of frequently asked questions can be found under "protocols" on our web site: www.inbio.com.

For research and commercial use in vitro: not for human in Vivo or therapeutic use.