SUPPLEMENTARY MATERIAL

EEC technical characteristics

1. **General conditions in this environmental exposure chamber (see Section 2.1)**

In the context of conducting research within an EEC, it is imperative to clearly define the environmental parameters under investigation and establish precise value ranges for each of these parameters. The primary focus typically revolves around temperature, relative humidity and differential pressure, which values must be kept constant and invariant in order to achieve reproducibility in these environmental conditions.

Once these parameters are explicitly defined, it becomes essential to implement a robust monitoring system within the chamber to enable effective environmental

Although temperature and relative humidity depend on the global hospital conditioning system, the EEC incorporates a control knob that allows to configure the ventilation rate within the exposure room, which makes it possible to choose among different options in order to achieve the desired particle dispersion and concentration, as well as the posterior EEC air cleaning (Table S1).

2. **Distributing pollens in an environmental exposure chamber**

In the solid aerosol generator SAG 410 (Topas© GmbH, Dresden, Germany) it is possible to configure two parameters: the speed of the blades (preparation) and the speed of the conveyor belt (feed rate). The dispersion of pollen in the exposure chamber must be controlled, so these two variables are set to a minimum. These parameters are modified manually throughout the exposure in order to keep a constant particle concentration within the chamber.

This ventilation system may be set at five different modes, modifying the room's flow rate and its differential pressure, which must be kept negative enough in order to prevent contamination from the EEC to the exterior (see Table S1). This differential pressure, along with temperature and relative humidity (which will depend on the hospital's global air conditioning system), may be live-monitored by a Honeywell© meteorologic station. In order to study the airflow inside the EEC (Environmental Control Chamber), various simulations were conducted for each of the ventilation system configurations. This allowed for an approximation of the allergen distribution indoors for each case, enabling the definition of the ventilation mode to be used (mode 3, 10 air renewals/h), as well as the arrangement of furniture inside.

To know the air flow inside the chamber, two simulations have been carried out using ANSYS software, version 2020, setting the air renovation system at Mode 3 (10 renewals/h). The aim of these simulations is to find the most suitable configuration for ventilation grilles to achieve a homogeneous distribution of pollen in the chamber. The conclusion is that the optimum placement of the supply grille is -5° towards the room and the extract grilles at -80° towards the room. This configuration results in fewer stagnation areas where pollen accumulates, and a higher airflow in the area where the patient will be placed (see Fig. S1, Fig. S2).

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3. Pollen counting

The samples obtained by the Burkard were analyzed by means of an optical microscope, with built-in software. For image acquisition, ZEN© Microscopy Software (*https://www.zeiss.com/microscopy/en/products/software/zeiss-zen.html*), was used, being a useful tool for real-time image acquisition and saving. On the other hand, ImageJ© software was used for image processing (which mainly consisted of noise removal, background subtraction, contrast improvement and image binarization) as well as for pollen counting. ImageJ offers the possibility of storing line commands in macros, which allows counting automation and would imply an improvement in the reproducibility of image processing (see Fig. S3).

4. Cleaning protocols

Before pollen dispersal begins, real-time measurements with an airborne particle counter (SOLAIR© Boulder Counter) ensure that no pollen-sized particles of any kind remain inside the chamber.

Once the exposure is over, the cleaning mode is activated in the chamber (-50 Pa) to expel all pollen. At the same time, the tube is purged to avoid any residues inside the tube. Finally, all the equipment inside the room is cleaned manually with a damp cloth, and pollen is removed from the walls and floor by cleaning equipment including disinfectants, which have rounded edges to facilitate cleaning.

The disperser is cleaned every five uses. Regarding the cleaning of the pollen disperser, it is carried out in a separate room with a ventilation system to avoid contamination of the EEC observation area and contamination of the room itself.

Firstly, the feeding area of the disperser is disassembled according to the instructions in the manual of the device. Once the disperser is disassembled, the remaining pollen is removed, the loose parts are cleaned with a damp cloth, it is possible to clean them directly with water, regardless of the way, the parts must be dried completely without leaving wet areas, as they contaminate the pollen and leave pollen stuck in these areas.

Regarding the static parts (not dismountable), the remaining pollen on the saw teeth of the feeding belt, where the pollen sticks the most, is cleaned with a brush. Afterwards, simply wipe all parts with a damp cloth and leave it to dry completely. Once the cleaning is finished, it is assembled according to the instructions in the manual.

Technical parameters results:

Regarding the monitoring of environmental conditions, the following results were obtained, as can be seen in Fig. S4. During the 17 simulations, a temperature of (23.30 ± 0.01ºC), a relative humidity of (37.46 ± 0.05%), and a differential pressure of (-12.63 ± 0.31Pa) were recorded, all of these parameters being within the expected ranges.

Clinical endpoints

Total nasal symptom score (TNSS), total ocular symptom score (TOSS) (5), specific asthma survey and a visual analogue scale (VAS) to assess nasal, ocular and asthma subjective symptoms. A peak nasal inspiratory flow (PNIF) (In-check, Clement Clarke International Ltd, Wales, United Kingdom) and a peak expiratory flow (PEF) (Mini Wright, Clement Clarke International Ltd, Wales, United Kingdom) meters were used (see Table S2).

References

S1. Hamada, T., Terry, I., Roemer, R., Marler, T. E. Potential Drift of Pollen of Cycas micronesica on the Island of Guam: A Comparative Study. HortScience horts. 2015;50(7): 1106-1117

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