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Technical standards in allergen exposure chambers worldwide – an EAACI Task Force Report

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ABBREVIATIONS

ACH	air change per hour
AEC	allergen exposure chamber
AIT	allergen immunotherapy
COVID-19	Coronavirus disease 2019
CV	coefficient of variation
EAACI	European Academy of Allergy and Clinical Immunology
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
FDA	U.S Food and Drug Administration

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HDM	house dust mite
HEPA	high efficiency particulate air
HVAC	Heating, Ventilation, and Air Conditioning
IQ	installation qualification
LPC	laser particle counter
OQ	operational qualification
PCT	particle containment tape
PDU	particle dispersal unit
PEF	Peak exspiratory flow
PLU	particle line unit
PNIF	peak nasal inspiratory flow
PQ	performance qualification
RH	relative humidity
RIS	rotating impaction sampler
SARS CoV 2	Severe acute respiratory syndrome coronavirus 2
SD	standard deviation
SOP	standard operating procedure
TNSS	total nasal symptom score
VOC	volatile organic compound
WHO	World Health Organization

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ABSTRACT

Allergen exposure chambers (AECs) can be used for controlled exposure to allergenic and non-allergenic airborne particles in an enclosed environment, in order to (i) characterize the pathological features of respiratory diseases and (ii) contribute to and accelerate the clinical development of pharmacological treatments and allergen immunotherapy for allergic disease of the respiratory tract (such as allergic rhinitis, allergic rhinoconjunctivitis, and allergic asthma).

In the guidelines of the European Medicines Agency for the clinical development of products for allergen immunotherapy (AIT), the role of AECs in determining primary endpoints in dose-finding Phase II trials is emphasized. Although methodologically insulated from the variability of natural pollen exposure, chamber models remain confined to supporting secondary, rather than primary, endpoints in Phase 3 registration trials. The need for further validation in comparison with field exposure is clearly mandated.

On this basis the European Academy of Allergy and Clinical Immunology (EAACI) initiated a Task Force in 2015 charged to gain a better understanding of how AECs can generate knowledge about respiratory allergies and can contribute to the clinical development of treatments.

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Researchers working with AECs worldwide were asked to provide technical information in eight sections: (i) dimensions and structure of the AEC, (ii) AEC staff, (iii) air flow, air processing, and operating conditions, (iv) particle dispersal, (v) pollen/particle counting, (vi) safety and non-contamination measures, (vii) procedures for symptom assessments, (viii) tested allergens/substances and validation procedures. On this basis, a minimal set of technical requirements for AECs applied to the field of allergology is proposed.

Key words: Allergen exposure chambers, allergen immunotherapy, technical standards, clinical trials, COVID-19

INTRODUCTION

Allergen exposure chambers (AECs) are clinical facilities enabling the controlled exposure of subjects to allergens in an enclosed environment, with the objectives to (i) characterize the pathological features of respiratory allergies and (ii) contribute to and accelerate the clinical development of pharmacological treatments and allergen immunotherapy (AIT) for allergic disease of the respiratory tract (such as allergic rhinitis, allergic rhinoconjunctivitis, and allergic asthma) (1, 2).

In Europe, according to the European Medicines Agency (EMA) guidelines for the clinical development of products for AIT (3), AECs are considered as a useful tool by reading primary end-points for dose-finding studies. However, their role in confirmatory Phase III trials is confined to secondary end-points, although AECs might represent a helpful marker in longterm studies when the natural exposure cannot be evaluated due to low allergen counts (3-5). According to regulatory authorities, as a prerequisite for the potential use of AECs in Phase III trials their validation in comparison with clinical symptoms induced by natural exposure is necessary (3).

In 2015, the European Academy of Allergy and Clinical Immunology (EAACI) initiated a Task Force with the goal of gaining a better understanding of how AECs can generate knowledge on respiratory allergies and can contribute to the clinical development of treatments for these conditions (6). Ultimately, to provide evidence, if any, for their use in pivotal Phase III double-blind, placebo-controlled, randomized clinical allergen immunotherapy trials. This position paper (6) will be followed by a series of Task Force reports over the coming years.

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In 2019, researchers working with AECs worldwide agreed to provide information on eight technical sections: (i) dimensions and structure of the AEC, (ii) AEC staff, (iii) air flow, air processing, and operating conditions, (iv) particle dispersal, (v) pollen/particle counting, (vi) safety and non-contamination measures, (vii) procedures for symptom assessments, (viii) tested allergens/substances and validation procedures. As this is the first in the planned series, this Task Force report provides an overview of these technical standards and as such provides comprehensive insights into the technical set ups of AEC facilities around the world. Due to the current situation with a pandemic, a short section on precautions concerning SARS CoV 2 safety measures has also been added.

TECHNICAL STANDARDS

Information on technical standardization was received from 11 AEC operators (Table 1) and grouped into eight sections.

SECTION 1: Dimensions and structure of the AEC

AARHUS: (7, 8) The facility at the University of Aarhus (Aarhus, Denmark) consists of two stainless steel chambers with a floor space of 29 m^2 (Volume 79m^3) and 12m^2 (Volume 33m^3), respectively. The AEC was designed to mimic an apartment, with a living room, bedroom, bathroom and toilet. A total of four persons can use the small chamber and a total of eight persons can be exposed in the bigger chamber for pollen exposures (Figure 1A).

ALL-MED: This facility (located in Wroclaw, Poland) consists of one room with a surface area of 12 m^2 and a ceiling height of 3.5 m. It has been designed to seat 15 participants comfortably (maximum: 20). The chamber floor, ceiling, and walls are covered with easy-to-clean, low-particle-emitting or –attracting materials. The furnishings (seats, etc.) are movable, to facilitate cleaning and to allow custom configurations. Viewing windows enable the chamber to be monitored from the control room during trials (Figure 1B).

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ALYATEC: (9). The 20-seat AEC (located in Strasbourg University Hospital, Strasbourg, France) was conceived as an ISO 8 clean room. It has a total surface area of 65 m^2 and a ceiling height of ~2.25 m. The chamber has non-staining, easy-to-clean, low-particle-emitting materials: Corian® on the walls and ceiling, vinyl on the floor (phthalate-free, and a total volatile organic compound (VOC) emission after 28 days of less than 10 $\mu g/m^3$; ISO 16000-9). The seats are movable in order to facilitate cleaning between allergen exposures and enable custom configurations. A viewing window enables all 20 seats to be monitored from the control room during trials. A built-in lavatory is present in the entry air lock and another in the exit one (Figure 1C).

BIOGENICS: (10-12) The Biogenics Research Chamber (San Antonio, TX, USA) was built using clean room technology. It consists of two chambers, a central control room, and a staging area. The large chamber has a volume of 325 m^3, and is designed to seat 50 participants (maximum: 70). The small chamber has a volume of 75 m^3 and is designed to seat 12 participants (maximum: 20). The furnishings (seats, etc.) are movable, to facilitate cleaning and to allow custom configurations. Viewing windows enable the chambers to be monitored from the control room during trials (Figure 1D).

CHIBAUNIV: (13) The AEC located at Chiba University (Chiba, Japan) has a total surface area of 71.8 m^2 and a ceiling height of 2.6 m, the whole capacity is 119 m^3.It can seat up to 50 participants. A viewing window enables the exposure chamber to be monitored from the control room during trials. The non-movable chairs are fixed to a floor made of fine steel mesh (Figure 1E).

CLIANTHA: (14-16) The Cliantha facility in Mississauga (Toronto area, Ontario, Canada) comprises two fixed chambers (one with a fixed seating area and the other with a modular seating area) and five mobile chambers (not described here). The larger chamber has a total surface area of 94 m^2 and a ceiling height of 3.03 m and can seat up to 60 study participants. The smaller chamber is approximately half the size of the former, with a total surface area of 49 m^2 and space for up to 30 participants. The facility features antechambers for accessing the experimental chambers. Both chambers have electrostatically dissipative walls, ceilings and floors, and the corners are curved or

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chamfered with surfaces optimized for cleaning and for switching the pollens or allergens studied (Figure 1F).

FRAUNHOFER: (17-19) The facility in Hannover (Germany) comprises three adjacent chambers of the same size on the first floor of a designated environmental exposure unit. Each chamber has a size of 47 m^2, a ceiling height of 2.80 m, a double-door lock for entering the chamber, and a built-in lavatory. The wall and the ceiling materials are made of stainless steel. The floor is covered with easy-to-clean ceramic tiling. Each chamber can seat up to 18 participants, who are monitored from the observation room through a large front window and via headset communication (Figure 1G).

GA²LEN: (20-22) This mobile facility consists of two cubic containers that can be transported by a truck equipped with a crane. The containers can be connected within 90 minutes. Once assembled, the containers form an interconnected compound with a 7.45 m × 4.90 m footprint (equivalent to 4 parking spaces). One container houses the observation room/office and a changing room, and the other houses the exposure chamber itself and the air processing installations. The test chamber is accessed through the changing room. The latter has an outside entrance that is independent of the entrance to the observation room. The ceiling height in the chamber is 2.86 m. The chamber is relatively self-sufficient because it can be equipped with independent fresh water and waste water tanks. Electricity can be provided by an external generator. The chamber can accommodate up to 9 patients simultaneously, plus a nurse or physician. The chamber's chairs are specified for a seated time of up to 12 hours and have adjustable footrests and surfaces that can be fully sanitized. Each chair has its own tablet holder. The chairs are always affixed in the same floor positions but can be removed for full cleaning. The seat height is adjusted so that the distance between the pollen outlet in the ceiling and the face is essentially the same distance for each participant (Figure 1H).

KINGSTON: The 297 m^2 allergen testing facility (located in Kingston, ON, Canada) comprises two self-contained testing rooms. The large room measures 12 m x 18 m x 2.9 m and can accommodate up to 120 study participants (plus research support staff, physicians, and nurses). The smaller testing room measures 6 m x 12 m x 2.9 m,

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including a rear ante-room, and can accommodate 35 study participants (plus staff). The use of smooth polyurethane seating facilitates cleaning between exposure sessions, and chair numbers are easily modified to accommodate groups of different sizes.
Several adjacent allergen-free rooms allow for participant staging, examination and training, pollen counting, data management, and food staging (Figure 1I).

RED MAPLE TRIALS: (23) The Red Maple Trials Allergen Challenge Theatre (Ottawa, Canada) measures 16 m by 8 m, giving a total surface area of 133 m^2). The ceiling height in the chamber is 2.2 m. The walls and floor were designed and fabricated using clean-room material that is repellent to particles for ease of cleaning. The adjustable theatre-like seating is arranged in elevated rows to optimize allergen exposure to the subjects' faces. The chamber consists of four independent quadrants, each of which has a dedicated allergen/particle supply. For consistency, the same allergen is always used in the same quadrant. The largest quadrant (Zone 2) has a 40-person capacity (five rows of eight seats). A viewing window enables the theatre to be monitored from the control room during trials. Dedicated lavatories are adjacent to the AEC (Figure 1J).

VCC VIENNA: The AEC located in Vienna (Austria, Vienna Challenge Chamber (VCC)) is an enclosed system that is loaded with filtered outdoor air. The total volume is 58 m^3, with a ceiling height of 2.70 m. Participants enter through an air gate of 2.40 m^2 in square footage.
The chamber seats up to 20 patients exposed to equal amounts of airborne allergen particles. The windows between the chamber and the surrounding working area enable the constant observation and investigation of the challenged patients. Communication is possible through these clear glass windows and via an intercom system. Smooth aluminium surfaces, antistatic measures and regular, thorough cleaning between challenge sessions reduce allergen adhesion, which could otherwise lead to irregular allergen concentrations (Figure 1K).

SECTION 2: AEC staff

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AARHUS: The facility has several dedicated staff: an engineer to set up and monitor the experimental operating conditions, and two health care workers with expertise in helping participants to perform studies under optimal conditions. The chamber staff also include physicians, and the facility collaborates closely with the Department of Chemistry at the University of Aarhus and the Department of Pulmonology at Aarhus University Hospital and with other specialists.

ALL-MED: The facility has three dedicated staff: an engineer to monitor the experimental conditions, and two health care workers (including at least one physician) to advise and care participants during studies.

ALYATEC: The facility has a dedicated team: a technician who sets up and monitors the AEC's technical operating conditions, and medical staff (including physicians and nurses) who closely supervise patients inside and outside the AEC and ensure their safety. The medical staff do not usually need to enter the AEC during an exposure session except in the studies designed for allergic conjunctivitis, when objective measurements are performed with a portable slit lamp. The patients are provided with instructions through the individual mobile tablet used to score clinical symptoms throughout the study session. Additional staff are available for electronic data processing and quality control of the different processes.

CHIBAUNIV: The facility has two dedicated staff members (engineers), who are responsible for keeping the room clean and for monitoring the operating conditions. For prespecified studies, the engineers prepare the allergen, the disposable clothing, and any other material required. During the study, the engineers monitor the chamber's operating conditions, whereas the study investigators manage the participants and record observations. In addition, there are several doctors on call in the control room in case of anaphylaxis or other problems.

CLIANTHA: This facility has specially trained, dedicated staff, including (but not limited to) the AEC specialists who operate and monitor the chamber and the allergen dispersal equipment. These staff monitor the computer interface that controls the temperature and

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the heating, ventilation, and air conditioning (HVAC) system. Experienced clinical research coordinators interact with the participants, e.g. performing standardized participant training on symptom reporting, bringing patients to and from the chamber (escorting them to the lavatory, for example), and monitoring participants in the chamber and their symptom inputs. Technologists monitor and record the pollen counts in the chamber (using microscopy) in real-time. A full-time principal investigator oversees the study and the participants in the chamber, with the assistance of other medical professionals (including nurse practitioners). All staff have up-to-date advanced cardiac life support training and certification. If required by a study's safety planning and/or the nature of the investigational product or the participant population, certified emergency paramedics are included in the study staff.

FRAUNHOFER: The facility's technical systems are operated by an aerosol physicist and an aerosol technician, while clinical operations (e.g. complex experimental medicine protocols) is supported by a dedicated team of physicians (pulmonologists and allergists), study nurses, documentation assistants and laboratory technicians. Staff members receive regular training in how to deal with anaphylactic reactions and other medical emergencies. The staff operate from outside the chambers, in accordance with study-site standard operating procedures (SOPs).

GA²LEN: The facility team consists of an engineer, two study nurses, two dermatologists, a lung physician and two allergists. The staff operate from inside the chamber in order to answer immediately to all questions from probands. All chamber members receive regular training in how to deal with medical emergencies including possible anaphylactic reactions.

KINGSTON: Within the facility's two testing rooms, physicians, nurses and qualified support staff remain with the study participants in order to closely monitor symptoms, answer questions, and provide training and instructions. A research study session leader guides the information presentations, time-sensitive symptom assessments, and testing procedures. The technical and data management staff remain in the testing rooms throughout the allergen exposure sessions.

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RED MAPLE TRIALS: The AEC has dedicated engineering staff to set up and monitor allergen dispersion for the duration of each challenge. The clinical team includes physicians and clinical research nurse coordinators who specialize in allergy research and are trained in emergency procedures.

VCC VIENNA: The AEC is operated by highly qualified, regularly trained staff working in their specific field of expertise and collaborating on the execution of clinical trials: experienced physicians conduct all medical procedures, technicians set up and maintain the electronic data processing system and network, and biologists and lab assistants process and analyse specimens. Additional staff is available for administrative tasks. For each process, SOPs are defined by the responsible experts, and respective documents are issued and maintained.

SECTION 3: Air flow, air processing, and operating conditions

AARHUS: The chamber has a fully up-to-date, computer-controlled air processing and monitoring system. The bigger chamber of 79m^3 has an air supply with clean air 0 to 1500 m^3/h. It can be used to study specific indoor and outdoor climate conditions, ranging from arctic conditions (dry cold; -15°C and 0% relative humidity (RH)) to humid, warm tropical conditions (+ 40°C and 100% RH) and dry, hot, desert-like conditions (+40°C, 1% RH). The smaller chamber of 32m^3 has an air supply wit clean air 50 to 800 m^3/h. It can have Tp. 15 - 28^{o}C & rH of10- 90% (24-31).

ALL-MED: The AEC's computer-controlled air system can be adjusted as a function of the occupancy. The temperature can be set to between 18°C and 27°C (typical temperature: 21°C ± 0.5°C), with the relative humidity between 40% and 58% and a CO_2 concentration below 800ppm. The air change per hour (ACH) ranges from 5 to 20: fresh outside air is drawn in through filtered intakes. Incoming air is constantly mixed under turbulent conditions, using floor- and ceiling-level swirl fans. Turbulent air mixing is a prerequisite for the homogeneous, spatially stable distribution of allergen particles, so

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that grains recirculate rather than accumulate on the floor. After each experimental run, the facility is cleaned thoroughly, in accordance with SOPs.

ALYATEC: The chamber has two airlock halls for entry and exit, and the airflow inside the chamber is totally controlled by a high efficiency particulate air (HEPA) air handling system. To prevent allergen loss from the chamber, the air pressure is lower in the AEC than in the airlocks and the control room. In the airlocks, overpressure is applied to prevent contaminating particles from entering the chamber. Prior to each experimental run, all the environmental parameters (air pressure, temperature, RH, flow, and extraction), and air inlets and outlets are checked and adjusted (if required), using SOPs. After each experimental run, the chamber is thoroughly cleaned -again according to a SOP. The chamber's operating temperature can be set between 18°C and 27°C (typical temperature: 21°C ± 0.5°C), with an RH from 40% to 57%. The ACH can be adjusted from 4.6 to 20.

BIOGENICS: Each chamber has a separate computer controlled air-handling system that can be adjusted as a function of the AEC's occupancy. The air handler has the capacity to control the temperatures of the large and small chambers when they are occupied by 70 and 20 individuals, respectively – even when the ambient temperature outside the AEC is high. In allergen delivery mode, fresh outside air is (i) drawn through highly filtered intakes, (ii) chilled in a single pass across the condenser, (iii) distributed from a duct system by powered, filtered diffusers from the ceiling, and (iv) exhausted by powered, filtering, floor-level vents to the outside environment. The ACH is approximately 12. The system creates a slight overpressure inside the chamber to reduce the entry of contaminants when the doors open. The facility has a back-up diesel-powered generator with enough capacity to power the chambers in full operational mode at maximum outside temperatures. The status of all filters, diffusers, and exhausts is computer-monitored. To check on airflow balance and uniform laminar flow through the chambers, the airflow at the diffusers and exhausts is measured with hot wire anemometers. The AECs' airflow characteristics can also be monitored using a MiniFog portable fog machine. The chamber's suction pumps are calibrated with a mass flowmeter.

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CHIBAUNIV: This chamber has an air supply with outside air 30 m^3/minute and the frequence of total exchange of air is 15 times per hour. Pollen that falls through the mesh on the floor is collected (so that grains do not accumulate on the floor) and is then recirculated for release from the ceiling. The chamber unit is controlled automatically, and the humidity and temperature are kept constant.

CLIANTHA: Air handling is controlled by an environmental control unit set to 23ºC ± 3ºC and 40% ± 5% RH. Prior to particle dispersal in the AEC, the air supply is cleaned (99.97% particulate free) with a HEPA filter. There are 8 air supply outlets located in pairs in each of the AEC's curved corners and 3 ceiling-mounted air return ducts. The typical ACH is 6, although the air exchange rate can be adjusted from ~850 to ~8500 m^3 per hour. Replacement parts are available for all critical equipment, as detailed in the facility's operating manuals. The ability to switch out aerosolization equipment (in the event of failure) is tested on a regular basis and can be performed within a short time period and with only a transient interruption in the pollen levels. All chambers and clinics have back-up diesel-powered generators. This facility has also been used to study dry eye disease, with the RH held below 2% during the exposure; this requires a high desiccating capacity.

FRAUNHOFER: Each chamber is ventilated with HEPA-H14-filtered air to achieve clean-room quality levels. Incoming air is constantly mixed under turbulent conditions using floor-level heating and ceiling-level swirl inlets. Turbulent air mixing is a prerequisite for the homogeneous spatially stable distribution of allergen particles (17). Each AEC has an independent air-conditioning system and calibrated sensors for controlling the temperature (-10°C to +30°C), humidity (20% to 95%) and ACH value (up to 20-fold). The standard chamber settings for allergen exposure sessions are 22 ± 2°C, 40 ± 10% RH, and an air flow rate of 500±50 m^3/h.

GA²LEN: The airflow in the test chamber was designed to be non-detectable at all seating positions, so as not to provoke any adverse reactions (e.g. irritation of respiratory tract, nose or eyes by the airflow). The air conditioning system allows the chamber temperature to be adjusted from 15°C to 38°C, and relative humidity levels can be set from 30% to 80% (non-condensing). Oxygen, carbon dioxide, carbon monoxide, and

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ozone levels are constantly monitored and recorded, using calibrated sensors. The outside air and chamber air are filtered using a multistage cleanroom filter system that removes almost all PM_{10} and $PM_{2.5}$ (21).

KINGSTON: A dedicated, oversized air handling system supplies 2400 cubic feet (68 m^3) per minute of fresh outdoor air to both AEC rooms. The entire volume of air within the AEC is replaced approximately every 10 minutes. Several banks of large prefilters filter outdoor air at the primary air handler; the final booster system provides added capacity and forces the air through high-efficiency filters. Humidification is performed with a dual manifold system just prior to the air entering the front of the AEC through several large ceiling-height wall-mounted adjustable directional airflow ducts. The oversized exhaust air handler can be set to pull air from the large room alone, the small (house dust mite (HDM)-only) room alone, or both rooms simultaneously. Air handling is balanced to provide the negativity in the allergen challenge rooms required for ante-room efficiency. The AEC's temperature and humidity are maintained at protocol-specific levels regardless of outdoor conditions, and (due to the over-capacity) react quickly to adjustments. The hospital-maintained automated Siemens R control system provides feedback to qualified hospital maintenance staff, and the entire system is inspected and cleaned (in line with SOPs) before each research study. Third-party air sampling and lab analysis testing (dust dilution, fungal, and VOC analysis) occurs during each research study, in order to qualify the sealed HVAC operation and ensure that the air is free of confounding allergens. Outdoor versus indoor samples are compared to ensure filtration efficiency and sealed system operation. The results are examined and ultimately approved by a third-party toxicologist. Air temperature, humidity, carbon dioxide, and carbon monoxide values are monitored and recorded during each allergen exposure session.

RED MAPLE TRIALS: Air flow through the AEC is controlled by a closed-loop dedicated HVAC system, which supplies HEPA-filtered air that is blown into the AEC through selected vanes at the front of the room. The air (i) exits the AEC through return air ducts behind the participants, (ii) is HEPA-filtered to remove pollen, and (iii) is recirculated after the addition of new pollen particles. The temperature can be adjusted between 18 to

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24 °C and the flow rate can be adjusted up to 3500 cubic feet (99 m^3) per minute. The AEC is maintained at a slightly positive pressure, to ensure that no other allergens enter the chamber during testing.

VCC VIENNA: An automatic air supply system guarantees permanent air conditioning. Filtered fresh air enters the chamber through fan coils in the ceiling; used pollen-loaded air is sucked out at floor level. Air change rate is 20 times per hour. The AEC's climatic conditions can be adjusted from 20 to 26°C and from 20% to 60% RH and stable climatic conditions can be maintained over a long period. Typical operating conditions for clinical trials are 24°C and 40% RH. A stable temperature is reached 15 minutes after the air supply system has started to operate. Six sets of temperature/humidity sensors are affixed to the ceiling at a height of 2.5 m.

SECTION 4: Allergen/particle dispersal

AARHUS: Pollen is dispersed using a turntable-type powder dispenser, with a target count of 1000 pollen/m^3. The dispenser is a modified TSI model 3433 with a disc with different size concentric v-shaped grooves. The disc is rotating one round every 40 minutes. A uniform supply of pollen is dispensed into the groove by means of a pipette system. Pollen in the groove is then sucked up and by a high-pressure air stream sent to a dispersion plate where a ceiling ventilator rotating reversely helps distributing pollen in the chamber.
In order to keep the pollen concentration in the chamber constant, settled pollen on the floor is removed by a push-pull air system. A narrow band of cool air flows at the floor level keeping the pollen moving towards an air outlet.

ALL-MED: The allergen is delivered by a computer-controlled feeder; an adjustable weight of allergen (in grams per minute) is injected into the air supply ducts and then blown into the AEC through selected vanes at the front of the room. Standardized, commercially available allergens are used. The pollen concentration can range from 500 to 10,000 grains/m^3(Figure 2).

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ALYATEC: Standardized, lyophilized commercially available extracts of HDM, cat or birch allergens (supplied by Stallergenes, ALK-Abello or Allergopharma) are used for nebulization. These extracts have been approved by the French Medicines Agency (*Agence Nationale de Sécurité du Médicament et des Produits de Santé*). Allergen particles are dispersed by spray-drying an allergen solution through a nebulizer composed of ultrasonic nozzles (SinapTec®, Lezennes, France). The nebulized solution is mixed under turbulent conditions in a mixing chamber, prior to diffusion into the AEC through six outlets (located 10 cm below the ceiling).

BIOGENICS: The allergen is delivered by a computer-controlled feeder that delivers an adjustable weight of allergen (in grams per hour) into a vortex driven by an adjustable blower. The blower is augmented by a specially designed eductor. The allergen is dispersed through a series of grounded stainless steel tubes with specially bored ports and machined joints that are locked by hand-cranked clamps to allow for the directional adjustment of allergen. The allergen feeders are calibrated before each study, utilizing a balance.

CHIBAUNIV: Pollen is released through the mesh ceiling at four locations in the room and then further dispersed using nine ceiling fans. The floor is double-layered and designed so that the pollen having fallen through the top floor layer (mesh) is collected.

CLIANTHA: HEPA-filtered air is introduced into the chambers either by inlets in the ceiling or via 4 to 8 inlets integrated in the AEC's corners. Pollen is released from a point-source within the chamber because this mimics natural pollen release from flowers. Lyophilized natural pollen provided by accredited allergen providers is aerosolized using a proprietary device. The pollen is mixed with fresh air within the chamber and then distributed through either free-standing fans (in the EEC-2) or by air jets in the corners and ceiling (EEC-3/mobile chambers). Airborne pollen concentrations are measured every 30 minutes using Rotorod™ impact samplers and assessed under the microscope. For non-pollen allergens (such as HDMs or cat allergens), air samples are collected hourly; the main allergen proteins are assayed after the session and then expressed in

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ng per unit volume of air in the chamber. Levels of HDM and cat allergens are also indirectly monitored in real time by counting the number of particles in certain ranges. Experiments performed during the development of the AECs showed that the airborne concentration of allergen is determined by the size of the largest effectively aerosolized particle. This is related to the physical nature of allergen particles, where the particle's surface area (and thus the allergen load on the surface) increases exponentially with its diameter.

FRAUNHOFER: Dispersion of allergen particles is achieved by either feeding natural pollen (grass or birch, GMP-grade material from Allergon, Sweden) into the chamber via four tubes just beneath an air outlet in the ceiling(17) or by spray-drying an allergen-lactose solution (HDM allergens from ALK, Germany) inside the chamber (18). A ceiling fan in reverse mode improves the radial dispersion of the allergen particles.

GA²LEN: The particle dispersion system consists of nine individual, independent particle dispersal units (PDUs) - one at each seating position. The particle disperse rate of each individual PDU and the overall control of the PDUs is centrally managed via dedicated software. Each PDU has its own dedicated particle source, called a particle containment tape (PCT). The PCTs are manufactured using a device called particle line unit (PLU). A PDU can release particles from a PCT through a nozzle located above the patient. The particles are released, the PDU guides the PCT through a scanning microscope.
Both, the PLU and the PDU are designed to handle particles between 0.1 µm and 100 µm in diameter. The particle concentration that a PDU can generate depends on the PCT's particle density and the speed at which the PCT is emptied. For grass or birch pollen, the concentration generated by the PDU ranges from 500 to 16,000 grains per m^3 of breathable air. When using HDM raw material, concentrations of 10 µg/m3 to 1000 $\mu g/m^3$ can be generated (21).

KINGSTON: Pollen is commercially sourced from Greer Laboratories (Lenoir, NC, USA). It undergoes third-party testing for fungal and bacterial contamination before use in AEC studies. Pollen/allergen dispersal incorporates a multiple metering process; the allergen is conditioned, metered and dispersed to ensure consistent output. In each AEC, allergen

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is dispersed from a single point source using a short-duct feeder system. The allergen level is monitored using industry standard methods and is controlled using custom software (32). This process does not require lengthy pressurized ceiling or ground feeder ducts to achieve stable airborne pollen levels.

RED MAPLE TRIALS: A pollen generator in the room above the AEC aerosolizes the pollen (sourced from Stallergenes Greer), which is injected into the air supply and fed into the room through vanes near the ceiling at the front of the room. The output from one laser particle counter (LPC) (the driving LPC) is fed back to a programmable logic controller, which adjusts (if required) the amount of pollen injected into the airflow and thus maintains the target level in the AEC.

VCC VIENNA: This patented AEC uses separate particle dispersion systems for the different types of allergen used (e.g. grass pollen, birch pollen, cedar pollen, and HDM-containing dust). Allergen particles are delivered from dispensers next to the fan coils in the AEC's ceiling. The HDM particles or pollen grains are continuously fed into the system from the outside working area. Vacuum pressure delivers the allergen to the chamber. The above-described dispensers and the slightly turbulent air flow in the chamber guarantee (as already described in (33)) a homogeneous dispersion of the allergen particles in the air. Slow, continuous sedimentation of the allergen particles can be achieved. Allergenic particles are exhausted at floor level without recirculation. During a longterm challenge of several hours, the allergen load is constant and has a small standard deviation (SD).

SECTION 5: Pollen/particle counting

AARHUS: In order to measure the pollen count online, we used an LPC (Lighthouse Worldwide Solutions, Inc., Fremont, CA, USA) running at a flow rate of 28 L/min. This is supplemented with three Burkard samplers collecting 10 L/min. The samplers' filters are weighed every 30 min, starting 15 min before the start and every 30 minutes during the 3½-hour session - giving a total of 21 measurements per session (Figure **3**).

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ALL-MED: The particle count and particle size are monitored throughout the experiment using an LPC (SOLAIR Boulder Counter, Lighthouse Worldwide Solutions). The pollen concentration (in grains per m^3) is counted and recorded every minute during the exposure (Figure **2**). The particle sizes that can be monitored range from 5 to 100 microns. During exposure sessions, the absence of contamination by allergens, VOC or endotoxins is confirmed.

ALYATEC: The particle count and particle size are monitored continuously during nebulization, using 10 optical counters placed at various points within the AEC. During studies with HDM, cat and birch allergens, it was confirmed that the nebulized particles are homogeneously distributed inside the chamber.

The inter-assay coefficient of variation (CV) in the particle count (particle size: 5-10 µm) was 15.2% for HDMs, 18.7 % (n=40 assays) for cat dander, and 11.2% (n=17 asays) for birch pollen. The airborne allergen is collected on five glass fiber filters, and then measured with an enzyme-linked immunosorbent assay (ELISA). For HDMs, the mean ± SD airborne concentration of Der p 1 was 25.5 ± 8.2 ng/m^3, with an inter-assay CV of 23.5% (n=25 assays). For cat allergen, the mean airborne concentration of Fel d 1 was 40.9 ng/m^3, with a CV of 10.4% (n=40 assays). For birch pollen, the mean airborne concentration of Bet v 1 was 73.8 ng/m^3 with a CV of 32.9 % (n=17 assays).

The mass median aerodynamic diameter of particles carrying the different allergens was measured with a cascade impactor coupled to an ELISA assay, and was around 10 µm. In the absence of exposure, no contamination with allergens, VOCs or endotoxins was found in the AEC (n=3 assays).

BIOGENICS: Pollen counts are measured using two manual Allergenco systems, MK-3 staged counters, and Allergenco-D Posi-track disposable cassettes powered by suction pumps running at 15 L/min. The Allergenco systems are mounted on a platform set 117 cm from floor level. Allergens (from HDM, animal dander, or cockroach extracts) are collected in special filters powered by suction pumps (again running at 15 L/min). Allergen content is then measured off-line using an ELISA. Prior to allergen exposure, the concentration of non-specific particles is measured with a Kanomax LPC. Between

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studies, microbial cultures of the AEC's air are obtained after samples are collected with an Andersen sampler.

CHIBAUNIV: A total of 56 automatic LPCs (Shinyei Technology, Kobe, Japan) are placed on the back of each chair and at fixed positions in the room. They measure pollen and dust counts in the air that is sucked into the device using a built-in fan. The scattered light intensity and degree of polarization yield the amount of airborne pollen. During trials in the AEC, the pollen count is continuously monitored so that it is maintained at a constant value (typically 8000 grains/m^3). The pollen count reaches the target value 15 minutes after the start of pollen dispersal, and can be held there (±12%) (13). Even when a participant changes the air flow by walking through the AEC, the pollen count immediately returns to the target value. When pollen is observed under the microscope, less than 1% of the grains are ruptured or damaged.

CLIANTHA: To ensure good sampling coverage in the seating area, 5 of the 7 rotating impaction samplers (RISs) are arranged in an X-configuration. Pollen levels in the seating area are monitored using RISs every 30 minutes. Non-pollen levels are monitored in real-time using an LPC, and key allergen proteins are quantified in ELISAs. For pollen levels, the AEC's technicians also count pollen grains under the microscope, in order to determine airborne concentrations. The mean pollen count from each RIS is monitored at each time point, enabling an overall average of all RISs at each time point to be calculated. If this average value is outside the target range, the system is adjusted accordingly. Temporary excursions from the target range may occur if there are disruptions such as patient movement, as would occur naturally in the field. Overall per-session averages are monitored and are documented as categorized deviations if they fall outside of the target range. In clinical trials, all pollen counts for each session are recorded and then archived as part of the study dataset.

FRAUNHOFER: The concentration of allergen particles (pollen or allergen-lactose particles, in this case) is measured online using an LPC (LASAIR 550, PMT GmbH, Heimsheim, Germany) at a defined flow rate in six particle size classes (from 1 to 100 µm). The particle concentration is used as a surrogate marker of the allergen

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concentration, which is maintained in the pre-specified target range (±10%). Pollen grains can also be counted and inspected visually offline under the light microscope, after capture using two RISs.

GA²LEN: A particle dispersal unit counts the number of particles or uses the line width and line density to calculate the dispersed amount. Hence, the amount of particles to be released (in ng per second or particles per second) can be controlled (Figure **4**). Each image generated by the scanning microscope is stored to enable post-validation analysis, if required. The used and re-spooled particle containment tapes are also kept on file for post-validation analysis, if required (21).

KINGSTON: Quantification of airborne pollen in the AEC is performed using Rotorod® samplers (seven Rotorod® samplers placed throughout the large AEC, and front, middle, and rear Rotorods® in the small AEC (Figure 1I)). Plastic sampling rods are inserted in the impact sampler arms and rotated at 2400 rpm (sampling 47.3L of air per minute). The sampling rod surface is greased using a custom machined greasing fixture, to maintain single-faced greasing consistency while greasing several rods simultaneously. During sampling periods, the Rotorod® sampling head (containing two rods) is spun for 30 seconds to capture pollen. The AEC studies frequently include one or more "zero spins" to confirm the absence of pollen in the AEC before the start of exposure. During the exposure session start-up, the Rotorod® samplers are activated 10 minutes after the onset of the exposure (to confirm that a steady state has indeed been reached) and at 30-minute intervals thereafter. Light microscopy is used to quantify the captured pollen. Based on the pollen counts from the Rotorod® samplers, a stable airborne pollen concentration is maintained during exposure sessions (Figure 5) by (i) adjusting the elevation and direction of individual fans in real time, and (ii) increasing or decreasing the pollen output rate. Third-party testing is performed to ensure that the pollen used in the AEC is free of bacterial and fungal contamination. The AEC also has an indoor air quality consultant, who checks the quality of both the air and pollen used in the AEC. The endotoxin content in pollen samples is measured to ensure that it is below the government-regulated acceptable level. Pollen grains (ragweed, grass, and birch) are used in the AEC, in order to mirror natural outdoor exposure as closely as possible.

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Previous AEC studies reported that the optimal target airborne pollen concentration for reproducing allergic symptoms typical of outdoor exposure was 3500 ± 500 grains/m^3 (34, 35). It takes approximately 5 to 10 minutes for the AEC to reach this target airborne pollen concentration. All devices used in the quantification process are validated/qualified prior to each allergen exposure session.

RED MAPLE TRIALS: Pollen concentrations are measured using LPCs (Model R5102, Lighthouse Worldwide Solutions, Inc.) and RISs (GRIPS-99M, Aerobiology Research Laboratories, Nepean, Canada) placed at multiple locations. The LPCs provide instantaneous assessment of the pollen concentration, the RISs provide confirmation that the particles are the pollen of interest. In a validation study, there was a linear relationship between LPC-derived and RIS-derived pollen counts over a wide range of concentrations of grass pollen (r^2: 0.85) and ragweed pollen (r^2: 0.93) (Figure 6)

VCC VIENNA: The allergen load during a challenge session is checked every 5 minutes: For routine procedures, a modified Burkard slide sampler is used for continuous volumetric recordings of the number of allergen particles per m^3 of air. The flow in the sampler is recorded by a thermo-anemometer, and the adhesive-coated slide is exposed for 5 minutes to the allergen-loaded air. The slide is changed every 5 minutes, and the particles are immediately counted under the microscope by trained staff. The consumption of mite allergen material is determined every 5 minutes by weighing, i.e. comparing the allergen container's current weight with that recorded before the exposure session (36).

SECTION 6: Safety and non-contamination measures.

AARHUS: Before entering the room, the participants put on coveralls and shoe covers. This is primarily to prevent participants from carrying pollen out of the chamber facility to their home after the exposure. During a study, the participants can communicate with the AEC staff if required via a window and a telecom system. There is always a person outside the facility who can be contacted through the telecom, and a physician on call. The facility meets the Danish Society for Allergology's standard safety requirements on equipment and personnel for the administration of subcutaneous allergen immunotherapy.

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ALYATEC: In the entry air lock, participants pull on a protective coverall with integrated socks, in order to avoid contamination of the AEC with outside allergens and contamination of the external environment with the test allergen. In the exit air lock, patients are asked to take off their protective coverall, wash their hands, and wash any mobile devices they took in the chamber to monitor symptoms. Patients inside the AEC can communicate with medical supervisors via wireless devices. To ensure maximum safety for participants, emergency care is available. Seats can be fully reclined if needed, and oxygen outlets, air outlets and vacuum inlets are placed between the seats. Moreover, the medical team is fully trained in emergency medicine. An emergency "crash cart" (containing all the supplies needed to handle any potential serious reactions) is present in the control room, next to the AEC. Lastly, the AEC is located less than 5 minutes away from Strasbourg University Hospital's intensive care unit.

ALL-MED: Study participants wear protective clothing to avoid taking non-allergen particles into the chamber. The study personnel in the control room communicate with study participants via the microphone system. A defibrillator and other resuscitation equipment are available in the AEC. The participants rate a number of safety parameters at home, after exposure: peak expiratory flow and a total symptom score. A safety follow-up call is made 24 h after the exposure session.

BIOGENICS: With regards to safety, the emergency equipment consists of oxygen, a crash cart, and a defibrillator. Audiovisual equipment is used so that the study participants in the AECs can communicate with the control room.

CHIBAUNIV: Before entering the room, the patients put on clean disposable gowns (including hair caps and shoe covers) so that (i) dust on participants does not interfere with measures and (ii) participants do not transport pollen out of the chamber facility after the exposure. Just after the participant has left the AEC, pollen is blown off the disposable clothing in an air shower, and the clothing taken is removed and disposed of.

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CLIANTHA: Study participants are instructed to change out of their own street clothes and are provided with clean hospital wear or scrubs. This is done to limit transfer of allergens into and out of the AEC. Furthermore, participants are issued disposable shoe covers, hair nets and a jumpsuit to wear over their scrubs. The disposable layer is removed in the antechamber entry area when exiting the chamber (for example when being escorted to the lavatory located outside the chamber). At the end of the chamber session and prior to exit, this disposable layer is discarded in the antechamber entry area and sequestered there aimed to limit pollen transfer to the rest of the clinical area/facility. For staff working in the chamber daily, health and safety standards dictate the use of appropriate personal protective equipment. For this reason, staff will also wear hospital clothing, coveralls and face masks during exposure trials. Trial participants are told that the staff have to wear masks because they work in the AEC on a daily basis, and are not being studied. Participants are viewed through large observation windows. If a participant needs to go to the lavatory during a trial, he/she is escorted there and back.

Staff have up-to-date advanced cardiac life support training and certification. If required by a study's safety planning and/or the nature of the investigational product or the participant population, certified emergency paramedics are included in the study staff. Participants with a known history of anaphylaxis are excluded from AEC trials, and those with asthma are monitored accordingly. For example, participants with mild asthma (< Global Initiative in Asthma step 2) can be included. Those with moderate asthma can be included in for very specific studies with one-by-one assessment by a physician and a paramedic on site throughout the exposure and follow-up period (37). The participants' health status is regularly monitored via direct observation, and the participants report their health status every hour. If an adverse event occurs, the physician can provide appropriate medical care. Emergency medical equipment (including oxygen and a crash cart) is available near the chamber facility. The equipment is monitored, checked and logged to ensure readiness in the event of an incident.

FRAUNHOFER: Study participants wear protective clothing to avoid taking non-allergen particles into the chamber; this is essential because the AEC's particle concentration is used as an online measure of the allergen concentration. The participants are seated in designated areas of the chamber. Study subjects with mild asthma are allowed to

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participate in allergen challenge trials and monitor their lung function on an hourly basis with a hand-held device. The study personnel in the control room communicate with study participants via wireless headsets. Emergency equipment is readily available, and staff members are regularly trained to deal with anaphylactic reactions and pulmonary emergencies. Participants are given specific instructions to be followed until the next morning with regard to lung function monitoring and rescue medication use. An on-call physician is available around the clock.

GA^{2}LEN: The study participants wear clean-room apparel to minimize the possible amount of allergens entering and leaving the chamber. The chamber's airflow design also prevents particles from refloating, even when people are moving within the chamber. Study nurses and the study physician are always present in the chamber.

KINGSTON: During all allergen challenge sessions, study physicians, nurses, and qualified support staff are constantly present with the study participants and monitor their symptoms. The site has detailed emergency SOPs that all staff members (including physicians) are required to review prior to every new study. To ensure that the self-reporting of symptoms during pollen exposure sessions is not influenced in any way, participants are not required to wear protective clothing; the goal is to mimic the conditions encountered outdoors as closely as possible. For HDM exposure, however, participants and staff/physicians do wear personal protective clothing to minimize the particle burden in the room. The AEC is located within a general hospital and therefore has access to safety and emergency resources. An emergency "crash cart" (containing all the supplies needed to handle potential serious allergen-induced reactions) is present in the AEC.

RED MAPLE TRIALS: Staff and subjects wear protective clothing while in the AEC, which they don and doff within an airlock hallway separating the AEC from the main facility. A physician and nurse coordinator, trained in emergency procedures and SOPs, are present at all times during challenges, and an urgent treatment room with beds, oxygen and a crash cart is adjacent. The AEC and adjacent patient areas are professionally cleaned before and after each use according to detailed SOPs.

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VCC VIENNA: During challenge chamber sessions study physicians and other medical staff are always present, taking care of the wellbeing of the study participants. The constant observation of and communication with the challenged patients is possible also from outside the chamber through clear glass windows and via an intercom system. The AEC staff has developed an emergency action plan and SOPs for the appropriate management of any medical emergencies. Emergency equipment is in place. A physician is available 24 hrs per day. All the staff members are familiar with the chain of rescue and receive regular training in basic life support. As bronchial adverse reactions are a realistic scenario in sessions with exposure to inhaled allergen, regular lung function testing is a standard assessment in all studies. Participants are provided with mobile devices and an asthma action plan for determining and managing any late-phase reactions at home. CO_2 level is monitored during the whole challenge session.

SECTION 7: Procedures for symptom assessments

AARHUS: The participants subjectively assess their own symptoms every 30 minutes on a visual analogue scale. Subjects are prompted by the tablet to provide symptom responses in order to input scores at the present times. Other measures such as acoustic rhinometry, FeNO, Nasal Lavage, PEF, non-specific bronchial challenge and spirometry can be tested outside the AEC before, right after and 24 hours after start of the provocation.

ALYATEC: During a study, each participant uses a mobile communication device to monitor symptoms severity in the AEC. The assessments can be tracked precisely by the medical team on a monitoring screen in the control room. An automatic procedure for symptom assessment can be adapted to suit the study design.

ALL-MED: Patients assess nose, eye and respiratory symptoms using symptom score and visual analog scale on paper and electronically at intervals as per protocol. Additionally, symptoms are monitored by means of acoustic rhinometry, peak nasal

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inspiratory flow (PNIF), peak expiratory flow (PEF) and spirometry. Additional study-specific assessments (for example nasal lavage) can be performed at a table located in front of the viewing window.

BIOGENICS: Participants self-assess nasal, ocular, and asthma symptoms on a paper diary or electronic device at intervals dictated by the protocol. Spirometry, peak expiratory flow, and peak nasal inspiratory flow are monitored according to the protocol.

CHIBAUNIV: During a study, each participant uses a mobile communication device to precisely record the frequencies of sneezing and rhinorrhoea and to subjectively assess symptoms

CLIANTHA: A validated, 21 CFR Part 11 compliant, electronic patient data acquisition tablet is provided to each subject and is used in the AEC to collect symptoms or patient reported outcomes (ePRO) at regular intervals. Symptoms can be recorded frequently every 2 minutes as is used for onset of action determination (14). Symptoms can be graded on discrete or on visual analogue scales. Subjects are prompted by the tablet to provide symptom responses to help to remind them to input scores. Other measures such as acoustic rhinometry, rhinomanometry, PNIF and spirometry can be tested at a table or station within the AEC. Subjects periodically move to a station to have these measures taken.

FRAUNHOFER: Study-specific assessments (such as rhinomanometry, spirometry and symptom scoring) are performed at a table located in front of the window separating the control room. The total nasal symptom score is captured with touch-screen computers using validated software (ClinBase™, Parexel, Berlin, Germany).

GA²LEN: Patients record eye symptoms (itching, foreign body sensation, redness, and watering), nose symptoms (itching, sneezing, running, and blocked), and bronchial symptoms (wheezing, shortness of breath, and cough). Subjective parameters are recorded at time zero and every 30 minutes during exposure. A visual analogue scale score, peak nasal inspiratory flow (PNIF), and peak expiratory flow are also recorded

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before entering the chamber, after acclimatization in the chamber (10-30 minutes, time zero), and every 30 minutes during exposure (22).

KINGSTON: All participants are trained in symptom rating assessments, and, if included in the protocol, use a PNIF meter prior to any study-related data being captured. Self-assessments are performed by the participants in their designated seats in the AEC. If procedures are required per protocol in addition to symptom or PNIF recording (e.g. blood or nasal samples), these are performed in a pollen-free assessment room adjacent to the AEC but still within the hospital.

RED MAPLE TRIALS: Rhinoconjunctivitis symptoms are assessed every 10 minutes with either paper case report forms or mobile phone technology. Pulmonary function is monitored by spirometry at a frequency determined by the study.

VCC VIENNA: Volunteers record their subjective symptoms every 15 minutes via touchscreen on a data capture tool. Standard assessments are nasal symptoms (sneezing, itching, rhinorrhoea and nasal obstruction), eye symptoms (tearing, itching, reddening, foreign body feeling), bronchial symptoms (cough, dyspnoea, wheeze) and other symptoms (e.g. itchy throat, itchy ears) which are scored on a categorical scale from 0 to 3 (0 representing no symptoms and 3 representing severe symptoms). Secretion weight is a surrogate of nasal secretion and is measured by weighing tissues every 30 minutes during the challenges. Nasal obstruction can be objectified by means of nasal airflow resistance using active anterior rhinomanometry and can be measured every 30 minutes as well. All other assessments not reflecting subjective symptoms (e.g. lung function testing, biomarker evaluation) are not described here.

SECTION 8: Tested allergens/substances, and validation procedures

AARHUS: The AEC has been tested in experiments with *Phleum pratense* grass pollen and *Betula verrucosa* birch pollen (both from ALK-Abello, Hørsholm, Denmark) (7) (8). The mean ambient conditions were kept stable (± 5%) at around 22°C and 35% RH.

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Experiments with various gases, vapours, and particulates have also been performed: examples include ozone, toluene, n-decane, indoor air mixtures, nitrogen oxides, house dust, passive electronic cigarette smoke, diesel particles, wood smoke, and candle emissions.

ALL-MED: The AEC has been tested in experiments with Timothy grass pollen, birch pollen and HDM particles (supplied by Allergopharma, Reinbek, Germany). The mean ambient conditions are as follows; temperature: 21.2 ± 0.40C; relative humidity: 52.4 ± 2.4%; CO_2 concentration: 805 ± 52ppm. The pollen count can be set to between 500 and 8000 grains per m^3. The count is known to be stable over time.

ALYATEC: Validation studies have been performed with different concentrations of HDM (38), birch and cat allergens (9,38. Non-allergic triggers (such as endotoxins and volatile organic compounds) have also been tested and measured in the AEC; these experiments confirmed the absence of contamination in the AEC and the supervision room after exposure.

BIOGENICS: Validation studies have been accomplished utilizing HDM, mountain cedar pollen, Virginia live oak pollen, Timothy grass pollen, and short ragweed pollen.

CHIBAUNIV: The AEC has been used to study tree pollen only (Japanese cedar and cypress). Based on a previous report, we compared 8000 and 12000 grains/m^3 of Japanese cedar and cypress pollen. We determined that 8000 grains/m^3 of Japanese cedar pollen and 12000 grains/m^3 of cypress pollen are sufficient to induce symptoms (39).

CLIANTHA: The AEC has been used to study grass pollen (mixtures and *Phleum pratense* pollen alone), tree pollen (oak and birch), ragweed pollen, HDMs, cat allergens (whole or pelt), and dog allergens. Non-allergic triggers (such as thermal shifts, dry air, ozone, and fragrance) have also been studied (40, 41).

Each pollen/allergen and each chamber are validated in line with WHO and FDA guidelines and a validation master plan (installation qualification (IQ), operational

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qualification (OQ), background counts, and performance qualification (PQ)). During IQ and OQ, all pieces of equipment are installed in compliance with the manufacturer's guidelines, relevant SOPs and/or ISOs, and includes documented calibration and service history/maintenance. After IQ&OQ, the chamber is tested to determine the ideal equipment settings and location(s) for each study's particular needs. The PQ demonstrates that the entire system is operating as required. All pre-execution validation protocols, system risk assessments and post-execution summary reports (including acceptance criteria) are developed and documented *a priori*. The AEC is validated when the acceptance criteria are met and demonstrated in triplicate. The spatial and time-domain acceptance criteria ensure that the airborne pollen concentration elicit allergy symptoms effectively and predictably. The validation summary report summarizes all the validation activities included in the master plan. All validation documents are developed, reviewed technically, approved by quality assurance and management, and made available for audit by sponsors or regulatory authorities.

The facility's quality assurance team has developed a series of SOPs in order to (i) validate or requalify the AEC's compliance with applicable in-house and regulatory requirements, (ii) validate tolerance testing, background particle counts, system risk assessments, final validation reports, and the traceability matrix, (iii) check allergen dispersal and review pollen count data, and (iv) perform in-process audits during a clinical study SOPs.

FRAUNHOFER: Using patented technology, the chambers have been validated for exposure to natural grass pollen (17), natural birch pollen (19), and HDM allergens (18). To avoid cross-contamination, the different allergens are studied in separate chambers.

GA^{2}LEN: The chamber has been technically and clinically validated (21, 22).

Phleum pratense, *Betula pendula*, *Dermatophagoides farinae*, and *Dermatophagoides pteronyssinus* allergens have been evaluated in the GA^{2}LEN chamber. The pollen concentration measured in breathable air showed a variation of ± 12.2% vs. the target concentration (Figure 4). The intra-seat variation was ± 13%, and the inter-seat variation was less than ± 7%. For HDM particles, the air variation was ±30% vs. the target, the intra-seat variation was +100%/−50%, and the inter-seat variation ±36.5%.

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KINGSTON: The AEC has been clinically validated for the distribution of and patient exposure to allergens from short ragweed, grass (orchard grass and perennial rye), European white birch, and HDMs (a mixture of Der p 1 and 2, Der f 1 and 2, in a 1:1:1:1 ratio). Ragweed pollen has also been successfully co-delivered with diethyl phthalate in the smaller (HDM-specific) AEC. Typical pollen concentrations during challenge sessions in the AEC of ragweed, grass, and birch pollen are shown in Figure 5.

RED MAPLE TRIALS: Technical validation has been performed for *Phleum pratense* grass pollen (timothy) and *Ambrosia artemisiifolia* pollen (ragweed) (23). The ragweed and grass pollen concentrations were found to be (i) stable over several hours (Figure 7A and B), (ii) responsive to changes in the pollen setpoint (Figure 7B), and (iii) sufficient to induce symptoms.
The distributions of *Phleum pratense* and *Ambrosia artemisiifolia* pollen in one quadrant of the AEC are given in Tables 2 and 3, respectively; the percentage deviation was 13% for ragweed and 17% for timothy grass.

VCC VIENNA: The AEC has allergen exposure models for grass pollen, birch pollen, Japanese cedar pollen, and HDMs. For all models, the allergen concentration that induces a moderate level of symptoms (comparable to the disease burden produced upon environmental exposure) was determined. For example, the grass pollen concentration established during the early 1990s is ~1500 pollen grains per m^3 of air. This value can be kept stable for the whole challenge period. Reproducibility and consistency have been proven (42-44). Higher concentrations increased the risk of bronchial adverse events in the exposed participants (data not published). The HDM model was also developed in the 1990s; in a recently published clinical validation study, the allergenic profile of the used natural material was described (Table 4), re-test reproducibility and inter- chamber variability were assessed and a high correlation ($r=0.961$) between the mean weight of allergen released and a target TNSS of ≥6 (Figure 8) could be determined (36).

DISCUSSION

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The regulatory environment for AECs varies across Europe, Asia, and the Americas (2). Allergen exposure chambers are not classifiable as medical devices (according to the Medical Device Directive (*Directive* 93/42/EEC) or medicinal products (according to Directive *2001/83/EC)*, and so a well-established regulatory framework is lacking (2, 4, 46, 47). Nevertheless, the Task Force members considered the definition of a set of minimal technical requirements for AECs used in allergy research and clinical development. The technical information contributed by the AEC operators is summarized in Table 5. Although it may not always be possible or desirable to set a single "correct" value for a given parameter, the ability to measure and then manage is the most important point.

It is unanimously agreed that the intensity of objectively or subjectively reported allergic symptoms is proportional to the degree of allergen exposure – even though researchers may disagree about the exact shape of the dose-response curve. For aeroallergens, this means the airborne concentration of allergen-containing particles. The Task Force members consider that the single most important minimum technical requirement in an AEC is a stable (intra- and inter-session), narrow concentration range of aeroallergen in the chamber in general and at the participant's head level in particular (inter- participant). Before operating a new AEC facility or employing a new allergen source in an established facility, a systematic technical validation process needs to be passed in order for AEC exposure to be accepted as an outcome measure in the clinical trial setting. It must be proven that the AEC system works within its specifications in terms of environmental conditions (e.g., temperature, humidity, air flow, air pressure) and target antigen concentrations. For reliable and robust study findings, allergen challenge conditions and the physical set-up must be reproducible and controlled. The allergen-containing particle being studied must be lofted, collected and analyzed in all potential areas of participant occupation using industry-acceptable methods. The airborne particle count in the chamber should be measured continuously and the allergen profiles and concentrations in sampled allergenic material should be monitored regularly, using established bioassays (e.g. ELISA, mass spectrometry). Most of the AECs described here use LPCs; It is noteworthy that an LPC count is not as accurate as visual identification, and is not allergen-specific. Many AEC operators consider that visual confirmation of the amount of allergen delivered (via an impact sampler or similar device) is optimal.

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Quality assurance of the lofting system is also recommended. Primarily, the particle lofting and dispersal system must not create conditions that might trap foreign matter and create microenvironments for potential irritants. The Task Force members also consider that the AEC's air supply/ air exhaust systems should be qualified regularly or after any major work that may affect their function, in order to prove that no outside-sourced particles bypass the HEPA filters. A comparison of the exterior air intake with the air inside the AEC will confirm the efficacy of HEPA filtering; spores and particles detected in the outdoor sample must not be detected in the chamber. If humidification is needed, the AEC's air should be humidified at the terminal end of the supply air ducting (i.e. close to the point of release into the chamber), since the wetting of internal ducting increases the risk of creating a spore-producing environment.

The quality of the lofted material is also a key concern. Even if all the air parameters are controlled and optimized, differences between materials used (e.g. extracts vs. non-extracted natural material) or the use of different batches of the allergen material in the course of a study will compromise (i) the comparison of multiple challenge sessions in a given chamber and (ii) collaborations between and comparisons of different AECs. Therefore, the identity and purity as well as the allergenic profile and quantitative composition of the allergens of the material must be documented

The impact of competing allergens, mould spores, Gram-negative bacteria, fungi, VOCs, and metals on clinical symptoms of allergic patients is not yet fully elucidated. Hence, some Task Force members use the advantage of the highly controlled climate in the AEC to perform toxicology testing of materials prior to lofting (in bulk form), with a focus on levels of government regulated toxics to gain some scientific evidence. Another safety concern relates to a potential build-up in CO_2 levels due to the participant load; again, local regulations on CO_2 levels (such as those applicable to hospital treatment rooms) can be applied. If different allergen sources are used from time to time in a given AEC (as was always the case for the facilities surveyed here), cross-contamination and the migration of airborne contaminants (e.g. foreign pollen) into the chamber must be prevented. In this respect, SOPs should be defined and qualified staff must ensure proper operation of the individual facility.

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After considering the AEC's air and released particles, some other technical aspects could be harmonized: the materials used for seating and other structures must be non-adhesive, a cleaning protocol must be in place, adequate service documentation must be maintained for all technical equipment discussed in this report. Poor designs or the use of excessive amounts of low- quality materials may trap allergens or spore-producing particles in lower/slower flow areas, seams, and grooves.

Yet, other requirements will concern the study participants and any staff who also remain in the AEC. Firstly, the participants' safety is paramount. All AECs must have diagnostic and interventional equipment (lung function test devices, patient monitoring equipment, communication systems, resuscitation equipment, "crash carts", etc.), SOPs and human resources (paramedics or other authorized medical professionals) in place to deal with not only severe allergic reactions but also other health events unrelated to the AEC or linked to failure of the AEC's control systems during a study. This aspect is part of the overall validation plan for hazards and mitigations.

Pandemic Measures for Allergen Exposure Chambers

The most important requirement during a pandemic (e.g. Coronavirus disease 2019 (COVID-19)) is to maintain the rights, safety, and well-being of site staff and trial subjects (referred to as participants). As different AEC are highly heterogeneous in their specifications, there is no overall guidance that is applicable to all, but rather each AEC operator must establish an appropriate approach taking chamber operations and local health authority guidances into consideration. Medical care and medical decisions made on behalf of subjects included in the AEC is the responsibility of the AEC site and Investigator. Certainly, the continuation of AEC operations during a pandemic is a complex decision to be made with reference to applicable competent authority positions on managing ongoing clinical trials during the COVID-19 Pandemic [EMA Guideline on the Management of Clinical Trials during the COVID-19 Pandemic and US FDA Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Pandemic, latest versions]. The safety measures must also follow local Public Health Authorities' restrictions and recommendations. Given the uncertainty of pandemics, local incidence rates and the associated impact on participants, the AEC site-specific changes and guidance may be of a regional nature and the final decisions of which measures to be

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taken is evaluated by the site, Investigator and their local public health authorities. The examinations and procedures must follow local guidance with respect to required use of personal protective equipment, SARS-CoV-2 virus testing, physical distancing, quarantine and/or other measures to ensure participant safety and should always be followed. If regional circumstances change, local guidance should be followed, but the personal safety comfort of participants should also be considered.

CONCLUSION

The data provided by the AEC operators emphasized both the similarities and differences between existing facilities. There appears to be room for useful progress towards the harmonization of chamber techniques e.g. determination of batch specific allergen profile and concentration of the used allergenic material or standardized symptom scoring. It is important to note that safety is a key concern for all AEC operators; all the facilities possess dedicated staff, equipment and procedures for protecting the well-being of study participants exposed to a test allergen. Furthermore, all the AECs appear to have validated procedures for producing stable, accurately measured levels of airborne allergen particles (mainly grass and weed pollens and HDM particles) for the duration of a typical challenge.

Disclaimer

Dr. Mahler and Dr. Kaul indicate, that the views expressed in this paper are the personal views of the authors as experts in the field of allergology and may not be understood or quoted as being made on behalf of or reflecting the position of the respective national competent authority, the European Medicines Agency, or one of its committees or working parties.

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FIGURES AND TABLES

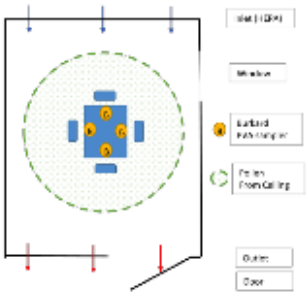
Figure 1. Schematic description of all AEC models, presented in clockwise order

1A. Aarhus AEC; 1B. ALL-MED AEC; 1C. ALYATEC EEC (20 seats and 10 particle counters (C1 to C10). Allergen particles are dispersed in the EEC through six outlets (green arrows). R1-R5 correspond to air extraction outlets); 1D. Biogenics AEC; 1E. CHIBAUNIV AEC; 1F. Cliantha AEC-2 and AEC-3; 1G. Fraunhofer Allergen Challenge Chamber (+ denotes pollen inlets from the ceiling, * denotes dispersion by ceiling ventilator, R denotes Rotorod samplers); IH. GA^{2}LEN chamber (Participants enter the chamber from the observation room (top right) crossing the changing room, where they are given protective clothing (top left). There are nine separate seats in the provocation room); 1I. Kingston EEU and HDM EEU; 1J. RED MAPLE TRIALS AEC (participants enter from a gowning area at the upper left and are observed through a large window at the upper right. The arrows at the top of the figure indicate allergen entry, and the arrows at the bottom indicate allergen exit through HEPA filters. Zone 2 is indicated by a blue rectangle); 1K. Vienna Challenge Chamber (VCC, AAR= active anterior rhinometry).

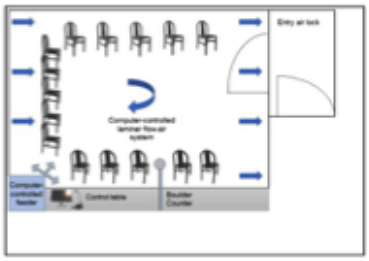
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1A



1B



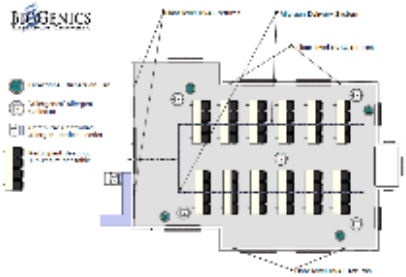
1C



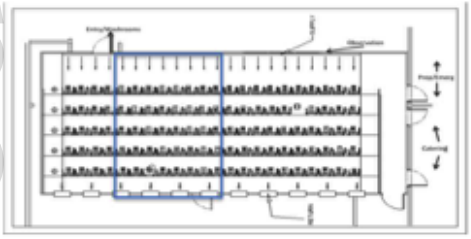
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1D



1J



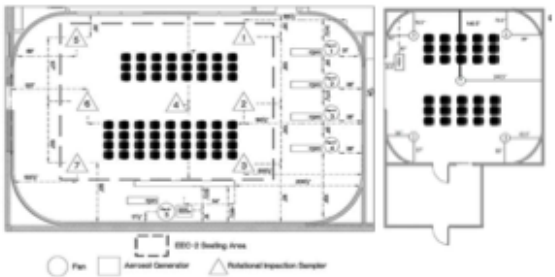
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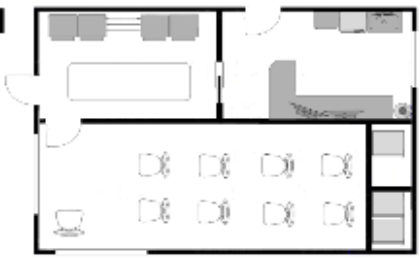
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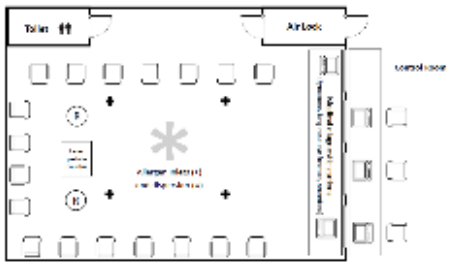
1F



1H



1G



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Figure 2. Pollen concentrations in the ALL-MED AEC

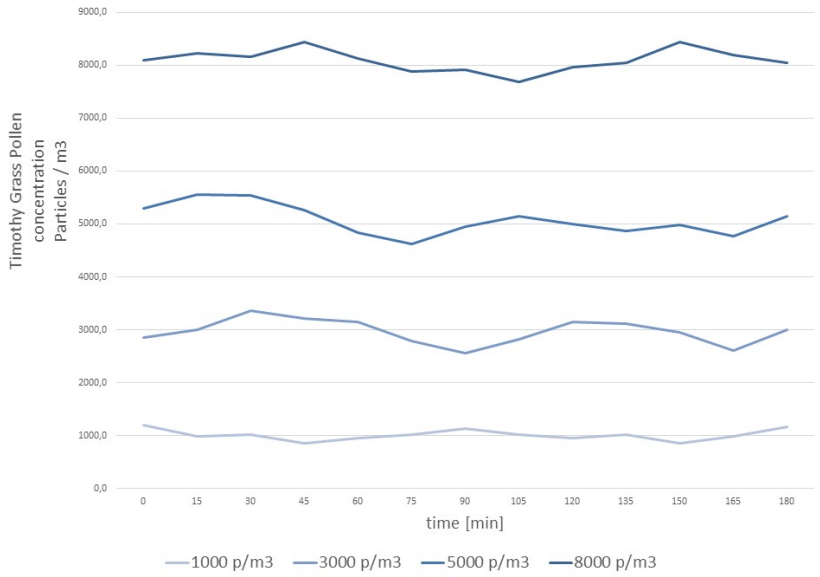
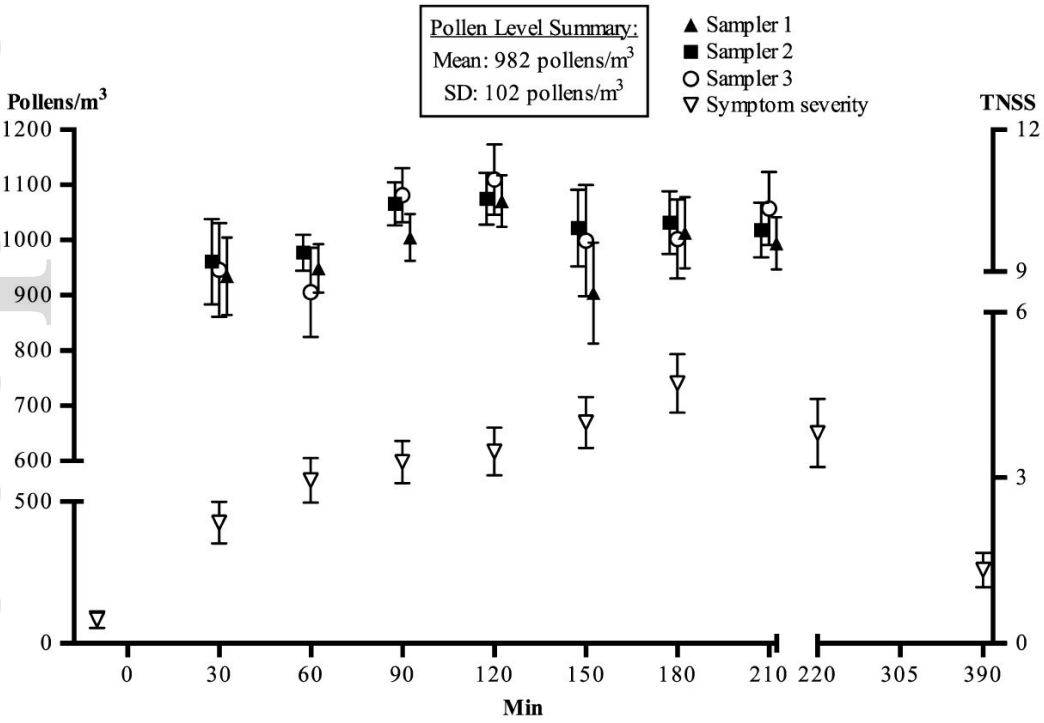
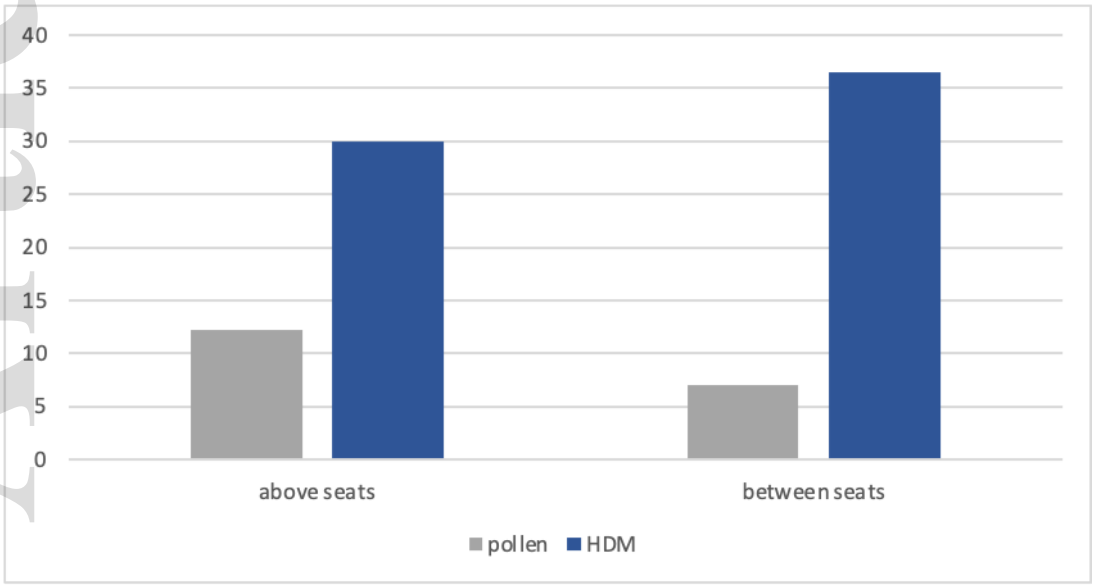


Figure 3. Pollen concentrations in the AARHUS AEC, measured at 21 time points during a study (7).



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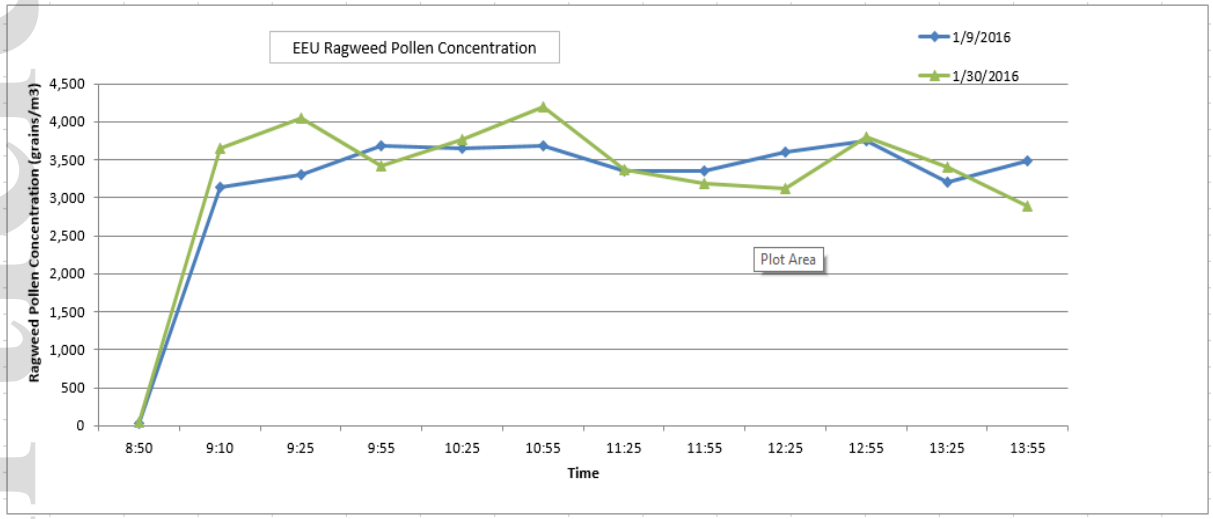
Figure 4: Variations (±%) with regard to the target concentration for pollen and HDM particles in the air at the level of the study subject's head during a 90-minute exposure in the GA²LEN AEC for a single seat and comparing different seats.

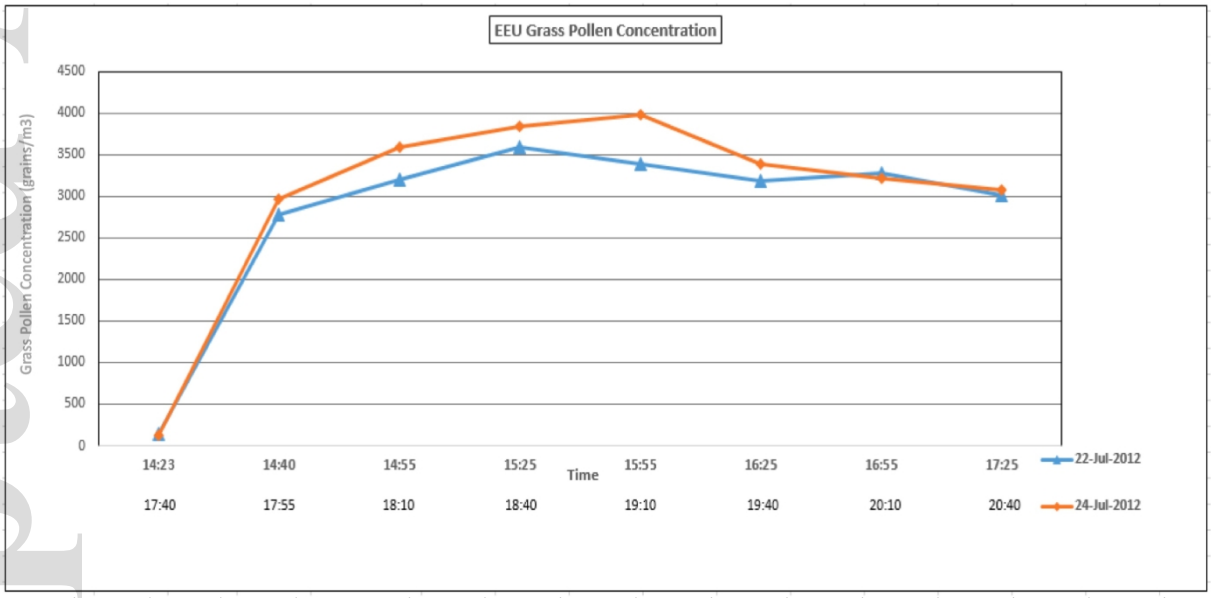


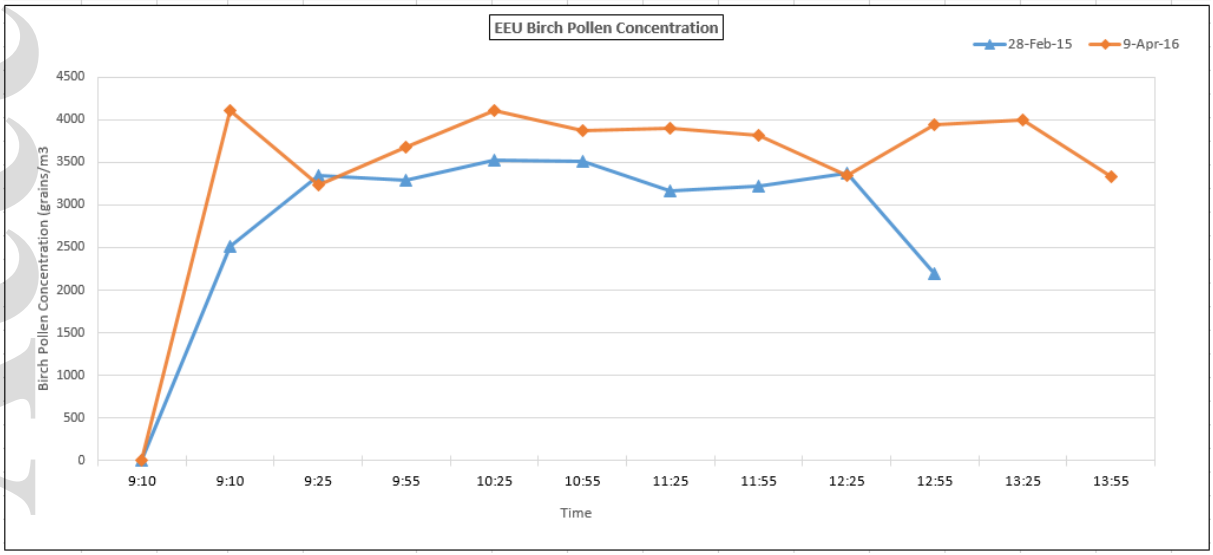
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Figure 5: Pollen concentrations in the Kingston AEC

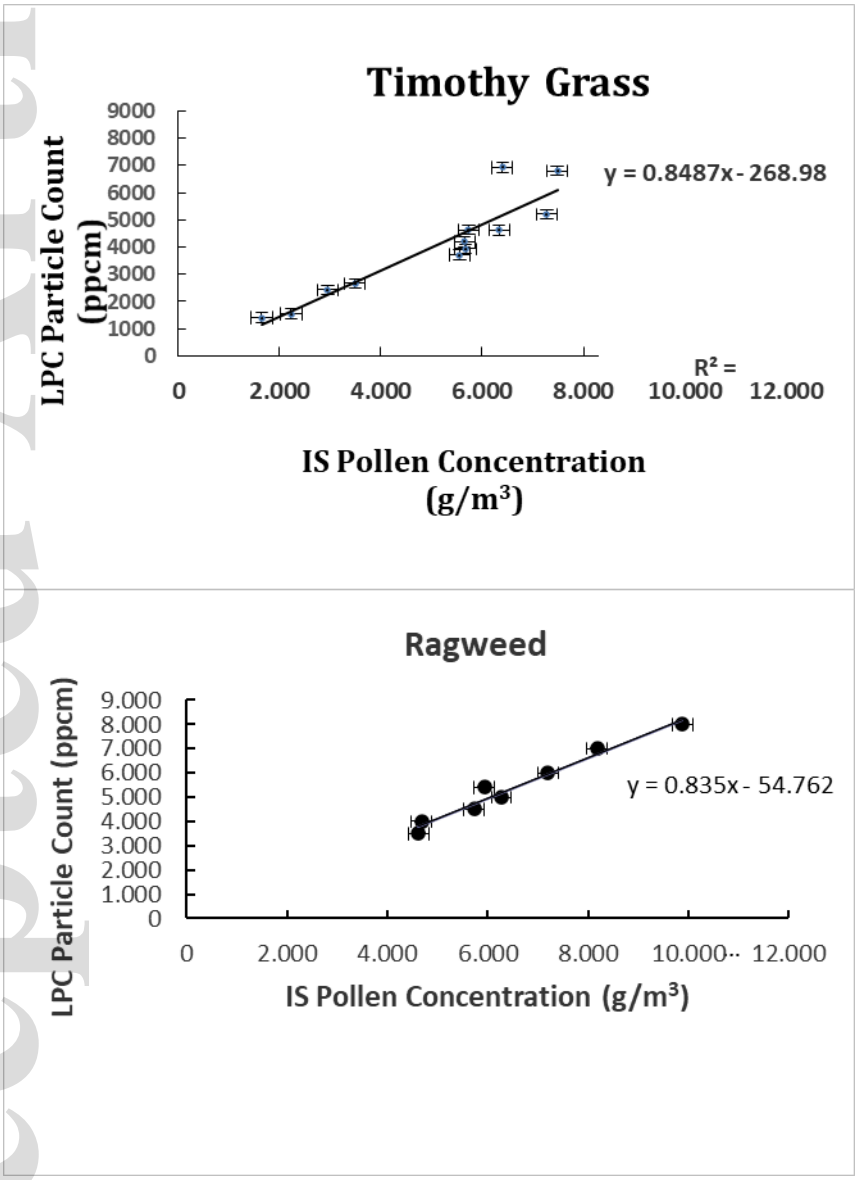






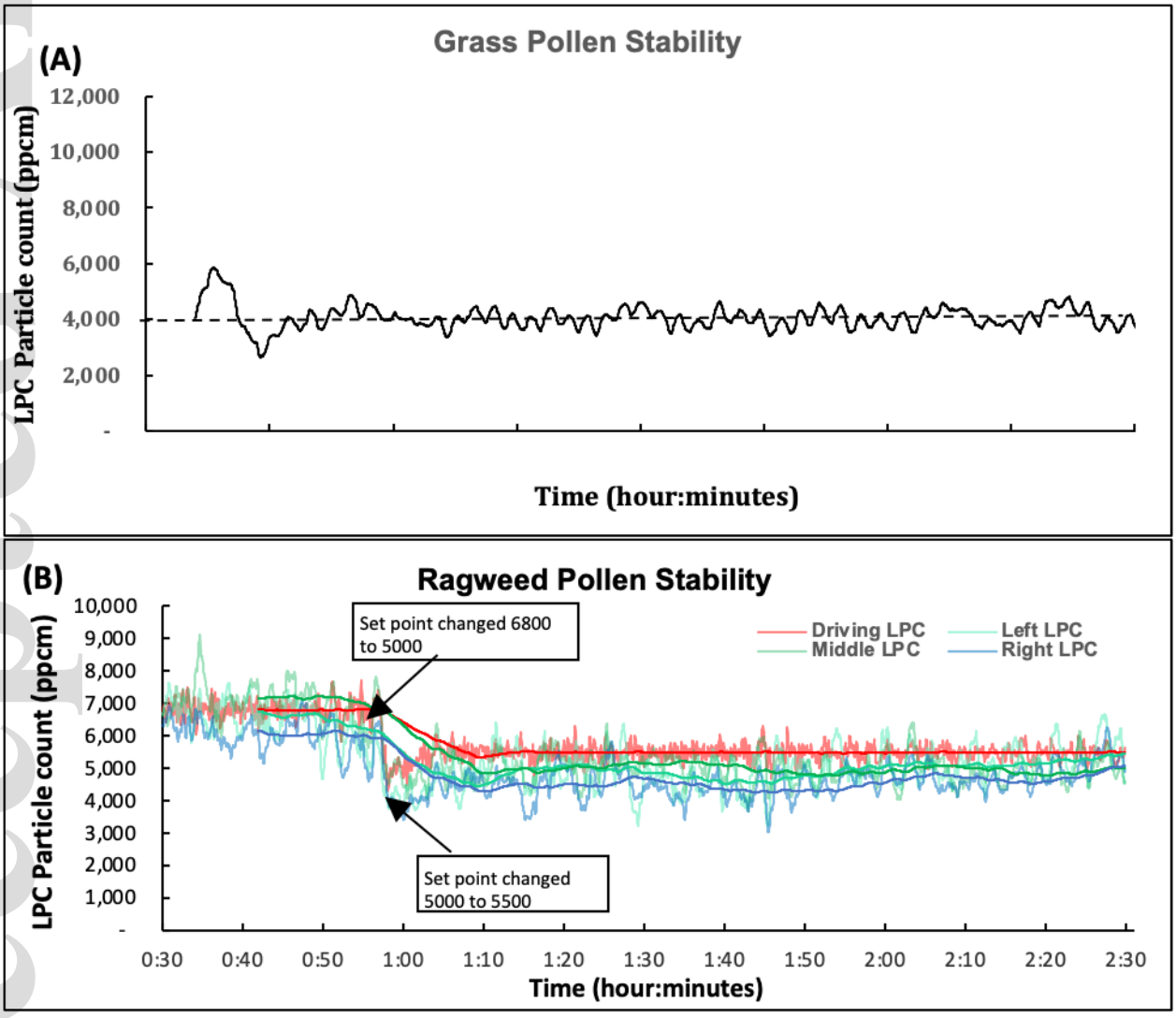
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Figure 6. In the RED MAPLE TRIALS AEC, the LPC-derived and RIS-derived particle counts are strongly and linearly correlated for both timothy grass pollen (top panel) and ragweed pollen (bottom panel) over a wide range of concentrations. This correlation allowed adjustment of the controlling LPC output to ensure correct pollen counts in the ACT.



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Figure 7. Panel (A): the grass pollen concentration in the RED MAPLE TRIALS AEC over 4 hours; the dashed line represents pollen setpoint. Panel B: Ragweed pollen concentrations over a 2-hour period. One LPC was designated as the driving LPC (red line) and the others were placed right (blue), middle (green) and left (aqua) in the centre of one zone. Solid lines: 12-minute running averages overlaying fainter lines which correspond to the raw data transmitted every 3 seconds. All LPCs were stable over the measurement period and were responsive to changes in set-point concentration. (ppcm = particles per cubic meter, LPC =laser particle counter, IS = impact sampler).

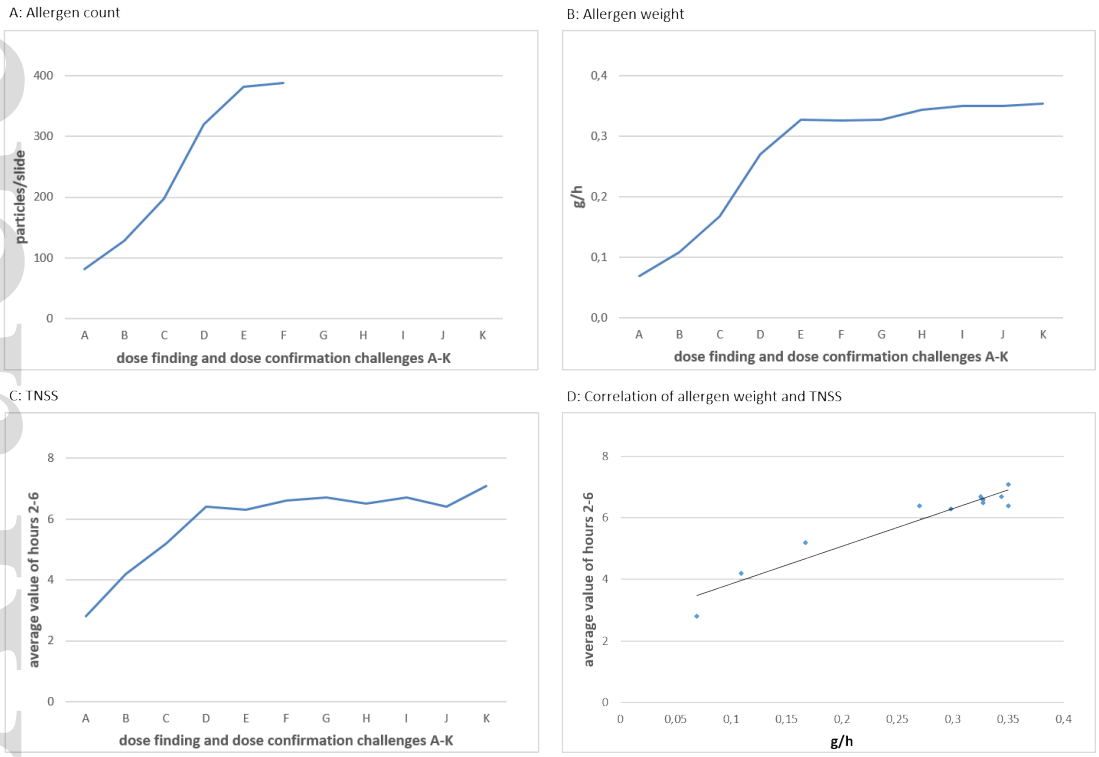


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Figure 8. Dose response correlation of the VCC Vienna HDM model (36).
A) allergen count (in particles/slide) B) allergen weight (in g/h) C) TNSS (average value of hours 2-6; 17 scores) during dose finding and dose confirmation challenges A-K and D) Scatterplot of the Correlation between mean allergen weight (g/h) and mean TNSS (average values of hours 2-6) r= .96

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Tables

Table 1: AEC operators having contributed to the present report.

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Abbreviated AEC name used in the present report	Name and/or operator	Location
AARHUS	Aarhus University	Aarhus, Denmark
ALL-MED	ALL-MED Medical Research Institute	Wroclaw, Poland
ALYATEC	ALYATEC at Strasbourg University Hospital (www.alyatec.com)	Strasbourg, France
BIOGENICS	Biogenics Research Chamber LLC	San Antonio, TX, USA
CHIBAUNIV	Chiba University	Chiba, Japan
CLIANTHA	Cliantha Research	Mississauga, Canada
FRAUNHOFER	Fraunhofer Institute for Toxicology and Experimental Medicine	Hannover, Germany
GA^{2}LEN	Global Allergy and Asthma European Network. European Centre Allergy Research Foundation.	Berlin, Germany
KINGSTON	Queen's University and Kingston Health Sciences Center, Kingston General Hospital	Kingston, Canada
RED MAPLE TRIALS	Ottawa Allergen Challenge Theater, Red Maple Trials Inc.	Ottawa, Canada
VIENNA	Vienna Challenge Chamber, Power Project GmbH (www.vcc.at)	Vienna, Austria

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Table 2: Distribution of *Phleum pratense* grass pollen in a single zone in the RED MAPLE TRIALS AEC. Data are quoted as the mean (SD) grains per m^3 for each RIS over the time period. The mean (SD) for all samples was 4000 (801) g/m3.

Position	Row 1	Row 2	Row 3	Row 4
Right RIS	4368 (867)			
Middle RIS	3363 (492)	5039 (585)	4302 (236)	3333 (338)
Left RIS	3597 (176)			

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Table 3: Distribution of *Ambrosia artemisiifolia* ragweed pollen in a single zone in the RED MAPLE TRIALS AEC. The mean ± SD for all samples is 4622 ± 578 ppcm. Abbreviations: AEC: allergen challenge chamber; LPC: laser particle counter; ppcm, particles per cubic meter.

Position	**Row 1**	**Row 2**	**Row 3**	**Row 4**	**Row 5**	**Mean (SD)**
Right LPC	4554	5166	4802	4238	3625	4477 (523)
Middle LPC	4947	5430	5508	4622	3988	4899 (559)
Left LPC	5401	4804	4229	3852	4163	4490 (550)
Mean (SD)	4967 (346)	5133 (257)	4846 (523)	4237 (315)	3925 (224)	
Overall mean (SD) all LPC data points						4622 (578)

Table 4: Semi quantitative analysis of mite allergen content by ELISA assay of mite material used in the VCC Vienna. Values indicate optical density (OD) (36)

Allergen	OD
Der p 1	3,581

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Der p 2	0,969
Der p 4	0,377
Der p 5	1,878
Der p 7	2,368
Der p 10	3,449
Der p 11	3,600
Der p 14	0,460
Der p 15	2,512
Der p 18	1,514
Der p 21	0,630
Der p 23	0,843

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Table 5. Summary of the AECs' characteristics

A: Abbreviation in this document

B: Chamber and/or Organization Name

C: City, country

D: Number of chambers

E: Max participants per chamber

F: Total floor space (m^2)

G: Ceiling height (m)

H: Allergens tested

I: Non-allergens tested

J: Temp. range (°C)

K: RH range (%)

L: Air change rate (volumes/h)

M: Allergen dispersion

N: Particle analyzers

A	B	C	D	E	F	G	H	I	J	K	L	M	N
AARHUS	Aarhus University	Aarhus, Denmark	2	*8 + 4*	29 + 11	*2.5*	grass (*Phleum pratens*) and birch pollen	toluene, n-decane, ozone, nitrogen oxides, house dust,	15°C to +40°C	0% to 100 %	0.5 to 20	turntable-type powder dispenser	LPC and Burchard samplers

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								cigarette smoke, diesel exhaust particles, wood smoke, candle smoke					
ALL-MED	ALL-MED Medical Research Institute	Wroclaw, Poland	1	20	12	*3.5*	grass (*Phleum pratense*) and birch pollen, and HDM	not reported	+18°C to +27°C	40 % to 58 %	5 to 20	Computer-controlled feeder	LPC
ALYATEC	Strasbourg University Hospital	Strasbourg, France	1	20	65	~2.25[a]	HDM, cat dander, birch allergen	endotoxins and volatile organic compounds	+18°C to +27°C	40 % to 57 %	4.6 to 20	nebulizer	Optical counters and impact samplers
BIOGENICS	Biogenics Research Chamber	San Antonio, TX, USA	2	70+20	*136 +25*	*3.07*	*HDM, Ragweed, Virginia Live Oak, Mountain Cedar, grass(Phleu*	None tested	*+15C to 25C*	*35 % to 60 %*	~12	computer-controlled feeder into a vortex blower	air sampling cassettes for aeroallergens and bioaeros

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							m pratense)						ols, also an LPC
CHIBAUNIV	Chiba University	Chiba, Japan	1	50	*71.8*	2.6	Japanese cedar and cypress pollen	None tested	+18℃ to 24℃	65 % to 85 %	15	computer-controlled feeder	LPC
CLIANTHA	Cliantha Research	Mississaugua, Canada	2	60 + 40	94 + 49	*3.03*	grass pollen mixture, *Phleum pratense* alone, oak, birch and ragweed pollen, HDMs, cat and dog allergens	thermal shifts, dry air, ozone, and fragrance	+20°C to +26°C	35 % to 45 %	3 to 30	aerosolized (within the chamber) using a proprietary device	Rotorod TM impact samplers
FRAUNHOFER	Fraunhofer Institute	Hannover, German	3	18	47 + 47	*2.8*	grass and birch pollen, HDM	ozone, ultrafine particles, and environmental	10°C to +30°C	20 % to	up to 20	feeder tubes or a spray-dryer	LPC plus Rotorod ® impact

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	for Toxicology and Experimental Medicine	y			+ 47		allergens	particles		95 %			sampler
GA2LEN	Mobile Chamber Experts/Global Allergy and Asthma European Network	Berlin, Germany	1	9	*36*	2.86	birch and grass pollen, HDMs	ozone	+15°C to +38°C	30 % to 80 %	5 to 30	particle dispersal unit	particle dispersal unit
KINGSTON	Kingston Health Science Center	Kingston, Canada	2	120 + 35	216 + 72	2.9	ragweed, birch, grass, HDM	diethyl phthalate	+15°C to +25°C	15 % to 60 %	6.5, 18	multimetered short-duct feeder system	Rotorod® impact sampler plus LPC

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RED MAPLE TRIALS	Ottawa Allergen Challenge Theater, Red Maple Trials Inc.	Ottawa, Canada	1*	40	133	2.2	*Phleum pratense* and ragweed pollen	None tested	+18℃ to +24℃	10 % to 60 %	up to 20	Programmable logic controlled particle dispersal unit	LPC and impact samplers
VIENNA	Vienna Challenge Chamber, Power Project GmbH	Vienna, Austria	1	20	21.5	2.7	grass, birch and Japanese cedar pollen, and HDM allergens	not reported	+20°C to +26°C	20 % to 60 %	15 to 20	dispensers next to the fan coils	Burkard samplers for allergens

[a] Calculated

[b]1 (but 4 independent quadrants)

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