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SPANISH AEROBIOLOGY NETWORK (REA)
Management and Quality Manual

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Spanish Aerobiology Network (REA): Management and Quality Manual

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1- Introduction. History of the Spanish Aerobiology Network (REA): Coordinating Centre and Participating Groups

In 1991, within the framework of a Hispano-British Integrated Action programme involving the University of Córdoba and the Pollen Research Unit at the Polytechnic of North London, now National Pollen and Aerobiology Research Unit, University of Worcester, attention was drawn to the need for a Spanish Aerobiological Monitoring Network, which could subsequently form part of the European Aeroallergen Network (EAN), based at the HNO-Klinik, University of Vienna, Austria.

A preliminary meeting held in 1992 in Zuheros, Córdoba, at the behest of Professor Eugenio Domínguez Vilches, was attended by research staff from several Spanish universities, together with representatives of a number of European aerobiological networks which were active members of EAN at the time, who were able to share their experiences in this field. Some of the Spanish research groups present at that meeting were working on various aspects of aeropalynology, and reported considerable public interest in the value of airborne pollen counts for preventing pollen allergies. At that time, however, findings could only be applied at local level, i.e. within the range covered by each monitoring unit.

The growing public demand for information, coupled with the need to coordinate and standardise the methodology used by each network member, prompted a move towards consolidating an aerobiological network covering a wide geographical area.

This first meeting saw the formal creation of the Spanish Aerobiology Network (Red Española de Aerobiología, REA), as a small network comprising just three sampling units, together with a work programme and a list of future goals. The University of Córdoba Aerobiological Monitoring Unit was also established as the REA's National Coordinating Centre, which was to be responsible for receiving, storing and processing data obtained at the various sampling sites, and for providing the media with data on airborne pollen counts.

Thereafter, the network grew rapidly, in terms both of the number of monitoring stations and the range of activities carried out by the Coordinating Centre and also by the various sampling units across the country. One of the first goals to be attained was the development of a standardised methodology to be applied by existing and newly-established sampling units. This methodology has been validated over the years thanks to extensive research by a number of specialists (Domínguez et al., 1992; Galán et al., 1995; Galán et al., 1997; Galán & Domínguez, 1997; Alcázar et al., 1999_a; Alcázar et al., 1999_b; Cariñanos et al., 2000; Alcázar et al., 2003; Galán, 2003). As a result, the pollen counts recorded at the current **47** Aerobiological Monitoring Units (**Figure 1: Map of REA Stations and Working Groups**) are fully standardised, thus enabling the generation of strictly comparable information. The Spanish Aerobiology Network is an academic network, comprising highly-qualified teaching and/or research staff specialising in various areas of Botany, Mycology, Palynology and Atmospheric Dynamics, all of which are essential elements in Aerobiology studies. The REA has been a Technical Network within the Spanish Aerobiology Association, since the Association was formed in 1995 (www.aea.uma.es/QueesAEA.html)

The creation of a growing number of Monitoring Units has been accompanied by an increasing range of activities intended to publicise the information generated, first at local level and then nationally; the REA now has the status of a National Pollen Allergy Prevention Service. The development and implementation of new technologies has also enabled members of the public to have full access to this service, through a number of channels, of which each person can choose the most suitable for his/her needs. Examples of joint activities by Network members include the regular publication of the journal *Rea*, which provides annual data from all the Monitoring Units, and the detailed analysis of the weather-related parameters most influencing results at each pollen station. At scientific level, REA researchers frequently publish papers in national and international journals. Outstanding among these are several joint papers by various research groups, whose findings have been striking in terms not only of their geographical scope but also of their comprehensive analysis of single species over a range of bioclimatic areas.

Although the main line of research among REA members concerns the application of airborne pollen data for the prevention of pollen allergies, the fact that many members are linked to Universities and Research Centres has also enabled them to focus on new areas of essential applied research within the framework of research projects sponsored by public and private bodies at national and international level. After an initial period devoted to the development of a suitable methodology and of prediction models for the major pollen types, members have started to address other topics which have enhanced the interdisciplinary nature of Aerobiology. These include research applicable to agriculture: pollen release data, studied in conjunction with phenological monitoring of the species concerned, provide valuable advance information regarding harvest prospects for commercial crops such as olives and grapes, and also for forestry-related species, such as the various *Quercus* growing in Spain. The expansion of the network of aerobiological monitoring units into various regions of Spain – mainland and islands – has enabled comparative studies of the behaviour of a single species in different areas. This research includes the establishment of flowering gradients for species growing over much of the country, so that the start of flowering in one area may be taken as a bioindicator for the start of flowering in other areas. Since most sampling units have been set up in urban areas, it has been possible to pay particular attention to ornamental species: a considerable amount of research into plane and cypress trees, for example, has provided valuable allergy-related and phenological information. Even more importantly, joint analysis of airborne pollen and atmospheric contamination data has highlighted the key contribution of airborne pollen counts to overall air quality levels. Samplers set up in rural areas have provided new information on the response of natural vegetation to adverse environmental situations, particularly where water shortages constitute a stress factor. The publication of numerous papers dealing with this aspect of aerobiology has made research results available to scientists working in other disciplines, prompting a growing interest in this field. This interest has constantly been made evident in congresses on aerobiology.

However, work on these applications has in no way diverted the REA from its overarching goal, and research into the prevention of pollen allergies remains a priority field, benefiting from the greatest investment of resources. The creation of the National Pollen Data Bank, housed at the Coordinating Centre, testifies to the full consolidation of the organisational structure, which has enabled the Centre to provide a service for which there is great demand. Over the years, and thanks to the incorporation of the

latest technology, the service has been improved and also made more accessible to users. Technological developments have been matched by ongoing research, focussing on the development of increasingly precise prediction models, which take into account all the factors involved in the complex process of release, airborne transport and deposition of pollen and spores. Detailed studies of the activity and allergenic potential of various pollen types have provided a great deal of new, valuable, high-quality information.

Whilst moving constantly towards consolidation at national level, the REA has also gradually become a major player on the international scene, enjoying the recognition of key world experts in this area of research. As a unified, active Network, it has taken part in all national and international Forums and other events, making amongst the greatest scientific contributions. Many of its individual members act as expert referees on numerous scientific panels, and some have even held leading offices in International Associations. Several REA working groups have been involved in Research Projects funded by the European Union; thanks to their multidisciplinary and international approach, these projects have generated knowledge which can only enhance the scientific value of Aerobiology as a discipline.

Relevant studies about Methodology carried out by REA Members:

1. Alcázar, P., C. Galán, P. Cariñanos & E. Domínguez. 1999. Diurnal variations of airborne pollen at two different heights. Journal of Investigational Allergology & Clinical Immunology, 9(2):89-95.
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6. Galán, C. 2003. Quality control in aerobiological networks. Postepy Dermatologii I Alergologii, XX(4):244-245.
7. Galán, G., P. Alcázar, E. Domínguez, F. Villamandos & F. Infante. 1995. Airborne pollen grain concentrations at two different heights. Aerobiología, 11:105-109.
8. Galán, C. 1997. Análisis y Control de Calidad en los Estudios Aerobiológicos. Revista Portuguesa de Inmunoalergología, 5:154-155.
9. Galán, C. & E. Domínguez. 1997. The capture media in aerobiological sampling. Aerobiología, 13:155-160.

Figure 1: REA Monitoring Stations. Working Groups.



ANDALUCÍA

Sampling Units: Córdoba, Priego de Córdoba, Baena, Hornachuelos y Ciudad Real

Responsible: Carmen Galán Soldevilla

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Responsible: Luís Ruiz Valenzuela

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2- Aerobiological sampling. Methods of Capture, Collection and Sample Preparation. Obtaining Results.

Given the close relationship between aerobiology and other sciences, one of the main aims is that information on airborne biological-particle counts should be of use in a wide range of disciplines and fields of application, including Biodiversity, Agricultural Engineering, Forestry, Phytopathology, Meteorology, Climatology, Forensic Science, Bioterrorism, and Pollinosis. Since the presence of biological particles in the atmosphere is closely related to the incidence of adverse reactions damaging to human health, it is essential to obtain data which may further the adoption of preventive measures. For that reason, sampling mechanisms have been designed to function in much the same way as the respiratory system, with a view to obtaining applicable data.

2.1 Type of sampler

Various monitoring methods, based on differing principles, have traditionally been used in aerobiological research.

Before selecting a method for capturing airborne biological particles, the aim(s) to be pursued must be clearly identified. These may include:

- a. To obtain a continuous record, or to take short, intermittent samples
- b. To obtain hourly, daily or weekly data
- c. To make a count of the total number of airborne particles, or simply of viable airborne particles
- d. To study either a particular taxonomic group or all airborne particles
- e. To obtain either a value for pollen rain or a value for the number of particles per volume of air

From the outset, the over-riding aim of the REA has been to create a database of airborne pollen and spore counts by continuous recording, using a sampler that facilitates detection of these particles.

As a standard, the REA uses volumetric suction samplers based on the impact principle (Hirst, 1952). These samplers enable standard data to be obtained regardless of the biogeographical and bioclimatic characteristics of the sampling area; they also allow hourly data to be recorded throughout the day. The suction flow rate is 10 litres air per minute, similar to the volume of air inhaled by the human lung.

This monitoring system is used by all the working groups in the various member countries of the European Aeroallergen Network (EAN), of which the REA forms part.

Hirst-type samplers offer a range of advantages: the robustness of the apparatus itself, which has to remain outdoors in adverse weather conditions; ease of use; efficiency; and minimum requirements – once in place, it requires only a permanent electricity socket and an anchoring system.

The two brands of Hirst-based equipment commercially available at present – the VPPS 2000 made by Lanzoni s.r.l., Italy, and the Burkard 7-day recorder spore-trap, by Burkard Manufacturing Co. Ltd., UK – can function continuously for one week without

attention, providing daily and hourly records. Detailed technical specifications are provided with the equipment.

2.2 Volumetric suction sampler units

As indicated above, all REA sampling stations currently use the volumetric suction sampler based on the impact principle, as initially designed by Hirst (1952). This is an essential requisite of the REA working protocol (Domínguez et al., 1992). Although these samplers were specifically designed for the capture of fungal spores, later modifications have enabled the highly-efficient capture of all solid airborne particles, of both biological and non-biological origin, whose diameter ranges between 1 and 100 micrometers.

The sampler consists essentially of three components: an impact unit, a wind vane and a vacuum pump.

The **impact unit** comprises an entrance orifice measuring 14 x 2 mm, and a circular support (drum) to which particles adhere. The drum is driven by a clockwork mechanism, enabling it to rotate 2 mm every hour, thus ensuring continuous air sampling and the provision of both hourly and daily data.

A length of Melinex® tape, coated with an adhesive substance, is wound around the drum; particles sucked in from outside at a certain speed adhere to the tape, minimising as much as possible the rebound effect.

The **vane**, fixed to the outer metal casing of the impact unit, ensures that the entrance orifice is always positioned according to the prevailing wind direction, thus enhancing the efficiency of capture of particles borne on air currents..

The **vacuum pump** is fitted with a mechanism to regulate air input volume. Throughput for airborne particles is 10 litres/minute, similar to volume of air inhaled by human lung.

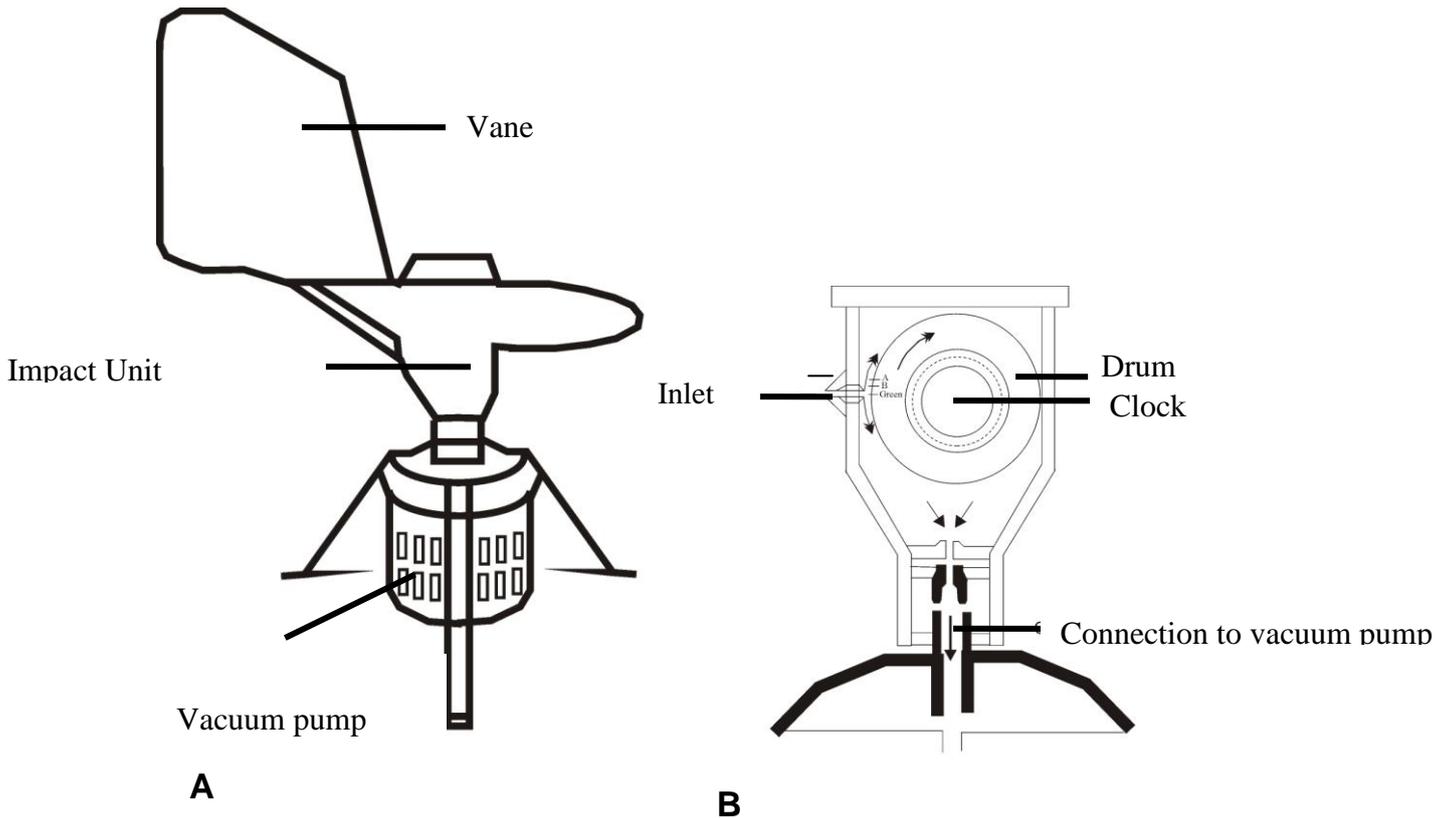


Figure 3: A. Hirst-type volumetric sampler (Hirst, 1952); B. Impact Unit.

2.3 Conditions for sampler positioning

In addition to the requirements outlined above, the installation of traps must comply with certain minimum requirements for aerobiological studies. The following regulations governing sampler positioning and installation have been adopted for all REA samplers:

1. The sampler should be placed on a readily-accessible, flat, horizontal surface.
2. Care should be taken to ensure that adjacent buildings do not screen the sampler or impede the flow of air. The sampler should be placed on the roof of a building; the height above ground level will depend on the city and on the height of neighbouring buildings.
3. The sampler itself should be elevated with respect to the installation surface, in order to avoid friction between air layers, which some studies have found to cause ground air turbulence. A tripod or small tower can be erected for this purpose.
4. Wherever possible, the sampler should not be placed in the vicinity of a fixed or mobile source of mass emission of biological or non-biological particles. The presence of single-species plant populations in the immediate surroundings of the sampler can lead to over-representation of a given pollen type, and thus to distorted data not representative of the species within the geographical range of the sampler. Proximity to non-biological particle sources may favour the massive presence of residues in samples, which will considerably hinder identification

5. The sampler should not be placed at the edge of a building, in order to avoid the turbulence generated by the impact of wind against the side of the building.

2.4 Setting up and running the samplers

Once in position, the sampler should be securely anchored to the ground, since it may at times be exposed to very strong winds.

Once installed and anchored, the sampler is connected to a permanent electricity socket, since the suction mechanism requires a constant electricity supply. The connecting cable should be well insulated.



Figure 4: Sampler installed on the flat roof of the Educational Sciences Faculty, about 15 metres above ground level. Raised from the ground using a metal tower. University of Córdoba.

As soon as the apparatus is plugged into the socket, the suction pump will start to function, and the sound of aspiration will be heard.

The first step in preparing the sampler for operation takes place in the laboratory: a length of adhesive-coated Melinex tape is wound around the drum. The drum can function continuously for one week.

The adhesive used to trap the particles should meet the following requirement: 1. It must be water-insoluble; 2. It should not dry up or evaporate; 3. The thickness of the spread film should not change over time, and should not be affected by temperature or humidity; 4. It should be highly retentive, thus ensuring that impacted particles do not bounce off again; 5. It must not support the growth of fungi or bacteria; 6. It should not be opaque under microscopy light; 6. It should be easy to use.

The REA working protocol uses LANZONI s.r.l. ® silicon fluid. This substance, a solution of pure silicone diluted in carbon tetrachloride has the advantage, amongst others things, that its physical properties remain unaltered over a range of temperatures from -20 to $+150^{\circ}\text{C}$, making it suitable for all the country's bioclimatic zones.

The adhesive is spread onto the Melinex tape using a soft brush whose diameter is similar to that of the tape. This operation should be performed in an extractor hood due to the volatile and toxic nature of the carbon tetrachloride.



Figure 5: Application of silicone fluid to the Melinex tape wound around the drum.

Once the adhesive-coated has been applied to the tape, the drum is carried to the sampler, in a hermetically-sealed metal drum-carrier, to avoid potential contamination in transit. This also minimises the risk of the Melinex tape rubbing against anything else.

Prior to operating the apparatus, the vane should be secured to ensure problem-free set-up.



Figure 6: Securing the vane.

With the head of the impact unit closed, the suction rate should be regulated to 10 litres/minute, adjusting the flowmeter to the suction slit. Alternatively, the regulating nut (outside on the Lanzoni sampler, inside on the Burkard sampler) can be used for this purpose. The flow rate should be checked every week.



Figure 7: Checking the flow rate.

The clockwork mechanism connected to the impact unit should be wound manually once a week. This is done by turning the nut or key (depending on the model) anti-clockwise as far as it will go, without forcing it. A typical clock sound will be heard when it starts to work. The drum adjustment device is fixed to the clock by a nut. It is essential to place the drum in the position indicated as the start of sampling (start bands), since this marks the sequence of sample collection over the whole sampling period; the length of tape immediately after the start bands corresponds to the first day of sampling.

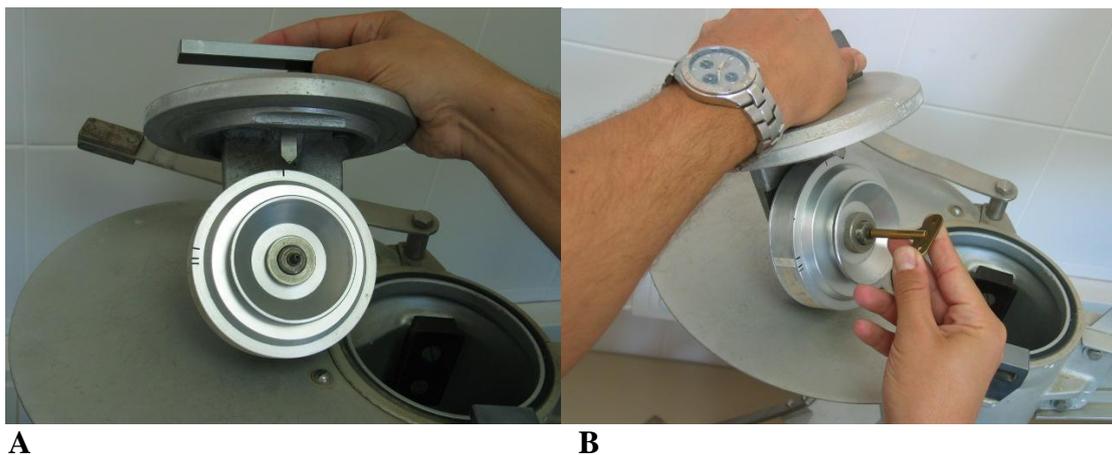


Figure 8 A: Correct positioning of the drum. B: Winding the clock on the impact unit.

Next, the head containing the impact unit is placed inside the metal casing using the guide-rail. It is hermetically sealed to prevent loss of vacuum and suction flow errors. At this point, the vane – previously secured by an anchoring screw – can be released.



Figure 9: Placing the head inside the metal casing of the impact unit.

After the sampling period (usually one week, or more often during peak pollen periods), a new drum is assembled in the laboratory and transferred to the sampling site, where it is exchanged for the used drum. The used drum, with the sampling tape, is taken to the laboratory for analysis, using the drum-carrier for this purpose. There, the sample preparation process can start, taking great care to avoid contamination.

The recommended time for the changeover of sampling drums is 12.00 UTM. Since, as indicated below, the sample-mounting process used 24-hour stretches of tape, each sample runs covers 12 hours on one day (from 12.00 UTM to 23.59 UTM) and 12 hours on the next day (from 00.00 UTM to 11.59 UTM).

2-5 Sample preparation

At the Aerobiology Unit laboratories in the REA member Centres, the following items are laid out on a clean table duly prepared for the process:



Figure 10: Items required for sample mounting.

- **Wad of blotting paper.** To protect the sample mounting surface, absorb any liquids that might be spilt and increase the sample/surface contrast, a white background enhancing sample visibility.

- **Perspex mounting ruler.** Included as an accessory with currently-available commercial samplers. The sampler drum rotates at 2 mm per hour; this transparent perspex ruler, which is over 1 cm thick, has notches every

48 mm. This enables the Melinex® tape, when placed over it (the ruler is wider than the tape) to be readily divided into 48 mm portions, each representing 24 hours' continuous sampling.

The Melinex tape is secured to the ruler at both ends, using a piece of adhesive tape which should never touch the impacted surface. In the case of the Lanzoni s.r.l. model, the ruler has a suction tube incorporated, which once connected to water flow allows fixing by doing a vacuum. The notches on the ruler facilitate tape cutting into 24-hour portions; one end of the tape is secured by a pin, and a cutter or sharp blade is used to make a clean transverse cut across the whole width of the tape.

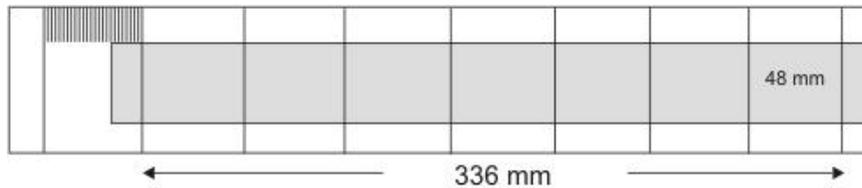


Figure 11: Melinex tape over perspex ruler on which each day of the week is marked.

○ **Microscope slide.** Before cutting the Melinex® tape into 24-hour portions, place on blotting paper as many slides as there are 48-mm (24-hour) portions of the whole impacted tape (i.e. a maximum of 7).

Each slide is identified by means of a sticky label bearing the name or initials of the sampling station, and the date; since each 48-mm portion contains data for two incomplete natural days, the sample should bear the date of the first of the two days. By this means, a series of samples for successive days is generated.

The procedure used to obtain data for natural days is described in detail below. The portions obtained by cutting up the Melinex tape are placed on the slide, on which a few drops of water have previously been placed to facilitate adhesion. At this stage, it is essential to respect the successive order of dates, both when mounting and at the start and end of sample preparation.



Figure 12: Preparation for a one-day sample, with identification label.

The sample must always be placed on the slide with the start-time to the left and the end-time to the right. To identify these positions, the identification label will always be placed on the left. Microscope sample reading will always be from left to right, i.e. from the first of the two sampled days to the second.

○ **Mounting daily samples.** The sample mounting medium should meet the following requirements: 1. It should be water soluble; 2. It should be compatible with the adhesive used; 3. It should allow selective staining of the material of interest (optional); 4. It should allow long-term storage of the material.

The traditionally-used medium is fuchsin-stained glycerin gelatin, whose composition is as follows: 50 ml glycerin, 7 gr gelatin, 1 gr phenol and a small amount of basic fuchsin, diluted in 42 ml distilled water, mixed – using an electric shaker – in an

extractor hood due to the toxic nature of phenol. The resulting mixture is pink in colour. This is the medium of choice, since it meets all the above requirements and is compatible with the adhesive used in the REA (silicone fluid). Use of basic fuchsin – a specific stain for plant material – facilitates pollen-grain identification and counting. All chemical products used in the mounting medium and in the silicone fluid should be stored in compliance with current regulations on Health and Safety at Work.

Glycerin gelatin is solid at room temperature, and needs to be liquefied before use. This is most swiftly done in a microwave oven, which takes only a few seconds.

Using a dropper, the liquefied medium is spread in an unbroken line on the slide cover, which is then placed on the slide containing the sample. The line should be continuous, and every effort should be made to avoid air bubbles, which would hinder identification and analysis. Any bubbles noted on the surface should be gently pushed to the edges, using a blunt instrument, before the gelatin resolidifies.



Figure 13: Application of glycerin gelatin to slide.

○ **Sealing medium.** Mounted samples should be sealed along the edges of the slide cover using a substance that will remain unaltered over time. Transparent nail varnish-lacquer is used for this purpose, since it is inexpensive, easy to obtain, easy to use, and of low toxicity; it dries rapidly, and is not impaired over time. Since it is transparent, it does not hinder sample identification. After microscopic examination (see below), sealed samples can be stored in a container known commercially as a Combi-box, designed specifically for optical microscopy samples.



Figure 14: Sample sealing. Alongside, collection of daily samples.

Prior to microscopy, mounted samples should be left for a while to allow the glycerin gelatin to solidify fully, and thus act as an adhesive between the slide and the slide cover. This interval also allows the various pollen grains to absorb the stain, thus enhancing their external morphological characteristics.

2-6 Sample Analysis and Counts.

Sample analysis is performed at a magnification of 40x10. Lower magnification would not enable identification of certain pollen types, while higher magnification would reduce the visual field. In principle, any microscope ensuring a good image and high resolution is suitable.



Figure 15: Microscopic reading of samples

Microscopic examination of aerobiological samples is essential for obtaining reliable results; it is also one of the most time-consuming stages in the process, due to the abundant material sometimes present on sample tapes.

As indicated earlier, the high-quality image obtained at the magnification recommended is ideal for identifying the various pollen types, largely distinguished by their external morphological features. The microscope should be well focussed, and a low-scatter white beam enables greater precision when identifying pollen types, thus minimising confusion of similar types.

Counting method:

Since it would take too long to count all the pollen-grains and spores on a slide, sub-sampling is essential in order to ensure the provision of timely information. The area selected should represent at least 10% of the whole slide (according to European Aeroallergen Network rules)

The Spanish Aerobiology Network counting method consists in 4 continuous horizontal sweeps over the whole slide with a 40x10 lens. This gives a subsample accounting for 12-13% of the total surface, depending on the microscopic field size at that magnification, which may vary depending on the microscope model.

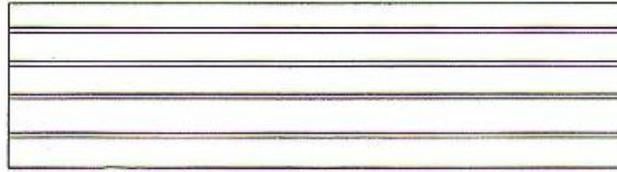


Figure 16: REA counting method: four continuous horizontal sweeps.

During these sweeps, the number of each pollen grain type identified is counted; this provides information on the pollen count throughout the day.

In order to ascertain the intra-diurnal variation, the REA uses a custom-made ruler: a piece of acetate cut to the size of the daily-sample slide is divided transversally into twenty-four 2 mm intervals, since the tape rotates 2 mm every hour. Divisions are marked in blue indelible ink using a superfine marker, since this offers the best light refraction. This ruler is placed over the slide, making sure that the first blue line flush with the start of the tape section to be analysed; the ruler is held in place with sticky tape.

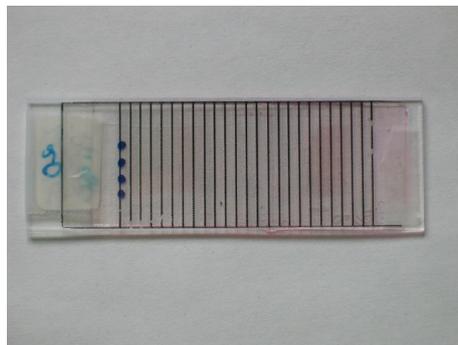


Figure 17: Placing the acetate ruler over the slide for a daily sample.

Pollen or spore counts for each hour can now be noted. The first hour is from 12.00a.m. to 13.00p.m. on a given day. The last hour is from 11.00a.m. to 11.59a.m. on the following day. The number of pollen grains per hour is noted on a data collection form, together with the sampling date and site. Each table represents one pollen or spore type, and comprises 4 horizontal rows, each containing 24 cells.

POLLEN TYPE				DAY												MONTH				YEAR			
13	14	15	16	17	18	19	20	21	22	23	24	1	2	3	4	5	6	7	8	9	10	11	12

Figure 18: Pollen-type data collection form.

Results for hourly counts are then added together to give total pollen counts for each pollen type for a given day.

2-7 Expressing the results.

Pollen counts should be expressed as the daily mean count per cubic metre of air, thus ensuring standardisation of data from all sites. For this purpose, the number of pollen grains counted is multiplied by a factor that takes into account the volume of air sampled (10 litres/minute), and the size of the microscope field of vision used (magnification 40x10). These measurements are included in the REA protocol. However, as indicated earlier, this factor will vary depending on the brand of microscope used.

- Calculating the correction factor:

The first step is to measure of the microscope field of vision at a magnification of 40x10.

Example: Let the diameter of the field of vision be 0.45 mm:

Air sampling rate: 10 l/min = 600 l/hour = 14400 l/day = 14.4 m³
 Mean diameter of the microscope field of vision: 0.45 mm
 Area of one horizontal sweep = 48 mm x 0.45 mm = 21.6 mm²
 Surface analysed = 21.6 x 4 sweeps = 86.4 mm²
 Total surface sampled = 48 mm length x 14 mm width = 672 mm²

Particle content per cubic metre of air = (672 mm²/86.4 mm²) x (1/14.4) x N

N = number of pollen grains in four sweeps.

Particle content per cubic metre of air = N x 0.54

2-8 Spanish Aerobiology Network database.

Once the total number of pollen grains per cubic metre of air has been ascertained for each identified and unidentified pollen type, for the whole 7-day week, these data are entered on standardised weekly data-recording sheets. This ensures availability on paper of a list of the minimum pollen types analysed. These weekly data sheets are then used as the basis for entering data into the computerised database.

SPANISH AEROBIOLOGY NETWORK

CITY: _____ RESPONSIBLE _____

PHONE N° _____ FAX: _____ E-MAIL: _____

TYPE OF SAMPLER: _____ DATE: From ___/___/200__ to ___/___/200__

Pgrains/m³/day	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY
Acer							
Alnus							
Apiaceae							
Artemisia							
Betula							
Brassicaceae							
Cannabinaceae							
Castanea							
Casuarina							

Cedros							
Compositae							
Corylus							
Cupressaceae/Taxaceae							
Cyperaceae							
Chenopodiaceae/Amarantaceae							
Ericaceae							
Fraxinus							
Helianthus							
Juncaceae							
Ligustrum							
Mercurialis							
Moraceae							
Myrtaceae							
Olea							
Palmae							
Pinus							
Plantago							
Platanus							
Poaceae							
Populus							
Quercus							
Rosaceae							
Rumex							
Salix							
Sambucus							
Ulmus							
Urticaceae							
Urtica membranaceae							
Unidentified							
TOTALES							

Figure 19: Daily data form for each pollen type.

The database designed by the REA Coordinating Centre is accessible to the various Monitoring Units belonging to the REA. Although Spain has a national Pollen Data Bank, located at the IT department of the REA Coordinating Centre, it is advisable for each Aerobiology Unit to have its own local database, since the Unit is responsible for managing and updating information at local level. The availability of a set of historical records not only ensures thorough knowledge of the incidence of local pollen types; it also facilitates the interpretation of results and the preparation of pollen forecasts, since mean-count curves can be drawn up for each pollen type, enabling inter annual variations from the mean to be analysed.

Essentially, any computer software incorporating a database function (e.g. Excel, Access Microsoft ®) can be used to create and maintain such a data storage system. The use of a statistical software package and of land-use maps is also recommended, as a basis for preliminary and advanced studies of airborne particle behaviour.

The spreadsheet of the database created by the Coordinating Centre is active in both Excel and Access, and contains a sheet for each day together with a list of potential

pollen types. This application facilitates the entering of the day’s pollen-grain counts. Days without records due to sampler malfunction will be deleted before statistical analysis.

The sheet comprises a set of columns, each headed with the first four letters of a pollen type (e.g. Poac for Poaceae). The first column indicates the date, in the format dd/mm/yy. The table of data for each pollen type and for every day of the year is thus generated by daily completion of the appropriate cell; a default value of 0 is automatically inserted in each cell, so that on every weekly update that value only needs to be replaced by the actual count; where no pollen was recorded, the default value 0 is retained.

Estación	Fecha	Acer	Alnu	Apia	Arte	Betu	Bros	Cann	Cast	Casu	Cedr	Comp	Cory	Cupr	Cypc	Chen	Eric	Frax	Heli	Junc	Ligu	
Ciudad	20/11/2006	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	1	0	0	0
Ciudad	21/11/2006	0	0	0	0	0	0	0	0	4	0	0	0	4	0	0	0	0	1	0	0	0
Ciudad	22/11/2006	0	0	0	0	0	0	0	0	2	0	0	0	5	0	0	0	0	0	0	0	0
Ciudad	23/11/2006	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	2	0	0	0
Ciudad	24/11/2006	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
Ciudad	25/11/2006	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0
Ciudad	26/11/2006	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	1	0	0	0

Estación	Fecha	Merc	Mors	Myrt	Olea	Palm	Pinu	Plan	Plat	Poac	Popu	Quer	Rosa	Rumc	Sali	Samb	Ulmus	Urta	Umen	Indet	Total	
Ciudad	20/11/2006	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	7
Ciudad	21/11/2006	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	0	12
Ciudad	22/11/2006	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
Ciudad	23/11/2006	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Ciudad	24/11/2006	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	3
Ciudad	25/11/2006	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	8
Ciudad	26/11/2006	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	8

Figure 20: Pollen Data Access Sheet.

Every week, each Regional Aerobiology Unit sends the latest update to the Coordinating Centre, by e-mail addressed to rea@uco.es. From there, data are automatically entered into the National Database, using a file created for each sampling site.



Figure 21: Introduction of pollen data in the application created.

Authorised staff at the REA Coordinating Centre are issued with passwords enabling them to enter data from the various sampling stations into the European Pollen Data Bank run by the European Aeroallergen Network (EAN <http://www.univie.ac.at/ean/>), based at the HNO-Klinik, University of Vienna, Austria. Information on the aerobiological situation in several European countries is updated weekly at www.polleninfo.org

A PC is therefore an essential tool for all Aerobiological Monitoring Units, in order to ensure the smooth operation of the Unit. The PC is a means of connecting the various Units and the Coordinating Centre, providing a fast channel for communicating data, news, general information and operating instructions for Units which have just joined. The Units use e-mail to send the weekly data obtained from aerobiological analysis to the Coordinating Centre, using the established format. The REA e-mail address is: rea@uco.es.

Bibliography:

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3- Interpretation of Results. Pollen Count Classes. Biological Air Quality. Publication of information.

3.1 Pollen classes

The REA classifies pollen counts from samples using a threshold system. The breaking down of counts into classes facilitates graphical expression of the results in the form of maps showing the current situation and possible forecasts, thus affording the user a fuller understanding of the information generated. In establishing a set of pollen count classes, the REA has sought to standardise aerobiological data over the whole country, bearing in mind the varying bioclimatic storeys and biogeographical units to be found.

However, it should be noted that at times, and for particular pollen types, type-specific classes and thresholds are required at local or regional level to reflect the numerous factors influencing pollen counts: abundance of a given species in a given area, presence of other species which might give rise to cross reactions, presence of atmospheric contaminants, specific weather conditions, among other factors, that are involved in the appearance of symptoms in pollen-allergy sufferers.

The cut off thresholds for each pollen-count class have been fixed to reflect: a) the anemophilous/entomophilous nature of the species concerned; b) the Annual Pollen Index; c) the daily pollen content in the air along the year, and d) the potential allergenic capacity of the species. For this purpose, pollen types have been assigned to four groups; for each group, there are four pollen-count classes: Nil, Low, Moderate and High. These terms refer to pollen-count thresholds required for a small, medium or large

percentage of the susceptible population to develop symptoms associated with the presence of this type of pollen.

The four pollen-count classes for each group of pollen types are as follows:

Group 1:

Classes: Nil: <1 grain/m³
 Low: 1-15 grains/m³
 Moderate: 16-30 grains/m³
 High: >30 grains/m³

Pollen types included: Apiaceae, Brassicaceae, *Cannabis*, *Echium*, Fabaceae, *Mercurialis*, Urticaceae, *Urtica membranacea*.

Group 2:

Classes: Nil: <1 grain/m³
 Low: 1-25 grains/m³
 Moderate: 26-50 grains/m³
 High: >50 grains/m³

Pollen types included: *Artemisia*, Asteraceae, Chenopodiaceae-Amaranthaceae, Ericaceae, *Helianthus* *Plantago*, Poaceae, *Rumex*.

Group 3:

Classes: Nil: <1 grain/m³
 Low: 1-30 grain/m³
 Moderate: 31-50 grains/m³
 High: >50 grains/m³

Pollen types included: *Acer*, *Alnus*, *Betula*, *Casuarina*, *Castanea*, *Corylus*, *Eucalyptus*, *Ligustrum*, *Populus*, *Ulmus*.

Group 4:

Classes: Nil: <1 grain/m³
 Low: 1-50 grains/m³
 Moderate: 51-200 grains/m³
 High: >200 grains/m³

Pollen types included: *Cupressus*, *Olea*, *Pinus*, *Platanus*, *Populus*, *Quercus*.

3.2 Biological air quality

At times, in addition to ascertaining whether the airborne pollen count is low, moderate or high, depending on the factors outlined earlier, air quality needs to be charted due to the potential simultaneous presence of one or more pollen types with allergenic capacity.

Over recent years, changes in normal climate conditions – a general increase in annual temperatures and marked alterations in the amount and distribution of rainfall – have delayed flowering in some grass species, and brought forward the flowering period of a number of spring-flowering tree species in the Mediterranean area. This has prompted some overlap in the flowering of a number of species with pollen-allergy potential. At

the same time, some species of agricultural or ornamental interest, introduced from elsewhere, are rapidly becoming naturalised, thus contributing to the already-high density of airborne biological particles. Given the constant increase in the pollen-sensitive population, i.e. the number of people likely to develop symptoms in response to a growing number of pollen allergens, the information provided aims to cover the greatest possible number of parameters responsible for impairing the quality of the air we breathe; for that reason, the REA seeks to offer not only a knowledge about the pollen and spores content in the air but also a precise assessment of Biological Air Quality (BAQ).

In general terms, and without taking into account local factors which may influence the established limits, Biological Air Quality in a given area is deemed:

Good, when airborne pollen counts remain at low levels;

Acceptable, when airborne pollen counts for most pollen types are low, but some types display greater allergenic potential (cypress, olive, plane, beech, grasses); or when counts are moderate, but the pollen types concerned are of low allergenic potential.

Poor, when pollen counts for pollen types with the greatest allergenic potential are moderate, or when sub-moderate counts are recorded for two or more pollen types with considerable allergenic potential;

Bad, whenever high airborne pollen counts are recorded for any pollen type with considerable allergenic potential, or when two or more highly-allergenic pollen types are recorded simultaneously. This tends to occur at specific times of year in the southern Iberian Peninsula, e.g. in early Spring, when still-moderate cypress pollen counts coincide with the start of plane flowering, and in late Spring, when the flowering of grasses is delayed and peaks at the same time as olive flowering.

3.3 Publication of information

One of the founding principles of the Spanish Aerobiology Network was that the general results of air monitoring should be made available to public via the mass media, so that anyone interested could have immediate access to information which might not only improve their quality of life but also enable them to adopt measures to prevent the symptoms associated with airborne pollen and spore allergies. From the outset, the REA has invested considerable resources in the publication of information, and in improving user access to it, using not only traditional media but also new technology developed over the last few years.

Today, aerobiological information is broadcast at various levels:

- Local level: each local urban or rural Pollen Monitoring Unit is responsible for publishing its results in the most appropriate media. This information is of interest for users whose activities take place in the area surrounding the Unit.
- Regional level: the Aerobiological Monitoring Units in a given biogeographical or administrative region usually belong to regional centres. This enables the acquisition of the resources required to maintain and publish information via regional media. Data available at this level more closely matches general user requirements.

- National level: thanks to the aerobiological coverage of most biogeographical areas in Spain, the Coordinating Centre is able to generate information at national level, based on data supplied by regional centres or local monitoring units, and available to users either via traditional media (press, radio and television), or via new technologies e.g. web pages, mobile phones). The opening of a commercial line supplying pollen-related information has enabled personalised information to be generated in response to an ever-growing demand.
- International level: as a member of the European Aeroallergen Network/European Pollen Information (EAN/EPI), the REA is committed to constantly updating its national data on the major allergenic pollen types during periods of peak airborne pollen counts. These data, like those of other European countries running a similar active network, is available at: www.polleninfo.org. Data obtained from the Aerobiological Monitoring Units forming part of the REA are also constantly updated at the European Pollen Data Bank, based at the University of Vienna (Austria). These data are occasionally used for scientific purposes, in studies related to pharmacy, meteorology or climate change at continental level.

One of the major activities of the Spanish Network is the publication of the journal *Rea*, which collects annual results from all active units with regard to particular weather-related features for the year. The multidisciplinary nature of aerobiology is evident in the increasing number of scientific papers produced in fields other than allergy studies, including agricultural engineering, biometeorology, climate change, air pollution, environmental studies, ecology, landscape gardening and even immunology, thanks to the recent application of immunoassay techniques for the detection of pollen allergenic proteins.

4- Management of the Spanish Aerobiology Network. Training Programme.

All REA staff is required to have the appropriate training to be able to undertake the work for which they are responsible. The REA has therefore laid down a set of criteria concerning the skills and qualifications required at each level of responsibility, in order for staff to implement properly the activities and processes involved.

The REA is also keen to motivate its staff, in order to enhance their job satisfaction; requests, suggestions and demands are therefore always welcome from all staff members.

Since the REA's needs are constantly changing, the skills and qualifications of the staff need to be continually reappraised and enhanced; this is done by in-house training activities, attending congresses and conferences, and generally improving staff self-awareness.

These activities are carefully programmed, to ensure the availability of appropriate resources, thus guaranteeing that staff at each level of responsibility are properly trained and acquire the best possible experience and awareness.

4.1 Levels of responsibility and required qualifications

The REA Coordinating Centre has drawn up a organisational structure flowchart, which includes a description of each level of responsibility together with the minimum entrance qualifications required; in all cases, these reflect the responsibilities involved, and ensure that the staff concerned are fully able to carry out their work. Each level of responsibility is clearly defined, and the functions and mandate of each post are clearly set out.

These requirements in terms of qualifications allow the REA:

- To institute appropriate training to met any change in professional functions or levels of responsibility within the REA.
- To tailor training programmes to meet the specific needs of new entrants at each level of responsibility.

4.2 Training programme

The aim of the Training Programme is to plan out all the training activities involving REA staff over a one-year period, and it is designed to meet the observed training needs of staff.

The term “training activities” covers all activities providing new or updated knowledge as well as scientific exchanges; activities include attending and speaking at scientific meetings.

Special emphasis is placed on training activities for new staff members, designed to provide them with an appropriate solid foundation as well as to promote their possible future specialisation in new areas or in various applications of aerobiology as a multidisciplinary science.

The following activities are part of the REA Training Programme:

- Attending national and international scientific congresses
- Presentation of scientific papers, holding of conferences and seminars to publicise aerobiology issues and the work of the Spanish Aerobiology Network
- Publication of annual results from each Aerobiological Monitoring Unit in the journal *Rea* and any other journals permitting the large-scale publication of results.
- Publication of research work in high-ranking scientific journals.
- Involvement in editorial and reviewing activities for scientific journals.

New staff are particularly encouraged to attend the courses in basic and advanced aerobiology organised on a regular basis by the various universities as part of doctoral and postgraduate degree programmes and masters’ degrees in expert training.

Each research group belonging to the REA has drawn up a training programme for new staff, aimed at ensuring they acquire the skills they need in order to carry out their tasks properly and without supervision. The training programme consists of the following sections:

- Theoretical training, designed to provide new staff with an introduction to the basic principles of botany, mycology, palynology and meteorology, thus enabling them to acquire general knowledge and learn the appropriate terminology.
- Practical sessions aimed at identifying the major pollen types and recognising distinctive morphological features. Staff is also taught how to handle the microscope, as well as learning how the pollen trap works, and how to maintain it and change the drum.
- In the laboratory, staff learns all the sample-mounting procedures detailed in chapter 2.
- After the theoretical training period, staff is asked to identify and count pollen types using historical samples, in order to check their precision. Samples containing few pollen types are recommended at the start; pollen-type diversity can gradually be increased as the staff becomes more proficient at working without supervision.
- After this practical training period, new staff work with control samples, i.e. samples for which no date or origin is given; staff are expected to identify the deposited material using the knowledge acquired during training. Staff scoring a success rate of over 90% is considered fit to analyse aerobiological samples without supervision, thus ensuring the quality of the results obtained.

The REA Training and Quality Plan also include peer updating and information-exchange sessions for experienced staff, with a view to maintaining high quality levels.

Scheduled activities include:

- Identification and counting of control samples both from the analyst's own biogeographical area and from other areas containing pollen types with which the analyst is less familiar.
- Identification of samples containing pollen types with similar morphology, in order to ensure correct identification
- Identification of new pollen types arising due to local climate changes or to the introduction of new ornamental species.

Other activities included in the REA Training Plan are concerned with basic and applied research. It is therefore essential that both the Coordinating Centre and the Regional Centres encourage staff to take part in national and international research projects, and that the centres run their own training programmes for research staff.

4.3 Staff qualification records

At all levels of responsibility within the REA, the following staff qualification records must be kept:

- *CURRICULUM VITAE*. All staff members should provide or draw up a CV, using a standard format.
- Documents related to training activities in which the staff member has taken part, e.g. records for external/in-house courses, diplomas or attendance certificates.

In general terms, and for guidance purposes, the minimum requirements for staff at the various levels of responsibility with the REA are as follows:

National Coordinator

- Required qualifications:
 - Postgraduate degree, and preferably university lectureship or Senior Researcher.
 - Must have been in charge of a research group in an aerobiology-related area.
 - Must have taken part in national and international research projects.
 - Fluent English.
 - Advanced computer skills.
- Remit:
 - Take responsibility for Network Coordination at national level.
 - Take responsibility for publication and dissemination of national pollen data generated by the REA Coordinating Centre.
 - Supervise activities carried out at all levels of responsibility within the REA.
 - Represent the REA at national and international events and scientific meetings.
 - Coordinate REA member groups.
 - Arrange for the provision of the financial resources required to maintain the REA's commercial activities
 - Draw up the Network Quality Policy, and ensure its implementation in all member centres.
 - Approval of all system-related documents: manual, procedures, annual work plan, among others.
 - Selection of Coordinating Centre personnel.
 - Supervise the management of human and financial resources.

National Organisation Secretary:

- Required qualifications:
 - Postgraduate degree, preferably doctorate.
 - At least three years' proven experience in aerobiology.
 - Must have trained at one of the REA's regional centres.
 - Must have taken part in research projects.

- Experience in management and administration, familiarity with Excel/Access databases and spreadsheets, and experience in resource-seeking.
 - Fluent English.
 - Advanced computer skills.
- Remit:
- Collate pollen data for commercial clients.
 - Deal with commercial clients.
 - Take responsibility for resource-seeking: draw up contracts, and apply to take part in R+D+I research projects, special actions, etc.
 - Manage the National and European Pollen Data Banks.
 - Take responsibility for the quality of the pollen data generated.

Research staff:

- Required qualifications:
- Postgraduate degree, preferably doctorate.
 - At least one year's proven experience in aerobiology, including supervision of research work, supervision of doctoral theses, involvement in research projects.
 - Fluent English.
 - Advanced computer skills.
- Remit:
- Oversee the functioning of the Aerobiological Monitoring Units for which the research staff member is responsible.
 - Take responsibility for the staff belonging to the Regional Aerobiology Centre or Unit for which the research staff member is responsible.
 - Draw up short and medium-term pollen forecasts for publication at regional or local level.
 - Oversee the publication of aerobiological information for the geographical area which the research staff member is responsible.

Technicians:

- Required qualifications:
- Degree or equivalent technical qualifications.
 - Must have trained at one of the REA's regional centres.
- Remit:
- Research support at the REA's regional centres and the REA Coordinating Centre.

New staff:

- Required qualifications:
 - Degree or equivalent technical qualifications.
 - Must have trained at one of the REA's regional centres.
 - Must have attended "Introduction to Aerobiology" courses.
 - Basic grasp of botany, in order to be able to understand the concepts and processes involved and interpret results correctly.
 - Intermediate computer skills.

These requirements will ensure the adequate implementation of the following tasks:

- Maintenance of Aerobiological Monitoring Units.
- Drum changing, sample mounting, sample analysis and counting and expression of results.
- Maintenance of local databases.
- Submission of data to the REA National Coordinating Centre.

5- REA Quality Plan and Quality Management.

5-1 Quality plan

The REA's geographical expansion programme is aimed at covering the largest possible number of bioclimatic belts and geographical units in Spain, by increasing both the number of Aerobiological Monitoring Units and the number of staff responsible for their management and maintenance. The social commitment assumed with regard to the users of aerobiological information thus requires that the data obtained at all centres be standardised and also submitted to very strict quality controls at all stages.

The Quality Plan designed by the REA covers the following:

- Staff responsible for maintenance, management and identification at the various Aerobiological Monitoring Units must have undergone training either at the REA Coordinating Centre or at one of the Regional Centres, in accordance with Staff Training Plan described earlier.
- Staff at the REA Aerobiological Monitoring Units must take part in the peer updating and information-exchange sessions scheduled in the REA Training and Quality Plan.

These activities will be run by coordinators at the various levels, and also periodically by staff at the Coordinating Centre. These staff is also responsible for checking the precision and reliability of the data provided to users. This can be done using various methods:

- Percentage matches between forecasts supplied and actual records, as tested by direct comparison. A match of over 90% is considered acceptable.

- Surveys to assess user satisfaction and assess the value of the data published. User feedback should be encouraged, in one section of the survey, as a means of improving the service.

With regard to technical specifications, the REA Quality Plan covers the following:

- Checking that sampler placement meets the requirements laid down in section 2.3 of this manual.
- Routine checking of sampler functioning, measuring flow rate, cleaning input orifices, checking clocks, electricity supply, etc.
- Checking sample transport and mounting procedures.
- Checking correct use of mounting adhesive and mounting medium.

5.2 Corrective and preventive measures. quality management.

The implementation of corrective and preventive measures by the REA is a key element in enhancing the effectiveness of the Quality System. For every event detected, a corrective/preventive action must be planned.

A corrective action or measure may be defined as any measure taken, following the detection of an unacceptable record, to eliminate the cause or causes which may have led to the appearance of that record, with a view to avoiding its recurrence in future. A preventive action or measure is one which is implemented prior to the detection of an unacceptable record, if a trend is observed which might lead to the future appearance of unacceptable records.

It is important to stress the difference between corrective and preventive measures:

- Corrective measures are implemented as a reaction to causes which may have given rise to a problem – an unacceptable record – which has occurred and which has prompted errors in a given process.
- Preventive measures are implemented in order to prevent the occurrence of a problem which could potentially occur in future.

Implementation of a corrective or preventive measure may be a short or a long process, depending on the complexity of the problem involved; it is therefore essential that the all such measures be monitored over a sufficient period of time, and that a member of staff be made responsible for monitoring.

Within the REA, an unacceptable record is most likely to be detected during the following:

- Monitoring of activities, documentation and filing.
- Inspection of the activities comprising the REA processes, including air sampling, sample preparation, equipment checks, expression of results and monitoring of documentation and filing.
- Monitoring of compliance with the requirements laid down in the Quality System section.

Once the likely cause of the unacceptable record has been ascertained, the appropriate corrective or preventive measure must be decided on. This involves:

- A precise description of the corrective or preventive measure to be implemented.
- Designation of the person(s) responsible for its implementation.
- Fixing a deadline for its implementation.