PD CEN/TS 16868:2015

BSI Standards Publication

Ambient air — Sampling and analysis of airborne pollen grains and fungal spores for allergy networks — Volumetric Hirst method

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National foreword

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Ambient air - Sampling and analysis of airborne pollen grains and fungal spores for allergy networks - Volumetric Hirst method

Air ambiant - Échantillonnage et analyse des grains de pollen et des spores fongiques aériens pour les réseaux aérobiologiques - Méthode volumétrique de Hirst

Außenluft - Probenahme und Analyse luftgetragener Pollen und Pilzsporen für Allergienetzwerke - Volumetrische Hirst-Methode

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European foreword

This document (CEN/TS 16868:2015) has been prepared by Technical Committee CEN/TC 264 “Air quality”, the secretariat of which is held by DIN.

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Introduction

Biological particles (pollen and fungal spores) are present in the air, causing health impacts at various levels. In Europe, nearly 18 % to 20 % of people suffer from pollinosis due to pollen and/or fungal spores. Pollen grains and fungal spores are considered in some Member States as an air pollutant as well as particles suspended in the air ($PM_{10,2,5}$). In Europe, European Aerobiology Society (EAS) in coordination with International Association for Aerobiology (IAA) manage problems of sampling, analysis, quality control, development and information.

For the sampling and analysis of biological particles different methodology and operating procedures are used.

Sampling equipment is diversified (see Annex A). Analysis is based on optical light microscopy for identification and counting pollen grains and fungal spores.

Elements and reagents used during sampling and analysis have very specific properties and require to be handled carefully.

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 aerobiology, biodiversity, agriculture, forestry, phytopathology, meteorology, climatology, forensic science, bioterrorism, and health (sensitization and allergy).

1 Scope

This European Standard specifies the procedure to sample continuously and analyse the concentration of airborne pollen grains and fungal spores in ambient air using the volumetric Hirst type sampler [1] [2] [3] (see Annex A).

This European Standard describes both the sampling and the analysis procedures for the purpose of allergy networks. For the other tasks mentioned in the introduction, other specifications may be required.

2 Normative references

Not applicable.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
accuracy
closeness of agreement between a measured quantity value and a true quantity value of a measurement

3.2
bench
long work table in a workshop or laboratory

3.3
clockwork
mechanism with a spring and toothed gearwheels, used to drive a mechanical clock, toy, or other device

3.4
combined standard measurement uncertainty
obtained using the individual standard measurement uncertainties associated with the input quantities in a measurement model

3.5
defatted
surface conditions after clearing with a fat removing substance

3.6
drum
cylindrical device for the mounting of a sticky tape

3.7
exine
outer layer of the wall of a spore or pollen grain, also called an exosporium

3.8
eyepiece
lens or combination of lenses in an optical instrument through which the eye views the image formed by the objective lens or lenses; ocular

3.9
flow meter
instrument for measuring the flow rate of a fluid in a pipe

3.10
flow rate
amount of fluid (e.g. air) that flows in a given time

3.11
fungal spore
reproductive unit capable of giving rise to a new individual with or without sexual fusion

3.12
hood
metal cover or canopy for a stove, ventilator, etc

3.13
impaction
sampling of airborne particles by inertial separation on any surface (e.g. of an adhesive)

3.14
magnetic stirrer
object or mechanical device used for stirring something

3.15
magnification
magnifying power of an instrument, e.g. this microscope should give a magnification of about x 100

3.16
microscope
optical instrument having a magnifying lens or a combination of lenses for inspecting objects too small to be seen or too small to be seen distinctly and in detail by the unaided eye

3.17
objectives
optics (in a telescope, microscope, camera, or other optical system), the lens or combination of lenses, that first receive the rays from the object and form the image in the focal plane of the eyepiece, as in a microscope, or on a plate or screen as in a camera

Note 1 to entry: Also called object glass, object lens, objective lens.

3.18
orifice
opening or aperture, as of a tube or pipe; a mouthpiece with a slotlike opening on the side of the trap

3.19
particle
minute portion of matter

3.20
pollen
male gametophyte of flowering plants, consisting of microscopic grain discharged from the anthers (*Angiosperms*) or from a male cone (*Gymnosperms*)

Note 1 to entry: Each grain contains two male gametes (only one can fertilize the female ovule).

Note 2 to entry: Pollen are transported by wind, water, animals (e.g. insects).

3.21
precision
closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions

3.22
repeatability
condition of measurement, out of a set of conditions that includes the same measurement procedure, same operators, same measuring system, same operating conditions and same location, and replicate measurements on the same or similar objects over a short period of time

3.23
reproducibility
condition of measurement, out of a set of conditions that includes different locations, operators, measuring systems, and replicate measurements on the same or similar objects

3.24
sensitivity
in aerobiology, measurement of the proportion of search particle which is correctly identified

3.25
slide
rectangular piece of glass on which an object is mounted or placed for examination under a microscope

3.26
specificity
in aerobiology, measurement of the proportion of non-searched particles which are correctly identified as different from the searched particles

3.27
standard measurement uncertainty
measurement uncertainty expressed as a standard deviation

3.28
suction
production of a negative pressure by the removal of air to force fluid into a vacant space

3.29
taxon
taxonomic group of any rank, such as a species, family or class

3.30
trap
container or device used to collect something

3.31
vacuum
space from which the air has been completely or partly removed

3.32
vacuum pump
pump or device by which a partial vacuum can be produced

3.33
wind vane
mechanical device attached to an elevated structure; rotates freely depending on the direction of the wind

4 Principle

Ambient air is sampled by a volumetric suction system and directed towards a suitably coated sampling surface through a specific orifice oriented towards the wind; the particles contained in the sampled air are deposited by impaction on a continuously moving adhesive acceptor surface. The sampling surface is then examined with an optical microscope in order to identify and count the allergy relevant particles per area (deposition rates). Using this method allows to count particles and subsequently calculate concentrations as a daily mean or a 2-hour mean. The low-volume sampler (10 l/min) allows a continuous sampling for up to seven days [4] [5] [6].

5 Sampling

5.1 Equipment

5.1.1 Apparatus

5.1.1.1 Motorised suction pump

The motorised suction pump shall work 24 hours a day and continuously throughout the year always at the same flow rate. The power supply may be either mains or battery driven (solar panels). The electric motor shall be capable of continuous operation.

The suction system, for instance, a vacuum pump, shall have a regular and continuous flow rate. The flow rate of suction may be adjusted by a flow control valve. The flow rate shall be 10 l/min (±1 l/min).

The flow rate shall be checked at every change of the impaction support with an adapted flow meter (i.e. the flow meter supplied by the same supplier of the sampler).

The flow meter shall be checked annually or less depending on the historical experience.

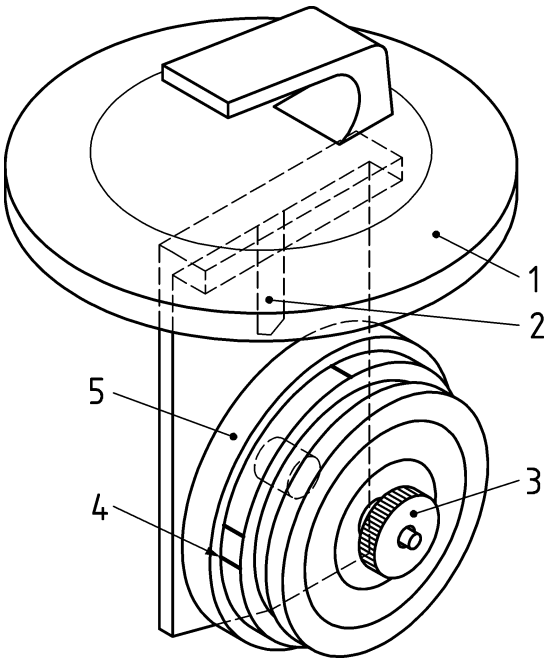
Sampling shall always be continuous and stable. The support shall scroll regularly in front of the back outlet of the orifice; its geometry and the scrolling speed depend on the duration of sampling period requested.

Example for the sampling area:

— a support of 48 mm (tape) with a speed of 2 mm/h: sampling period = one day;

— a flexible support of 336 mm with a speed of 2 mm/h: sampling period = seven days.

The scrolling speed may be adjusted to other sampling period durations. The trap can work with two different impaction supports: a drum (see Figure 1a) covered with a coated transparent tape or a glass slide (see Figure 1b) coated.



Key

1 lid

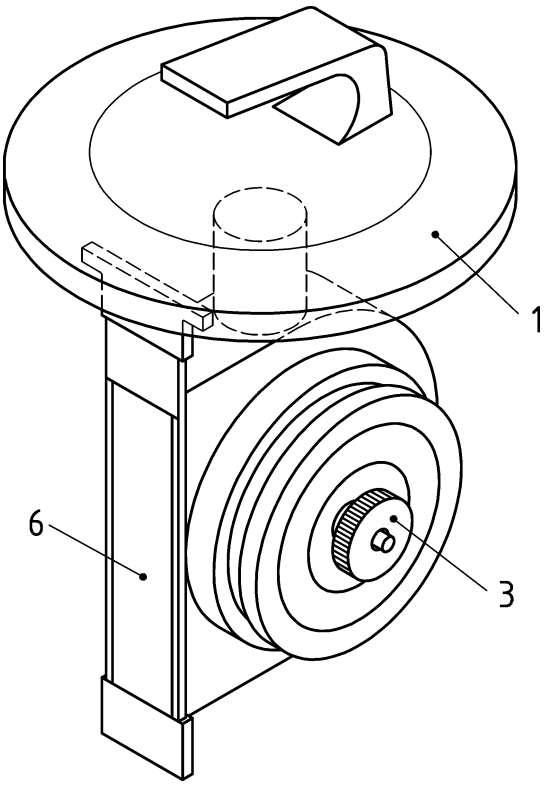
2 start reference pointer

3 lock nut

4 orifice start position

5 trapping surface

Figure 1a — The Hirst volumetric trap showing 7-day lid assembly with drum [5, modified]



Key

1 lid

3 lock nut

6 trapping surface on slide

Figure 1b — The Hirst volumetric trap showing 24-h lid assembly with slide [5, modified]

5.1.1.2 Specific orifice

The orifice shall have the following dimensions (with associated tolerances):

— rectangular opening: 14 mm (±0,1 mm) × 2 mm (±0,1 mm)

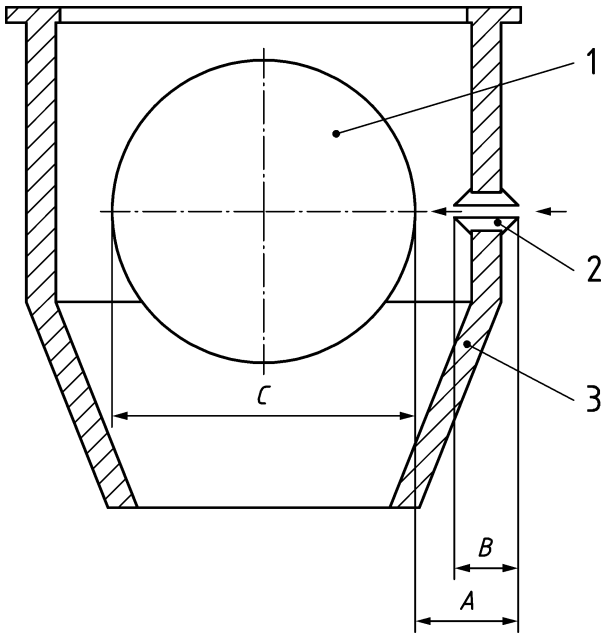
— depth of the orifice: > 19 mm

— distance from the inside orifice to the drum without the tape: 0,70 mm (±0,1 m)

The depth allows the non-turbulence of laminar flow and directs the mixture of air and particles towards the coated support. In consequence, an efficient particle impaction for pollen grains and fungal spores, induced by the laminar flow, is ensured.

The outlet of the orifice shall be 0,70 mm (±0,1 mm) of the coated support (see Figure 2 – distance A-B). The distance allows efficient particle impaction for pollen grains and fungal spores. It shall be controlled with a ruler [7] [8] [9].

The orifice should be directed into the air-stream using a wind vane.



Key

1	drum
2	orifice
3	cover
A	20,5 mm or 22,5 mm, depending on supplier
B	19,8 mm or 21,8 mm, depending on supplier
C	drum diameter 110 mm to 112 mm
A-B	0,7 mm (±0,1 mm)

Figure 2 — Pollen trap (Head of Hirst system) [9, modified]

5.1.2 Scrolling speed of support

The scrolling speed of the support is from 2 mm/h to 14 mm/h (±0,01 mm/h).

It shall be consistent throughout the scrolling period and can be ensured by a clockwork system or by an electric motor that shall not be stopped during the sampling period.

5.1.3 Impaction support

5.1.3.1 General

Only two options are allowed, both clockwork driven:

— a glass slide (76 mm x 26 mm) on which a transparent tape is fixed (48 mm x 19 mm) coated with specific reagents (see Figure B.1 in Annex B);

— a 110 mm to 112 mm diameter drum on which a transparent coated tape is wrapped around (see Figure B.2 in Annex B). The length of this transparent tape is 345 mm (±0,5 mm) to 350 mm (±0,5 mm).

5.1.3.2 Transparent tape

The transparent tape is coated with an adhesive in order to fix the particles.

The following requirements shall be fulfilled:

— The transparent tape shall be water-insoluble.

— The thickness of the whole transparent tape shall not be changed over time, and should not be affected by operational conditions (temperature between – 20 °C to + 60 °C or humidity between 20 % and 100 %).

— It shall be perfectly transparent to allow the passing of microscopic light.

— The length is > 336 mm.

5.1.3.3 Reagents

Two transparent coating products (solubilised in specific solvents or not) are useable: Vaseline (petroleum jelly) or silicone.

5.1.3.3.1 Vaseline (petroleum jelly)

The main characteristics of Vaseline (petroleum jelly) (n° CAS: [8009 03 8], see Annex C) are the following:

— odourless;

— colourless;

— viscous liquid;

— boiling point : 68 °C;

— purity > 99 %;

— risk of diarrhoea and other stomach problems if absorbed or in case of repeated exposure.

NOTE Consult Safety Data Sheet or MSDS to obtain special instructions before use.

Solubilise the Vaseline (petroleum jelly) with toluene (n° CAS [108 88 3], see Annex C). It is also possible to use unsolubilised Vaseline (petroleum jelly).

The purity of Vaseline (petroleum jelly) and toluene shall be > 99 %.

5.1.3.3.2 Silicone

The main characteristics of silicone (n° CAS [90337 93 2], see Annex C) are the following:

— odourless;

— colourless to white;

— pasty;

— high viscosity stable from – 20 °C to +150 °C;

— flammable over 400 °C;

— irritating to eyes;

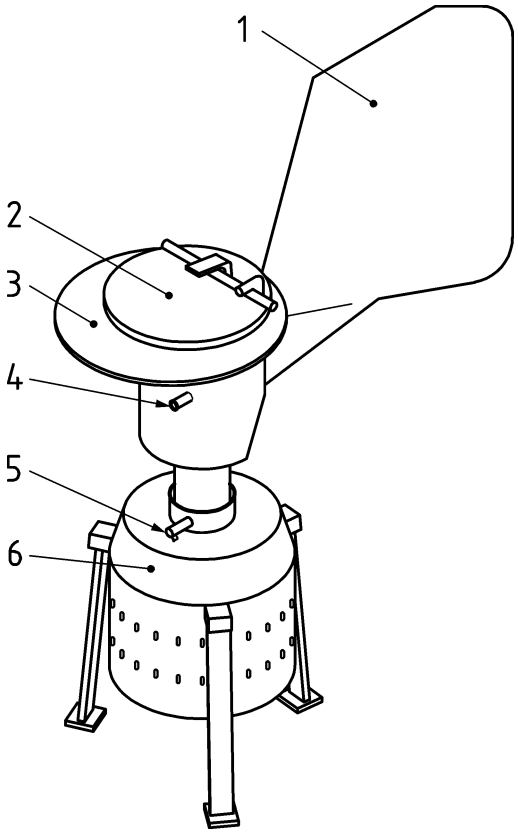
— non-biodegradable.

Solubilise the silicone with a specific solvent (see Annex C).

The physical properties of the adhesive medium remain unaltered at temperatures between – 20 °C and + 50 °C, making it suitable for the majority of bioclimatic zones.

5.1.4 Wind vane and rain shield

The wind vane allows permanent rotation of the trap head so that the orifice faces the wind. The rain shield ensures a weather protection for the orifice (i.e. rainfall) (see Figure 3).



Key

1 wind vane
2 lid assembly
3 rain shield
4 orifice
5 rotation lock
6 motor cover

Figure 3a — Schematic picture of a Hirst seven day volumetric spore sampling system [5, modified]

Figure 3b — Photo of a Hirst volumetric spore sampler [Source: RNSA]

5.1.5 Complete sampling system

The complete sampling system (so called “trap”) containing the motor, the vacuum pump, the orifice, the rotating drum, the wind vane, the clockwork system, the impaction support shall be

— resistant to corrosion;

— well attached (i.e. resistant to wind-blow, …);

— always horizontal (at the head level).

The commercial devices that meet the requirements are presented in Annex A.

For the different purposes, refer to the specific publications.

For allergen monitoring purposes, the following requirements and conditions for sampler positioning of the trap shall be fulfilled:

— The sampler shall be placed on a readily accessible, flat, horizontal surface. It should be on the roof of a building, and away from the edge of the building in order to reduce the effects of turbulence.

— Care shall be taken to ensure that adjacent buildings do not screen the sampler or interfere with the airflow. The sampler should be ideally placed on the roof of a building at more than 2 m from the edge; the height above ground level depends on the city and on the height of neighbouring buildings.

— The sampler itself shall be elevated between 100 cm to 150 cm from the roof in order to avoid turbulence between air layers and possible re-suspension of particles from the roof.

— The sampler shall not be placed in the vicinity of fixed or mobile sources of mass emission of biological or non-biological particles. Proximity to non-biological particle sources may favour the massive presence of residues in samples, which considerably hinder identification.

5.2 Operating procedure

5.2.1 Preparation of the coating medium [12]

Prepare the coating medium as follows:

— Vaseline (petroleum jelly (18 g (± 1 g)) and toluene (1l) (purity > 99 %);

— under the hood, add Vaseline (petroleum jelly) to toluene stirring until completely dispersed and leave it to stand for about 48 h with periodic shaking to obtain a homogenous fluid solution.

Or use pure Vaseline (petroleum jelly).

Or

— silicone fluid (20 g (±1 g)) and a specific solvent;

— under the hood, add silicone to the solvent stirring until completely dispersed and leave to stand for about 48 h with periodic shaking to obtain a homogeneous fluid solution;

— spread the solution on the tape with a brush and let it dry under the hood for at least 1 h.

The brush should be a pure Ox-hair brush N°14.i.e.: ref 922 N°14 OMEGA, made in Italy[1)]

WARNING — For the use of silicone or Vaseline (petroleum jelly) solutions:

— Keep the transparent tape dust free and always work with closed windows to avoid contamination.

— Always keep the coating medium under an extractor hood due to the release of solvents (toxic nature of the solvents).

— Wear protective gloves/clothing/equipment for eye and face protection. Wash thoroughly after handling.

5.2.2 Support preparation

Fixation of the coated or uncoated tape on the drum.

Be careful to

— always clean the drum or the glass slide with ethyl alcohol (70 %) in order to avoid dust and/or biological particles;

— fix the coated or uncoated transparent tape on the drum.

If it is an uncoated tape, the tape fixed on the drum shall be coated under the hood if a solvent reagent is used.

The capture surface shall be covered with a thin homogeneous layer of coating medium in order to retain the targeted particles.

The support shall be protected from ambient air during transportation from the laboratory to the trap and return and shall remain in a metal box for protection against shocks (see Figure 4). Conservation at ambient temperatures shall not exceed 12 months for silicone and one month for Vaseline (petroleum jelly).

1) Brush: ref 922 N°14 OMEGA is (are) an example(s) of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

Figure 4 — The drum and its protection box [Source: RNSA]

5.2.3 Changing of the drum

The person in charge of changing the drum shall

— mark the tape with a sharp instrument through the orifice at the end of the registered drum;

— measure and record the flow rate of 10 l/min (±1 l/min);

— remove the drum, clean the orifice and the etancheity gasket;

— wind the clock;

— put in the new drum;

— measure the flow rate; make a note of it and correct if necessary after cleaning the orifice and checking the sealing;

— note date and exact time (hour and minute) as well as flow rate and initials of the operator;

— mark the tape with a sharp instrument through the orifice at the beginning of the new drum.

6 Analysis

6.1 Equipment required

— microscope;

— magnetic stirrer;

— hot plate or microwave;

— bench;

— utensils to use (tweezers, scalpel);

— winding support;

— cutting rule;

— extractor hood;

— brush;

— glass slide;

— cover glass or cover slip;

— ethyl alcohol (70 %);

— reagents for coating medium;

— reagents for mounting medium;

— transparent tape;

— drum (with its box).

6.2 Operating procedure

6.2.1 Support

6.2.1.1 Slide

The size of the microscope glass slide is ≥ 76 mm × 26 mm.

The requirements for slides are the following:

— clean;

— defatted;

— disposable (single use).

6.2.1.2 Microscope cover glass

The size of the microscope cover glass shall be longer and larger than the size of the fraction of tape which is under the cover glass.

Properties that shall be respected:

— transparent;

— disposable (single use).

6.2.2 Mounting medium

The reagents used for colouration and fixation of the preparation are the following:

The colouring agents may be fuchsin (N° CAS [632 99 5], see Annex C) or safranine (N° CAS [477-74 6], see Annex C). The fixation agents may be glycerine/gelatin or simple glycerine.

Specific mixtures containing Gelvatol[2)]/Mowiol[3)] or other ready to use products may be used.

6.3 Methodology for counting

6.3.1 Glass slide preparation for microscopy analysis for drum tape [13] [14]

The preparation of the slides for the microscopic analysis shall be performed at room temperature and in less than 14 days after removing the drum from the trap (to keep the migration of pollen grains as low as possible).

Operating procedure:

— If necessary, put the mounting medium on the hot plate or in a microwave oven.

— Clean bench, hot plate and utensils before use (tweezers, scalpel).

— Take one slide per day (seven to eight if weekly monitoring) and stick labels (location, date and initials of the operator) onto one side of the glass.

— Degrease the glass slide with ethyl alcohol (70 %) if necessary.

— Fix the drum on the winding support (see Figure 5).

— Cut the tape at the junction point (double slide tape).

— Take the tape with the tweezers (do not touch the tape with your fingers) and place the tape on the cutting rule (see Figure 6).

— Match the beginning or the end of the deposition of pollens and place it taking into account the hour.

— Cut the tape into daily portions (see Figure 6). Put three drops (0,05 ml/drop) or a line (0,15 ml) of liquid mounting medium (with or without colour) on the glass slide (see Figure 7 and Figure 8).

— Using the tweezers, gently deposit the first tape section in the centre of the glass slide on the mounting medium. The beginning of the recording shall be on the label side.

Place three drops (0,05 ml/drop) or a line (0,15 ml) of coloured mounting medium on this tape or on the cover glass.

Place slowly cover glass on the preparation, avoiding bubbles and movement of particles.

— If necessary, leave the glass slide on the hot plate from 50 °C to 60 °C to remove air bubbles.

— Leave the slide to cool at room temperature for at least 30 min.

— Use the same procedure for the other daily sections of tapes.

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2) Gelvatol is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN to this product.

3) Mowiol is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN to this product.

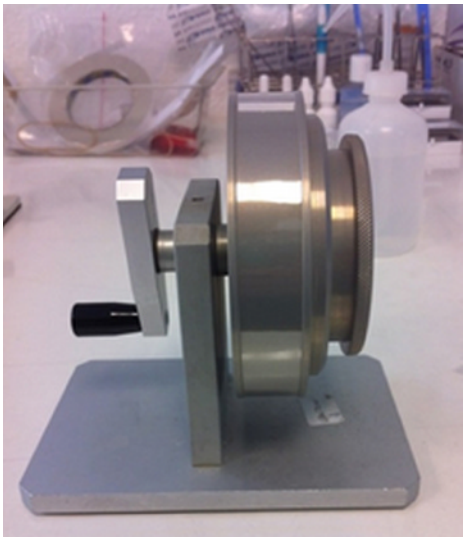


Figure 5 — Drum on the winding support

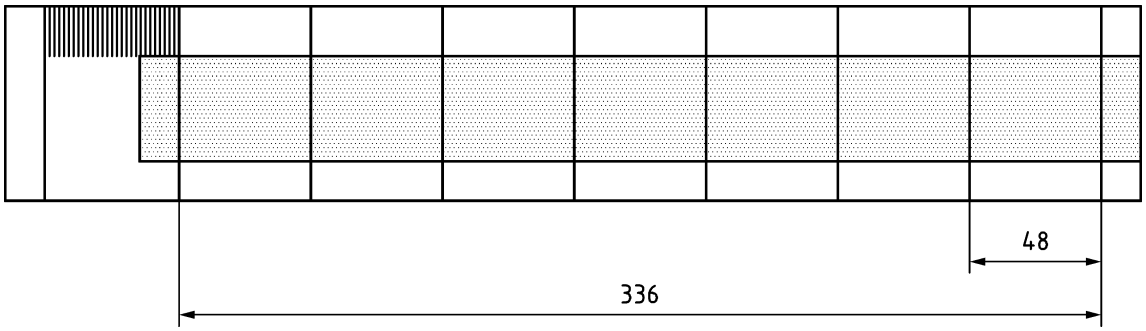


Figure 6 — Transparent tape on the cutting rule

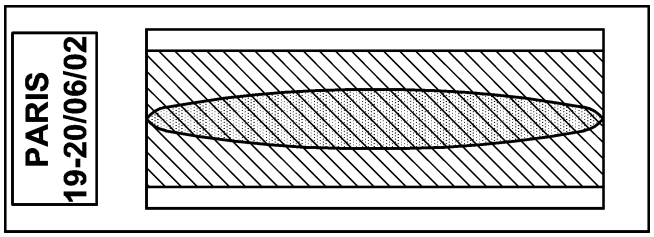


Figure 7 — Glass slide preparation – method 1

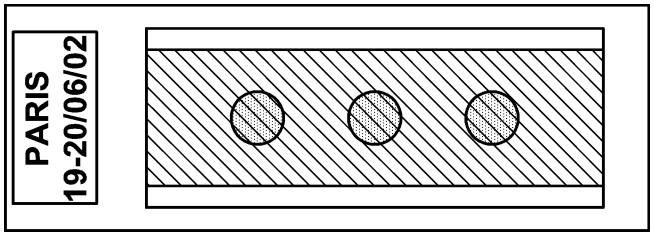


Figure 8 — Glass slide preparation – method 2

6.3.2 Optical microscopy [14] [16]

The slides are examined under an optical microscope at variable magnification [1].

The samples analyses are performed with different objectives ranging from X20 to X100.

The eyepieces are from X8 to X12,5.

In a routine mode, for pollen and fungal spore identification a magnification of at least 400X shall be used.

NOTE Lower magnification might increase the error of identification, but a higher magnification requires a greater reading area not inferior to 10 % of the deposit area.

6.3.3 Identification [15] [16]

The identification shall be made by a specifically trained operator.

The identification takes place according to some key of determination.

The classification is achieved thanks to a key of determination which is based on size, form, number and types of apertures on the surface, ornamentation of exine, etc.

Example: The most widely used key of determination is the one provided by RNSA (see Annex D) [14].

More details and information about this key of determination are given in Annex D [15].

The Spanish Aeropalynology Atlas offers the pollen types description [16].

6.3.4 Counting method

Horizontal or vertical parallel sweeps can be used for reading slides. Due to the area of slides examined depending on the size of the microscope's field of view and amount of magnification, a minimum area of the slide recommended for counting shall be defined rather than the number of transept (horizontal or vertical) used. A minimum surface of 10 % for pollen analysis is recommended.

NOTE Horizontal sweeps, i.e.sweeps running parallel to the time axis are strongly preferred, following the same drum rotation continuously, to provide better results.

In the examined area, the number of each pollen and fungal spore identified is counted; this provides information on the pollen and fungal spore count throughout the day.

6.3.5 Data recording

— manual recording;

— semi-automatic recording (voice recognition and incrementing in a database).

The minimum information that is collected is:

— location;

— year;

— month;

— day;

— hour;

— counter's name;

— number and type of counted pollen grains and fungal spores;

— conversion factor.

6.3.6 Conversion factor

Pollen or fungal spore counts should be expressed as the daily average pollen or fungal spores counts per cubic meter of air. For this purpose, the number of pollen or fungal spores counted is multiplied by a factor that takes into account the volume of air sampled (10 l/min), the sampling area and the size of the microscope's field of view used.

NOTE It is also possible to express the results as bi-hourly data/m^3 of air, in accordance with the needs.

Calculate the conversion factor (CF) for the unit of time chosen by using Formulae (1) and (2)

$$CF = \frac{S\ total\ sampled}{S\ analysed} \times \frac{1}{V} = \frac{L \times l}{L \times d \times N} \times \frac{1}{V} = \frac{l}{d \times N} \times \frac{1}{V} \quad (1)$$

$$Pollen\ or\ fungal\ spore\ concentration = n \times CF \quad (2)$$

Where

S surface (total sampled or analysed) (mm^2)

V volume of sucked air ($V = D \times t$) (m^3)

D flow rate of the equipment (l/min converted in m^3/unit of time)

t duration of the sampling period (min)

L length of the line (mm)

l width of the line (mm)

d used diameter of the microscope's field of vision (mm)

N number of sweeps

n number of pollen or fungal spores counted in the analysed area of the microscope slide

The first step to calculate the conversion factor is to measure the microscope's field of vision at the magnification which is used.

Example for daily concentration [2]:

If the diameter of the field of view is 0,45 mm:

Air sampling rate: 10 l/min = 600 l/hour = 14 400 l/day = 14,4 m^3/day

Length of the tape (or line): 48 mm

Width of impaction area on the tape: 14 mm

Area of one horizontal sweep = 48 mm × 0,45 mm = 21,6 mm^2

Surface analysed = 21,6 × 4 sweeps = 86,4 mm^2

Total surface sampled = 48 mm length × 14 mm width = 672 mm^2

Conversion factor (CF) = (672 mm^2/86,4 mm^2) × (1/14,4) = 0,54

n = number of pollen grains in four sweeps = 250 average particle content per cubic metre of air = CF × *n* = 250 × 0,54 = 135 pollen grains/m^3

Accumulated daily data: annual, seasonal, monthly, or weekly pollen or fungal spore counts should be expressed as an index without any unit. The index is calculated by adding the daily concentrations during the studied period.

7 Performance characteristics [10] [11] [17] [18] [19]

7.1 General

The performance characteristics shall be assessed by calculating precision and accuracy indicators.

In aerobiology, the precision (under repeatability and reproducibility conditions) is usually quantified by the variation coefficient.

In aerobiology, accuracy is usually quantified by the absolute error or the relative error. The error depends on the dimension of the samples.

7.2 Performance requirements

7.2.1 Repeatability

The performance of each counter for pollen and fungal spores shall be controlled once a year by measurement under repeatability conditions:

— one slide;

— same counter;

— same method;

— minimum three replicates per counter.

The acceptable coefficients of variation (CV), calculated only for taxa with a true value > 10 shall be:

— for an accepted true value between 10 and 30 pollen: CV ≤ 30 %;

— for an accepted true value between 30 and 300 pollen: CV ≤ 20 %;

— for an accepted true value > 300 pollen: CV < 10 %.

7.3 Performance recommendations [18] [19]

7.3.1 Reproducibility and accuracy

When there is more than one counter in a team of laboratory staff, it is recommended to assess the intralaboratory reproducibility. The acceptable coefficients of variation are the same of those for repeatability (see 7.2.1).

When the laboratory is involved in a network, it is also recommended to assess the interlaboratory reproducibility and the accuracy.

Precision and accuracy should be assessed through interlaboratory exercises following the proposed methodology [18] [19].

7.3.2 Sensitivity and specificity

Sensitivity measures the proportion of searched particles which are correctly identified. It can be expressed as:

$$sensitivity = \frac{number\ of\ true\ searched\ particles}{number\ of\ true\ searched\ particles + number\ of\ false\ non\ searched\ particles} \quad (3)$$

Specificity measures the proportion of non-searched particles which are correctly identified as different from the searched particles. Specificity can be expressed as:

$$specificity = \frac{number\ of\ true\ non-searched\ particles}{number\ of\ true\ non-searched\ particles + number\ of\ false\ searched\ particles} \quad (4)$$

Both, sensitivity and specificity depend on the operator's ability to properly recognize a certain type of pollen grain in the sample. Assuming that the operator is well-trained, sensitivity and specificity are considered of 1 [16].

According to the regional circumstances, the most relevant taxa should be analysed *Alnus*, *Amaranthaceae*, *Ambrosia*, *Artemisia*, *Betula*, *Castanea*, *Casuarinaceae*, *Corylus*, *Cupressaceae*, *Fagus*, *Fraxinus*, *Olea*, *Parietaria*, *Plantago*, *Platanus*, *Poaceae*, *Populus*, *Quercus*, *Salix*, *Tilia*, *Urticaceae*.

NOTE Current studies classified the species of former *Chenopodiaceae* as a new subfamily of *Amaranthaceae*.

Annex A
(informative)

Hirst type volumetric trap

The three brands of Hirst-based equipment commercially available at present are:

— The VPPS 2000 made by Lanzoni s.r.l., Italy.

— The VPPS 2010 made by Lanzoni s.r.l., Italy.

— The Burkard 7-day recording volumetric spore-trap, by Burkard Manufacturing Co. Ltd, UK.

— The Burkard scientific 2000 made by Burkard scientific Co. Ltd, UK.

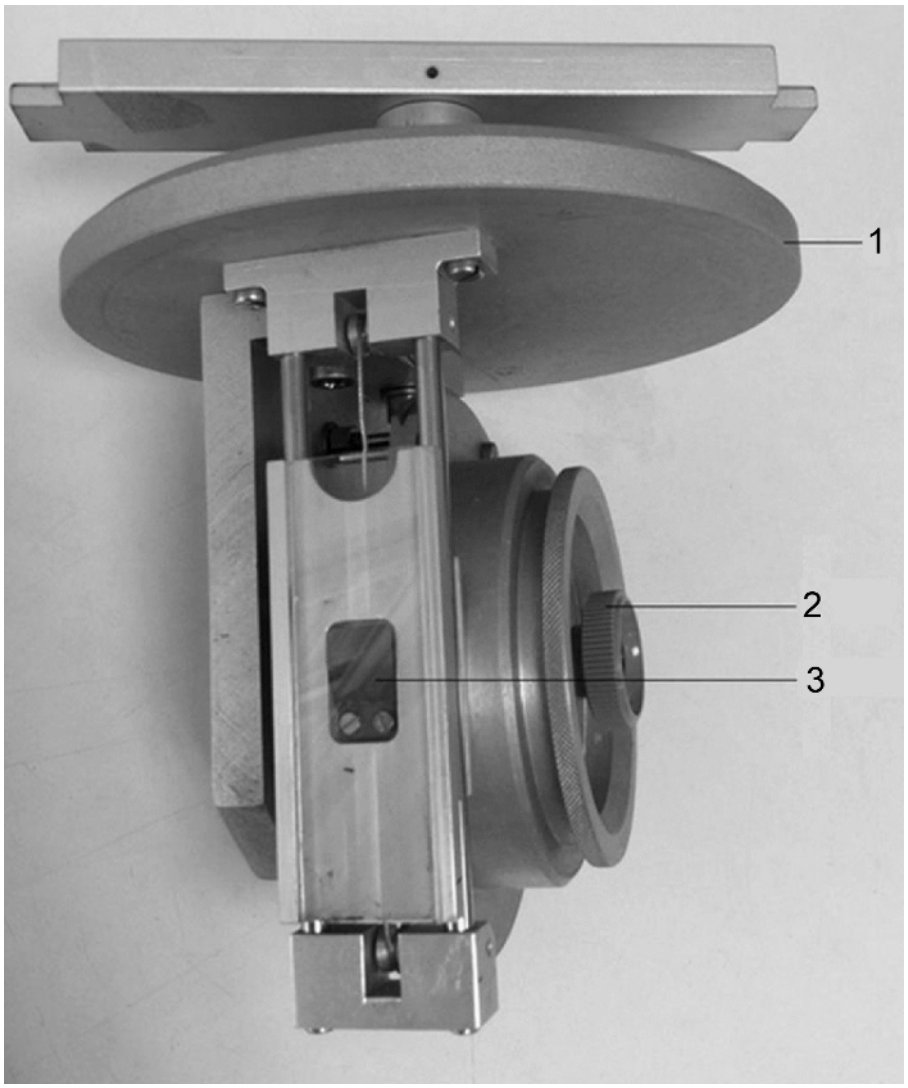
  — Hirst volumetric spore sampler (7 days and 24 hours recording versions).

  — Sporewatch, electronic spore and pollen sampler.

They can function continuously for one day to one week.

Annex B
(informative)

Pictures of impaction support



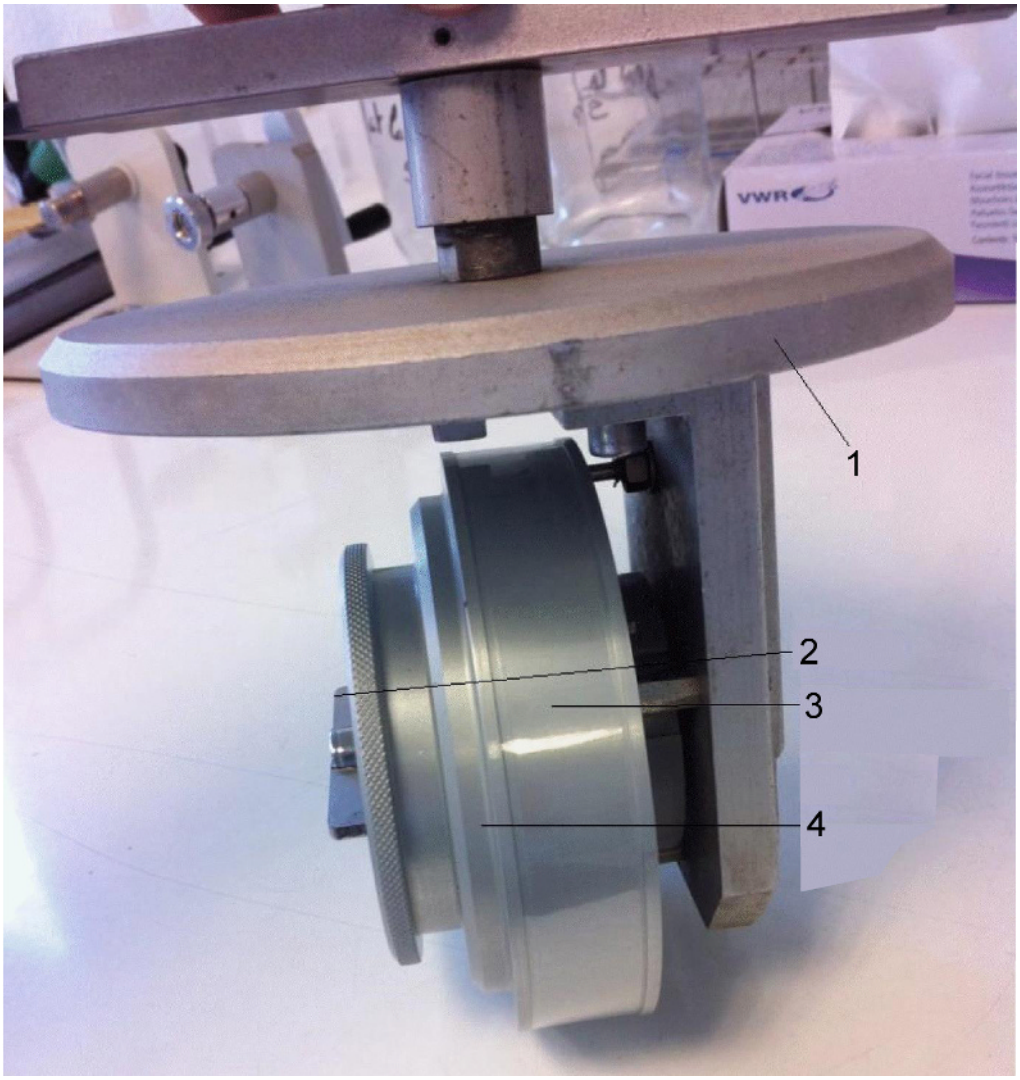
Key

1 lid

2 lock nut

3 trapping surface on slide

Figure B.1 — Impaction support using a glass slide



Key

1 lid

2 lock nut

3 trapping surface with transparent strip

4 drum

Figure B.2 — Impaction support using a drum with a transparent tape

Annex C
(informative)

Safety data sheet

— Vaseline (petroleum jelly): http://www.sciencelab.com/msds.php?msdsId=9927388

— Silicone: http://www.guidechem.com/reference/dic-536643.html

— Toluene: http://www.sciencelab.com/msds.php?msdsId=9927301

— CCl_4: http://www.mathesongas.com/pdfs/msds/MAT04310.pdf

— Fuchsin: http://www.sciencelab.com/msds.php?msdsId=9923017

— Phenol: https://www.sciencelab.com/msds.php?msdsId=9926463

— Safranin: http://www.csst.qc.ca/prevention/reptox/pages/fiche-complete.aspx?no_produit=151365&nom=Safranine

Annex D
(informative)

Key of determination

For example: The English version of CD Rom "The pollen content of the air: Identification Key" of RNSA member of International Association for Aerobiology (IAA) [14].

The different chapters of the CD are:

— "Identification key for the pollen content of the air". This key takes into account those taxa most frequently found in the atmosphere as well as the main pollen types useful during the identification of pollen grains.

— "Identification key for atmospheric pollen". This key has been constructed to allow the identification of "fresh" samples, without acid treatments, of the main pollen types commonly present in the atmosphere as well as those belonging to the large botanical families who generally use vectors other than the wind for pollination but who possess a particular palynological interest.

— "Specification sheets and glossary" with 114 specification sheets detailing the characteristics of the pollen of typical species including some biological and botanical information.

In this CD there is a glossary with "English word", "French word", "definition", and "illustration":

GLOSSARY

Gérard SULMONT

ENGLISH WORD	FRENCH WORD	DÉFINITION	ILLUSTRATION
AIR	Air	Pollen grains found in suspension in the air have generally come from plants that use the wind as a pollination agent (anemogamous or anemophilous plants). E.g.: the stamens of cocksfoot (*Dactylis glomerata*) are held outside the spikelets to disperse their pollen in the air.	
AIR SACK or VESICULE	Ballonet ou Vésicule	Extensions on some types of pollen grain (vesiculate* pollen grains of the Gymnosperms*). The grain is composed of the main body with one or several air-sacks* or vesicules filled with air. These extensions result in the separation of the endexine* from the ectexine*. Suspension of the grain in the air is assisted by the air-sacks and this is an adaption for anemophily. E.g.: pine pollen grains (*Pinus sylvestris*).	

A B C D E F G H I J K L M
N O P Q R S T U V W X Y Z

Figure D.1 — Glossary example

In this CD there is also an index with the details of all the species:

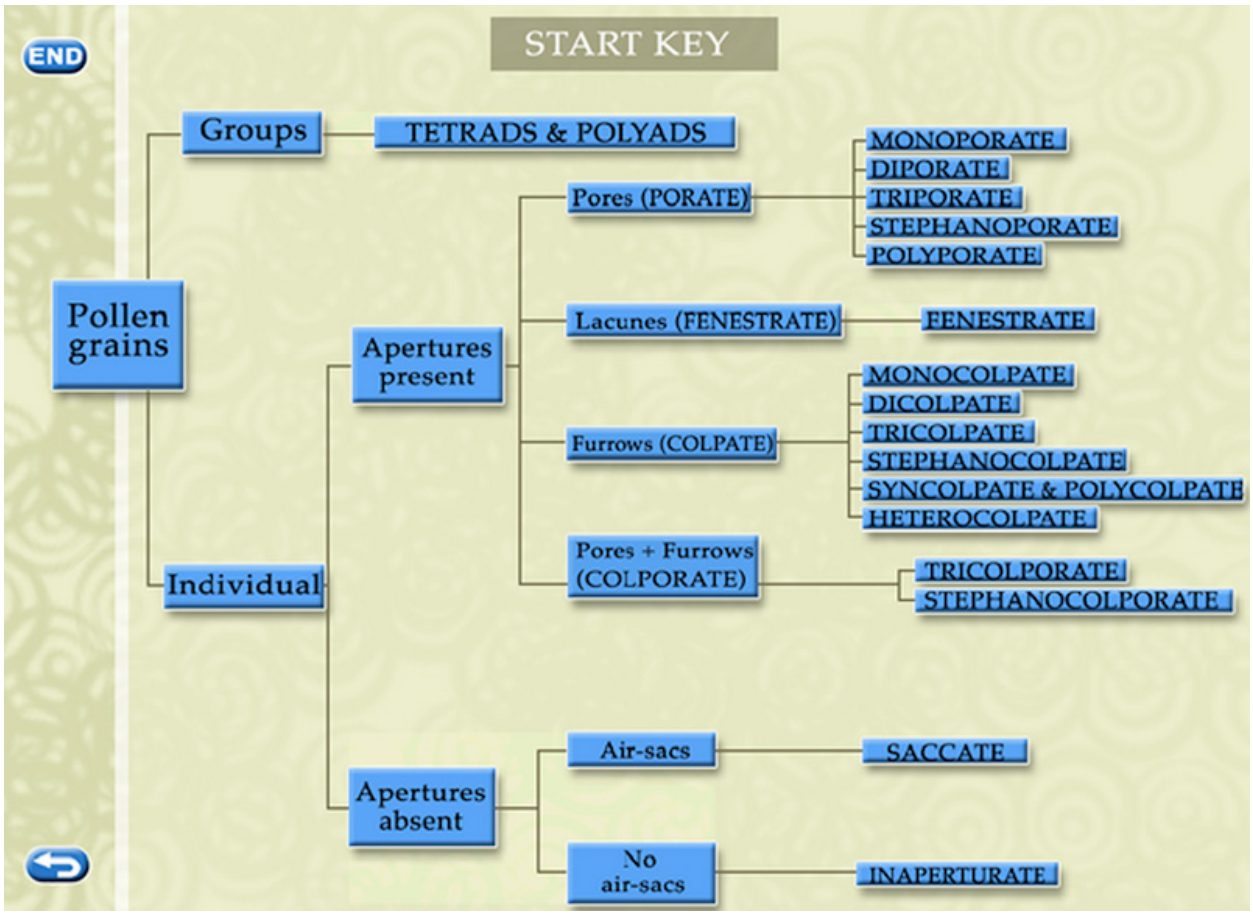


Figure D.2 — The different types of pollen

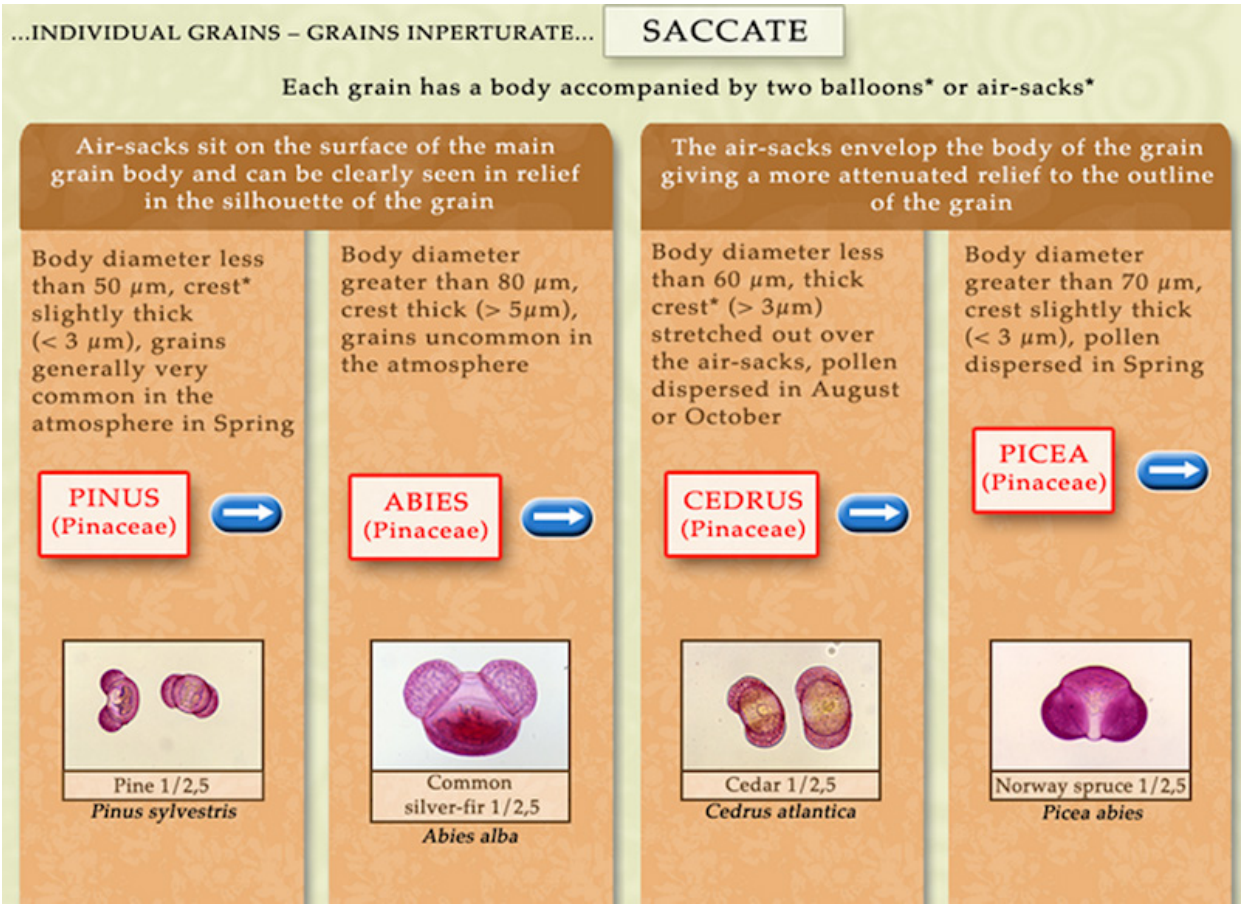


Figure D.3 — Index example

Non exhaustive list of books to help for identification of pollen and fungal spores:

CHARPIN J – SURINYACH R (1974), book, « Atlas of European allergenic pollens », Sandoz Editions, 229 Pages

NILSSON SIWERT TAGE – PRAGLOWSKI JOSEPH – NILSSON LENNART (1977), book, « Atlas of airborne pollen grains and spores in northern Europe », Natur och kultur, 159 Pages

IVERSEN JOHS – FAEGRI KNUT (1989), « Textbook of pollen analysis », IV edition, 328 Pages

ELLIS MARTIN B – ELLIS J PAMELA (1985), « Microfungi on Land Plants An Identification Handbook », book, 818 Pages

JENSEN KNUD WILKEN – GRAVESEN SUZANNE (1984), book, « Atlas of moulds in Europe causing respiratory Allergy », Foundation for Allergy in Europe, 110 Pages

IWANAMI YOZO – SASAKUMA TETSUO – YAMADA Yoshio (1988), book, « Pollen: Illustrations and scanning Electronmicrographs », 198 Pages

MULCAHY DAVID L – MULCAHY GABRIELLA BERGAMINI (1986), book, « Biotechnologie and Ecology of pollen », 528 Pages

FALAGIANI Paolo (1990), book, « Pollinosis », 267 Pages

MISKOVSKY RENAULT JOSETTE – PETZOLD MICHEL (1989), book, « Spores et pollen », edition « La Duraulie », 360 Pages

REILLE MAURICE (1992), book, « Pollen et spores d'Europe et d'Afrique du nord », laboratoire de botanique historique et palynologie URA CNRS 1152 Marseille, 520 Pages

Internet links:

EAS: http://eas.polleninfo.org/

IAA: https://sites.google.com/site/aerobiologyinternational/

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[9] LANZONI SRL (2000) COD 200000, technical report. «Technical data sheet». Bologna, Italy

[10] LANZONI CARLO (2009) Technical report, «Calibration and adjustment for pollen trap VPPS 2000 and 2010». Workshop «Quality Control». Bologna, Italy

[11] VDI 4252, *Part 4 (under way) «Bioaerosols and biological agents; Investigation of airborne allergy relevant pollen and mould spores using a volumetric method». Germany*

[12] MERCK. (2010) Data sheet. «Fiche de données de securité Silicone». Nogent sur seine, France

[13] POILANE SOLENE (2011) Data sheet. «Préparation de bandes pré-enduite» and «fabrication du milieu d'enduction» and «préparation tambours». Brussieu, France

[14] SINDT CHARLOTTE (2012) Technical report. «Préparation des lames» and «Lecture de lames». Brussieu, France

[15] SULMONT GERARD, LAINE CATHERINE, SULMONT DOMITILLE, DUPUY NADINE, LACHASSE CHARLOTTE, THIBAUDON MICHEL (2006) «The pollen content of the air: Identification Key». CD Rom V2, RNSA and IAA. France

[16] TRIGO MARIA DEL MAR, JATO MARIA VICTORIA, FERNANDEZ DELIA, GALAN CARMEN (2008) Atlas Aeropalinologico de España. Secretariado de publicaciones de la Universidad de León. Spain

[17] COMTOIS PAUL, ALZAZAR PURIFICACIÓN, NERON DANIEL (1999) Pollen counts statistics and its relevance to precision. Aerobiologia, 15:19-28

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