Basic Aeroallergen Course

Establishing and Operating an Air Sampling Station

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THE UNIVERSITY of TULSA

Disclosure

- No conflicts to disclose

VIRTUAL ANNUAL MEETING
Incorporating New and Emerging Therapies into Allergy/Immunology Practice and Research
#AAAAI21

Establishing and Operating an Air Sampling Station

What to consider:

- Sampling plan or objective
- Samplers: Rotorod, Burkard spore trap (i.e. Hirst-type spore trap)
- Location
- One-day head or 7-day head for Burkard spore trap
- Preparing samples
- Slide analysis
- Identification
- Calculating the data
- NAB certification

Sampling Objective

- Pollen only or both pollen and spores
- Sampling frequency
  - 7 days a week
  - 5 days a week
  - 3 days a week
- What is the time commitment

Rotating Arm Impactors: *Rotorod® and GRIPST Samplers*

Rotorod® Samplers ⇨

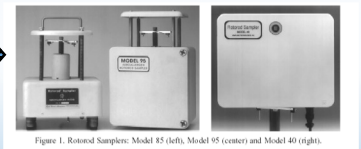


Figure 1. Rotorod Samplers: Model 85 (left), Model 95 (center) and Model 40 (right).

⇦ GRIPST-2009 Sampler

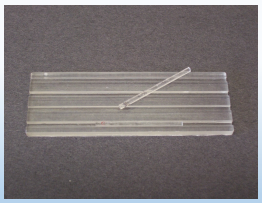
Rotorod® Samplers

- Models typically used have retracting rods
- Head rotates at 2400 rpm, leading edge of rod coated with silicon grease
- Pollen and spores impacted on greased surface
- Generally operated at 10% sampling time
- Efficient for pollen and spores >10 μm

Rotorod® Sampler

Rotorod® Analysis

- Collector rods placed in a special adapter for microscopic examination
- Rods stained with Calberla's pollen stain
- Entire surface of each rod counted unless pollen/spore load very high (then a subset of the surface is analyzed)
- Atmospheric concentrations determined



Rotorod® Calculations

C = N / V

C is concentration, N is the number of pollen or spores counted on both rods*, V is the volume of air sampled by the rods

V = Rod area (m^2) x D x π x RPM x t

Rod area = width of rod (1.52** mm = 0.00152* m) x length of the rod (23 mm = 0.023 m) x 2 (both rods), D is the diameter of the Rotorod head (8.5 cm = 0.085 m), RPM is 2400, t is minutes sampled per day

With a 10% sampling time (144 min) V = 6.452 m^3

Concentration = N/6.452 m^3

With a 5% sampling time (72 min) V = 3.226 m^3

Concentration = N/3.226 m^3

*Adjust calculations if only one rod is counted

**NOTE: Width of rods may vary slightly

Hirst-Type Spore Traps:

Burkard or Lanzoni

Advantages

- High efficiency down to less than 5 µm
  - Allows for greater accuracy for small fungal spores such as basidiospores, small ascospores, and *Penicillium/Aspergillus* conidia
- Time discrimination
  - Permits analysis for diurnal rhythms
- Permanent slides for future reference

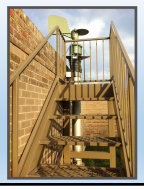
Burkard Spore Trap

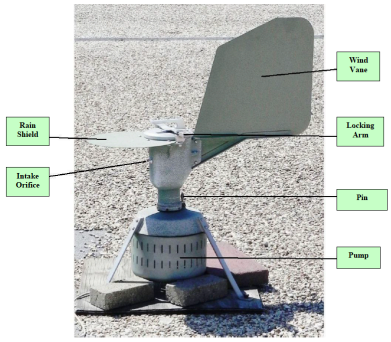
Location

- Roof of a building - ideal 3 to 6 stories above ground (30 to 60 ft)
- Not close to overhanging vegetation
- Air flow not obstructed by nearby buildings, walls, or other structural features
- Sampler should be level

Parapet around roof requires platform to elevate the orifice above the wall

Telescoping mast elevates sampler above local vegetation.





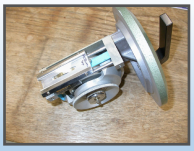
Close-up of Intake Orifice

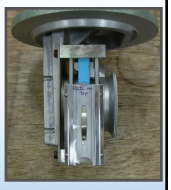
Burkard 7-day Sampler Head

- Standard is the 7-day sampling head
- Sampler drum mounted on 7-day clock
- Drum moves by orifice at 2 mm per hour
- Melinex tape mounted on drum and greased
- Air is brought in at 10 l/min and particles impact on greased Melinex tape
- Drum changed each week
- Melinex tape is removed and cut into one day segments, which are mounted on slides, stained, and examined

One-Day (24-Hour) Sampling Head

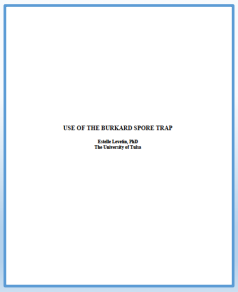
- Alternate head is the 24-hour head
- Standard glass microscope slide is greased and placed in the slide carrier
- Slide is changed daily, carrier realigned





Preparing and Changing Burkard Spore Trap

- Handout provides step-by-step instructions on using the Burkard spore trap sampler


USE OF THE BURKARD SPORE TRAP

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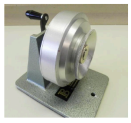
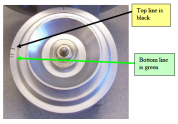
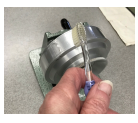
Preparing a Sampling Drum

- Place drum on mounting stand
- Clean drum with alcohol swab
- Place double-sided tape on drum between green line and top black line
- Place Melinex tape around the drum
- Grease tape: Lubriseal, Dow-Corning High Vacuum Grease, silicon oil, other
- Only handle drum by knurled rim
- Place drum in canister or other container for transport to roof





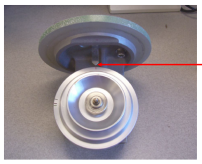


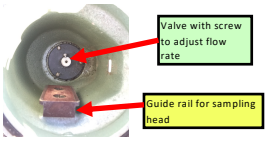
Secure the head with pin

Swing the locking arm 180º

- Lift out 7-day head
- Clean the intake orifice
- Remove the nut on the drum
- Lift drum by knurled rim
- Wind the clock

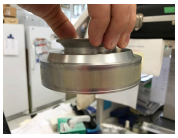
- Place a fresh drum on the clock
- Replace the head in the sampler (on guide rails)
- Swing locking arm back to seal the chamber
- Check the flow rate with flow meter
- Adjust if needed
- Mark the tape if desired
- Remove pin so head swings free



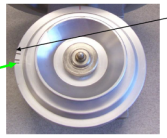


Processing the Samples

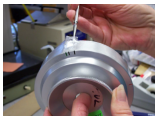
Exposed Burkard drum

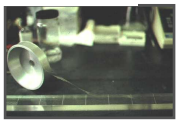


Green line is at the beginning of day one



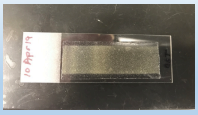
- Melinex tape removed from drum and placed on the ruled cutting board
- Use care so that day one is place on the left side of the cutting board and day seven on the right side



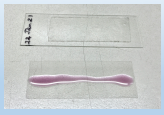


- Melinex tape cut into seven 24-hour segments each 48 mm long
- Segments mounted on microscope slides in 10% Gelvatol with begin of the 24 hours on the left
- Allow the Gelvatol to dry
- For permanent slides glycerin-jelly mounting medium added with a 50 mm or 60 mm cover slip
- Mounting medium contains a pollen stain: basic fuchsin or phenosafarin
- Alternatively, Calberla's stain can be used but the slide will eventually dry out

Slide warmer speeds drying



Slide from the peak tree pollen season

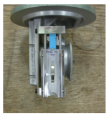


Glycerin-jelly application

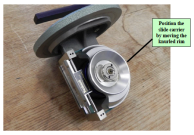
24-Hour (One Day) Sampling Head

- Write the date on a standard microscope slide
- Lightly grease slide below the date
- Place the slide in a slide box for transport to the roof
- Use same procedure to remove sampling head
- After 24 hour the slide carrier has moved up
- Use knurled rim on the slide carrier to reposition the carrier to the start position
- Remove the exposed slide and place a fresh slide in the carrier
- At least once a week wind the clock and check the flow rate

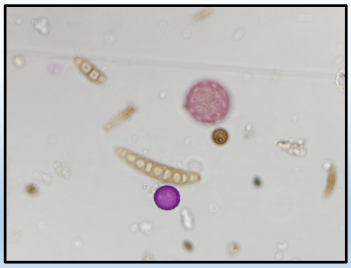
Slide carrier at start position



Slide carrier after 24 hours



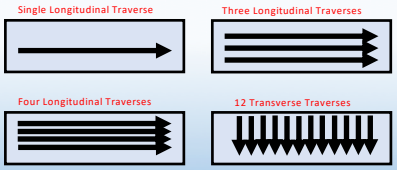
Outdoor Air Sample from Tulsa



Slide Analysis

- Microscopy - 400X for pollen; 1000X (oil immersion) for fungal spores
- Different methods of microscopic analysis are used
  - Average daily concentration - Single longitudinal traverse or multiple longitudinal traverses
  - Hourly or bihourly concentrations which can then be averaged to obtain a daily average - 12 transverse traverses

Burkard Slide Counting Methods



Comparison of Methods

Single Longitudinal Traverse

- Quicker
- Produces average daily concentration
- Good for routine monitoring
- 2, 3, or 4 longitudinal traverses can increase accuracy

12 Transverse Traverses

- Takes longer
- Can determine diurnal rhythm of airborne allergens
- All traverses can be averaged to determine average daily concentration

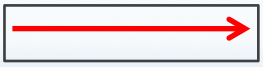
Cell Counters are Essential Tools

Counting Apps

Computer keyboards can also be programed to enter counts directly into a database

Counting Practices

- On the Burkard slide – for the single longitudinal traverse count near the center, not in the exact middle, when you are using the single longitudinal traverse



- Counting partial pollen (spores) on edge of the field – count only bottom edge or top edges, not both

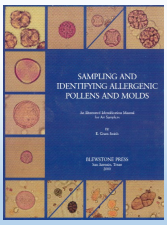
Identification

- AAAAI Aeroallergen courses
- Other aerobiology courses such as the New Orleans Aeroallergen Course
- Reference slides
  - **Reference slides from local specimens**
  - Consult a botanist at a local university
  - NAB/AAAAI Pollen Slide Library
- Identification Manuals

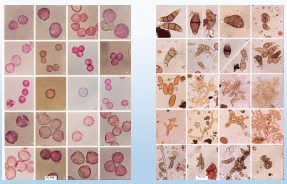
Identification Manuals

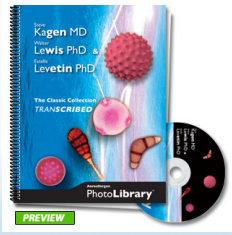
- Grant Smith. 2000. *Sampling and Identifying Allergenic Pollens and Molds*, AAAAI, Milwaukee
- R.O. Kapp, *How to Know Pollen and Spores* - originally published in 1960s - new edition
- Richard Weber. 1998. *Pollen Identification* Ann Allergy Asthma Immunol 80:141–7.
- Lacey, Maureen and J. West. 2006. The Air Spora: A Manual for Catching and Identifying Airborne Biological Particles, Springer.
- Lewis WH, Vinay P, Zenger VE. 1983. *Airborne and Allergenic Pollen of North America.* Johns Hopkins University Press, Baltimore, MD.
- Aeroallergen Photo Library, Steve Kagan, http://allernet.net/
- Jelks M. 2003. Pollen Key

Essential Reference: Grant Smith's Sampling and Identifying Allergenic Pollen and Molds



Sample Pages





http://allernet.net/

Username: guest
Password: guest

On-Line Sites

PalDat - Palynological Database
- https://www.paldat.org/

Science & Plants for Schools - Pollen Image Library
- http://www-saps.plantsci.cam.ac.uk/pollen/index.htm

Missouri Pollen Project
- http://www.davidbegler.com/Pollen/glossary.html

Pollenwarndienst – Austrian database
- https://www.pollenwarndienst.at/en/aerobiology/pollen-atlas.html

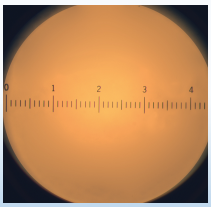
Australian Pollen and Spore Atlas
- http://apsa.anu.edu.au/

Converting Raw Counts to Concentrations

- Microscope counts are entered into a database such as Excel
- Formulas added to convert counts into concentrations
- Information needed
  - Field diameter of objective lens - ***Variable***
  - Flow rate (10 liters/minute) and exposure time (normally 24 hrs.) for a total volume of air sampled of 14.4 m^3

Stage Micrometer

- Use a stage micrometer to measure the field diameters of your objective lenses
- To measure the size of pollen grains or fungal spores, you need to use an eyepiece (ocular) micrometer



Field diameter in the image above is approximately 0.45 mm (450 microns)

Calculating Pollen Concentrations for Single Longitudinal Traverse at 400X

C = N/V

- C = Concentration - pollen grains/m^3
- N = number of pollen counted on traverse
- W = Width of entire sample - 14 mm
- *F = field diameter of* ***my 40X*** *objective lens - 0.48 mm*
- V = total volume of air sampled - 14.4 m^3

C = N x W/F x 1/V

C = N x 14mm/0.48mm x 1/14.4m^3

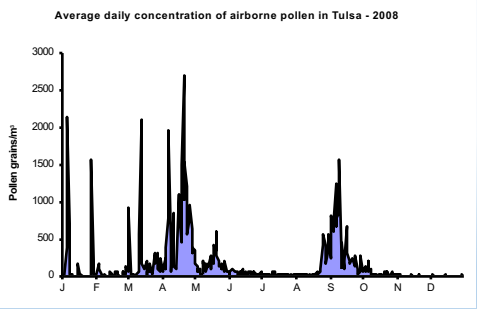
C = N x 2.025

Example of a Spreadsheet

Date	*Acer*	*Alnus*	*Ambrosia*	*Artemisia*	*Betula*	*Carpinus*	*Carya*	*Celtis*
25 Mar 2020	4	0	2	0	58	4	0	49
26 Mar 2020	8	0	0	0	75	2	0	51
27 Mar 2020	12	0	0	0	140	6	0	106
28 Mar 2020	2	0	0	0	10	0	0	12
29 Mar 2020	0	0	0	0	20	0	0	14
30 Mar 2020	0	0	0	0	32	2	0	20
31 Mar 2020	0	0	2	0	28	0	0	19
1 Apr 2020	2	0	0	0	41	2	0	68
2 Apr 2020	4	0	0	0	18	2	0	24
3 Apr 2020	2	0	0	0	22	0	0	20
4 Apr 2020	0	0	0	0	4	0	2	4

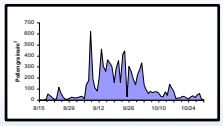
How the Data Can Be Used

- Average daily concentrations can be graphed to look at the seasonal and yearly pollen levels
- Develop regional pollen calendar
- Data can be compared with patient symptoms, peak flow readings, office visits, emergency room visits
- Prepare for peak seasons - staffing, etc.



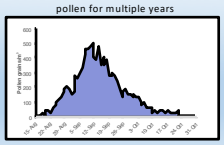
Single vs Multiple Years of Data

Airborne *Ambrosia* pollen in Tulsa 1999

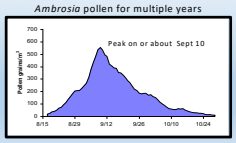


- Pollen data from one year reflects the current weather conditions
- Data from several years can be averaged to produce a graph reflecting pollen release period
- Smoothing techniques such as 5 day running mean can be used to generate a smoother curve and better estimate of the typical peak period

Mean daily concentration of *Ambrosia* pollen for multiple years



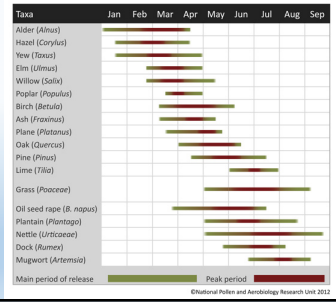
Five day running mean concentration of *Ambrosia* pollen for multiple years



Pollen Calendars

- Graphs or charts depicting the annual pollen release period for the major airborne pollen or spore types in a given area
- Several methods for showing the pollen seasons
- At least 5 years of data (10 years are better) are needed to develop the pollen calendar

Pollen Calendar for England



NAB Certification

1. Take an aeroallergen course
   - Contact Melissa Ramsey at the AAAAI
2. Take the on-line exam
   - 80% correct is passing
3. Take the pollen test (and/or spore test)
   - 80% correct is passing

Knowledge Base for Counters

1. Good microscope skills
2. Knowledge of air sampling and analysis
3. Knowledge of pollen morphology
4. Knowledge of pollen seasons
5. Knowledge of spore morphology
6. Knowledge of spore dispersal and fungal biology

Conclusion

- Air sampling allows the allergist to get a first hand understanding of the local aeroallergens, their concentration, and seasonal occurrence
- Several years of sampling will allow for the development of a pollen calendar which can benefit the physician and his or her patients

Real-time Samplers