Pan-American Aerobiology Association

Standardized Protocols

COMPREHENSIVE GUIDELINES FOR THE OPERATION OF HIRST-TYPE SUCTION BIOAEROSOL SAMPLERS

By Christine Rogers and Michael Muilenberg

1. Purpose

These standard methods establish uniform procedures for monitoring airborne particulate matter using Hirst-type suction slit impaction samplers. The standard methods provide a means to compare and evaluate data generated from these samplers under general use. The standard methods are not intended to restrict application of other methods for specific purposes and should not inhibit the development of improved techniques.

2. Samplers

These guidelines are applicable to Hirst-type suction slit impactors operating continuously with a flow rate of 10 liters/min.

Burkard 7-day Recording Volumetric Spore Trap

Burkard Manufacturing Company Limited
Woodcock Hill Industrial Estate
Rickmansworth
Hertfordshire WD3 IPJ
England
Phone: 0 44 0923 773 134
Fax: 0 44 0923 774 790

Volumetric Pollen & Particles Sampler (VPPS 2000)

Lanzoni S.R.L.
Via Michelino
93 - 40127 Bologna
Italy
Tel: 0 39 51 - 504810 - 501334

Fax: 0 39 51 - 6331892

3. Location

Sampler location determines directly the kind of results obtained with these samplers. Care must be taken to evaluate a proper location for the sampler keeping in mind that the closer the sampler is to a particular source of pollen or spores, the greater the results will be influenced by that source. Pollen and spore concentration also varies with height in the atmosphere, and so a sufficient vertical distance should also be considered. For general aerobiological reporting, a flat rooftop at a height above local tree tops would be optimal. The sampler must be placed as far as possible away from any rooftop obstructions or abutments. If sampling at ground level, raising the trap to 1.5 m is desirable in order to sample above the local grassy and weedy vegetation.

4. Time of year to sample

Many tree taxa release pollen very early in the year, and airborne pollen can be detected prior to any evidence of spring, therefore start sampling at least one month prior to the usual start of the season. If a complete record of all pollen and spore taxa is desired, then consider year round sampling.

5. Adhesives used as a sampling surface

Many adhesives have been used in aerobiological sampling with varying success. Important considerations are 1) thermal stability 2) hygroscopicity 3) compatibility with mountant.

Lubriseal -stopcock grease - Thomas Scientific Melting point 52^{o}C
Vaseline - white petroleum jelly
Vaseline & wax -
18 g Vaseline
2 g White Paraffin wax (congealing point 68-74^{o}F)
Melt wax, melt vaseline and combine. Mix thoroughly and let cool.
Mix thoroughly before each application onto tape.
Silicone fluid - 1 part silicone grease : 2 parts hexane

6. Mountants

With stain

Glycerin Jelly

20 g Gelatin
70 ml water
60 ml glycerin (same as glycerol)

2.4 g Phenol * be very careful with Phenol*

Boil water. Measure 70 ml and add to gelatin. Boil again and mix. Add glycerin and phenol and mix. Add a small amount of stain and mix again.

Gelvatol - (also used to adhere tape to the slide in a 1:9 dilution in water)

35 g Gelvatol (grade 40-20 available from Burkard Manufacturing Co.)
100 ml distilled water
50 ml glycerol (or 40ml lactic acid)
2 g Phenol * be very careful with Phenol*

Gelvatol is added to the water and left to stand overnight. Glycerol (or lactic acid) is then added and the solution warmed slightly. Add phenol, and let stand. A small amount of stain can be added.

Stains

Basic Fuchsin as crystals or in Calberla's Solution

Phenosafranin

About 0.5 ml stain (5mg/ml DW) per 100ml jelly is a good starting point. A good intensity of stain would be the colour 7B on Pg 12 of the Methuen Handbook of Colour.

Without Stain - these mountants do not act as a good vehicle for stains.

Lactophenol with PVA

7.5 g polyvinyl alcohol powder
50 ml distilled water
22 ml lactic acid
22 g Phenol * be very careful with Phenol *

Heat water in a boiling water bath, add polyvinyl alcohol and dissolve. It may take several minutes of mixing, and the mixture can be quite thick. Add lactic acid and mix. Place phenol in a large test tube. Heat the test tube under hot tap water until phenol has melted. Add phenol to mixture and mix. Store in dark dropper bottles.

7. Preparing the Drum

Place the drum on the tape mounting stand and clean the sampling surface (ethanol works well). Place a small piece of double sided tape (3M Scotch Double Stick Tape) on the sampling surface of the drum, centered between the black notches on the side of the

drum. Attach the end of the Melinex tape to cover 1/2 of the double sided tape. Wind the tape around the drum tightly by turning the drum with one hand and applying resistance on the tape with the other hand. When the drum has rotated 360^{o}, press the Melinex tape onto the remaining exposed double sided tape and cut the Melinex tape so that the ends of the tape just touch with a minimum of overlap. Make sure the tape lies flat against the drum. Lightly wipe the tape clean (with ethanol). Apply adhesive to the tape with a flat 1/2" to 3/4" wide artist's brush. Make sure the entire tape is uniformly covered with a very thin layer. Rotating the drum while holding the brush to the surface seems to even out the adhesive quite well. For a very thin layer with no brush marks, a lint-free tissue can be placed over one finger, and with the covered finger laid across the drum, the drum can be rotated as before.

8. Changing the drum

Place the drum over the timer mechanism of the sampler with the red notch on the drum at, or slightly to the right (clockwise) of, the pointer. Tighten the locking nut securely. Wind the timer counterclockwise and place the drum and timer into the trap. Note that, when the drum is in the sampler, the beginning of the Melinex tape is aligned with the intake orifice. To mark the exact start of sampling, a small, thin spatula can be inserted into the orifice at the front of the trap. A horizontal mark is made by running the spatula along the **top** of the orifice while the end is in contact with the tape. A similar mark is made at the end of sampling on that drum after one week. The spatula is inserted into the orifice, but the mark is made by running the spatula along the **bottom** of the orifice.

9. Sampling and flow rate

Once the power cord has been fitted with an appropriate plug, the vacuum pump is started by plugging in the power cord. The flow rate of the sampler should be 10 liters/min and can be checked by fitting a specially adapted flowmeter completely over the intake orifice (note: flowmeters must also be calibrated to be sure of accurate measurement). The flow rate can be adjusted by an adjusting screw located inside the sampling chamber at the bottom. Turning the screw a 1/4 turn and replacing the drum and lid and rechecking the flow rate will adjust the flow. It should read as close as possible to 10 liters/min.

It is possible to operate the drum for up to seven days between drum changes. It is advisable to establish one set time each week to change the drum. Changing the drum on Tuesday, Wednesday or Thursday reduces the chances of forgetting to change the drum due to long weekends. The time that the drum is changed should remain constant with respect to standard time. For example, if the drum is normally changed at 11:00am Standard Time, the drum should be changed at 12:00noon during Daylight Savings Time. This ensures that each segment of tape has been exposed for exactly 24 hours.

Alternative sampling heads and timers are available which allow for 24 hour samples onto glass microscope slides or 24 hour samples onto a drum with a rotation rate at 14 mm per hour (2 mm per hour is standard).

10. Cutting the tape

Use a razor blade, dissecting needle or fine forceps to remove the exposed tape from a drum by peeling the beginning end of the Melinex tape from the double sided tape, taking care not to scratch the drum. Lift the entire Melinex tape from the drum, and carefully pull the trailing end from the double sided tape. When removing the tape from the drum it is essential not to confuse the orientation of the tape. Place the tape on the cutting block (or glass plate) aligning the mark at the start of the tape as the edge of the first segment. The end mark should align with the last edge on the seventh day of the cutting block. Using a sharp razor blade or thin pair of scissors, cut into 48 mm or "24 hour" long segments. Care must be taken to make cuts as straight as possible.

11. Preparing the slides

Prepare the slides by writing the date of the samples (and sampler # if using more than one trap) onto cleaned slides using an ultrathin permanent ink Sharpie or diamond-pointed scriber. Lay a bead of 10% Gelvatol in distilled water, about 45 mm long, down the center of the slide. This works well as an adhesive to hold the tape on the slide. The exposed segment of tape can then be rolled onto the Gelvatol, using fine pointed forceps, taking care not to get bubbles under the tape. Once in place, the tape can be readjusted to make sure it is adhered perfectly straight. The start of the tape should consistently be placed at the same end of the slide. The Gelvatol should be allowed to dry for 24 hours before further processing. At this stage, the slides can be stored for later mounting.

12. Mounting

Glycerin Jelly

Place 5 to 7 drops of melted (in a 50^{o}C hot water bath) glycerin jelly on the exposed tape and carefully cover with a 22mm x 50mm cover glass (No. 1 thickness). Warming the slides on a slide warmer at a moderate setting (48^{o}C) will help the glycerin jelly to spread without melting the adhesive. The cover glass will have to be gently pressed down to spread the glycerin jelly with a slight back and forth movement of the cover glass (try using a pencil eraser) to spread the jelly evenly. It is imperative that this be done GENTLY so as not to disturb particles on the tape. Practise is required to determine the best technique and amount of glycerin jelly needed.

Gelvatol or PVA

Place 5 to 7 drops on the exposed tape and cover with a 22mm x 50mm cover glass (No. 1 thickness). Allow to dry.

13. Microscopy

A research grade compound light microscope, with a stage Vernier scale, is required for the analysis of pollen and fungal spores. It should be fitted with 10X ocular lenses, a 40X

objective (with a n.a. not less than 0.65), and an oil immersion 100X objective. A magnification of 400X is normally used for identifying pollen (a 40X objective lens with 10X ocular lenses), and 1000X magnification for identifying spores (100X oil immersion objective with 10X ocular lenses). A stage micrometer is needed to determine the size of the field of view. An eyepiece micrometer (reticule), calibrated with the stage micrometer, is needed for measuring particle sizes.

14. Identifications

Identification of particles should proceed to the most specific classification possible. However, rarely can pollen and spores be identified to species level, and some may have to be placed in groups of multiple genera or families. Consult proper identification manuals for descriptions of distinctive features (a list of relevant sources is found in Appendix I). One of the most valuable sources for identifications is a reference slide collection made from pollen or fungi found in the local area, or from expertly identified collections. Slides should be prepared using the same media as used for sampling.

Pollen grains or spores that are in good condition, but where taxonomic identifications cannot be determined should be recorded in the category "Unknown". Pollen grains or spores that cannot be identified due to degradation, destruction, or obstruction should be recorded as "Indeterminable".

15. Counting technique

Two counting techniques are in common use in aerobiology. One single longitudinal traverse, or twelve bi-hourly transverse traverses. It is up to the individual investigator to decide which method suits the investigation at hand.

1 Longitudinal traverse near the middle of the trace

Advantages

Provides "average" prevalence over 24 hours and requires less time to examine a daily slide.

Disadvantages

Due to uneven particle deposition across the tape, a single traverse may not be representative. Care must be taken to position the transect to avoid blank areas due to bugs sliding across the tape. A smaller area of the tape is examined using a single longitudinal traverse than with 12 transverse traverses and therefore the estimated concentration is less accurate. Depending on the timing of the changing of the drum (usually mid morning), a "daily" slide does not correspond to a calendar day. If using the data to correlate with weather parameters, care must be taken to either a) separate the count before and after midnight or b) re-adjust weather parameters to correspond with the sampling day.

12 Transverse traverses at 4mm (2hr) intervals

Counting the same hours each day is desirable, and is easy if the drum is changed at the same time each week. For the first trace, find the edge of the tape on the slide, then move in at least 2mm (using the scale on the stage). This avoids an edge effect of cutting and the 2mm strip at the beginning and end of the 7 day tape that is not exposed for a full hour.

Advantages

This method examines a larger area of the slide surface and therefore estimates of concentration are more accurate. By providing 2 hourly concentrations, the diurnal periodicity of particles can also be studied. The 2 hourly values can easily be recombined to correspond to the calendar day. An average of the 12 two hourly concentrations gives an average daily concentration that can be directly compared with weather parameters.

Disadvantages

Because traverses are at intervals, important peak or low concentrations may be missed in between the sampled hours. This method requires examining 3.5 times the area of the slide than with a single traverse, and therefore generally takes 3.5 times longer to examine a daily slide.

16. Count sheets

Examples of count sheets for the two methods of counting are included in Appendix II. The sheet should include date of sample, location of sample, times covered by the sample, person counting the sample, field diameter and magnification. Care should be taken to clearly distinguish between two strokes for individual counts (||) and the number eleven (11). Multiple cell counters are handy for enumerating large numbers of particles of different taxa.

17. Conversion to concentration

Longitudinal Traverse

The volume of air represented by a longitudinal sweep of the tape can be calculated as follows:

Note: the "trace" refers to the area of the particle deposit on the tape segment. The trace width (14mm) corresponds to the sampler's intake-slit width.

Volume (m^3) = fraction of the trace counted x flow rate x exposure time

$$= \frac{\text{field diameter of objective}}{\text{trace width}} \times \text{flow rate} \times \text{exposure time}$$

Under normal operation, the following are constants

trace width = 14mm = 14,000 μ m
flow rate = 10 liters/min = 0.01 m^3/min
exposure time = 24 hours = 1440 min
field diameter = X μ m * each objective is different, therefore the field diameter must be measured directly with a stage micrometer

Therefore the volume (m^3) is:

V = (X μ m / 14,000 μ m) x 0.01 m^3/min x 1440 min

To derive the concentration of particles counted, divide the number counted by the volume sampled.

Particles/m^3 = (# counted / V)

This is an average daily concentration

Twelve Traverse

Similarly, the calculations for each transverse traverse would be:

Volume (m^3) = $\frac{\text{field diameter of objective}}{\text{drum rotation rate}}$ x flow rate

where the drum rotation rate = 2mm/60min = 2,000 μ m/60min = 33.33μ m/min

Therefore :

V (m^3) = (X μ m / x 33.33μ m/min) x 0.01 m^3/min

To derive the concentration of particles counted, divide the number counted by the volume sampled.

Particles/m^3 = (# counted / V)

This is the average hourly concentration

Average the twelve hourly concentrations to obtain an average daily concentration.

No conversion should be applied to take into account the efficiency of the trap.

If entering the data into a computer, it is recommended to have a different person check the entered data against the count sheets.

18. Units

Concentrations of enumerated particles should be reported with the following standard units:

Pollen/m^3

Spores/m^3

19. Reporting

Average daily concentration plotted over time is the most commonly reported statistic. Other useful indices are the yearly sum of average daily concentration, and peak concentration.

20. Referencing

Referencing of this protocol for a description of methods in research articles is permissible. The location and height of the trap, adhesive, mountant and counting technique used should be specified as well as any deviation from these recommendations.

21. Maintenance

Each of the samplers are virtually maintenance free, however, the orifice slit, and the hole to the pump, should be cleaned out periodically using alcohol on a cotton swab.

22. Safety

Personnel should practise general lab safety at all times, and Material Safety Data Sheets should be readily available.

Appendix I List of relevant Identification Manuals

Barnett, .L., & Hunter, B.B., 1975. Illustrated Genera of Imperfect Fungi (3rd ed.). Burgees Publ.Co. Minneapolis.

Bassett, I.J., Crompton, C.W. & Parmelee, J.A. 1978. An Atlas of Airborne Pollen Grains and Common Fungus Spores of Canada. Thorne Press Ltd.

Carmichael, J.W., Kendrick, W.B., Conners, I.L., & Sigler, L. 1980. Genera of Hyphomycetes. Univ of Alberta Press, Edmonton

Dennis, R.W.G., 1978. British Ascomycetes. J Cramer, Germany.

Ellis, M.B. 1971. Dematiaceous Hyphmycetes. International Mycological Institute. CAB International, Surrey, UK.

Ellis, M.B. 1976. More Dematiaceous Hyphmycetes. International Mycological Institute. CAB International, Surrey, UK.

Ellis, M.B., & Ellis, M.P. 1985. Microfungi on Land Plants. Macmillan Publishing Co., New York.

Hawksworth, D.L., Kirk, P.M., Sutton, B.C., & Peglar, D.N., 1995. Ainsworth & Bisby's Dictionary of the Fungi. International Mycological Institute, CAB International, Surrey UK

Hyde, H.A., & Adams, K.F., 1958. An Atlas of Airborne Pollen Grains. St. Martin's Press, New York.

Kapp, R.O., 1969. How to Know Pollen and Spores. Wm C Brown Co., Dubuque Iowa

Lewis, W.H., Vinay, P. & Zenger, V.E. 1983. Airborne and Allergenic Pollen of North America. John's Hopkins University Press, Baltimore.

Martin, G.W., & Alexopoulos, C.J. 1969. The Myxomycetes. University of Iowa Press, Iowa City.

Pegler, D.N., & Young, T.W.K., 1971. Basidiospore Morphology in the Agaricales. J. Cramer, Germany.

Smith, E.G., 1990. Sampling and Identifying Allergenic Pollens and Molds. Blewstone Press, San Antonio.

Watanabe, T., 1994. Pictorial Atlas of Soil and Seed Fungi. Lewis Publishers, Boca Raton.

Wodehouse, R.P., 1959. Pollen Grains. Hafner Pub. Co. New York.

Wodehouse, R.P., 1971. Hayfever Plants (2nd ed.). Hafner Press. New York

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