USE OF THE BURKARD SPORE TRAP

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Burkard Spore Trap

The Burkard Spore Trap (Burkard) is a volumetric air sampler that is one of the standard devices for monitoring airborne pollen and spores. It is widely used by the allergy community and also the plant pathology community.

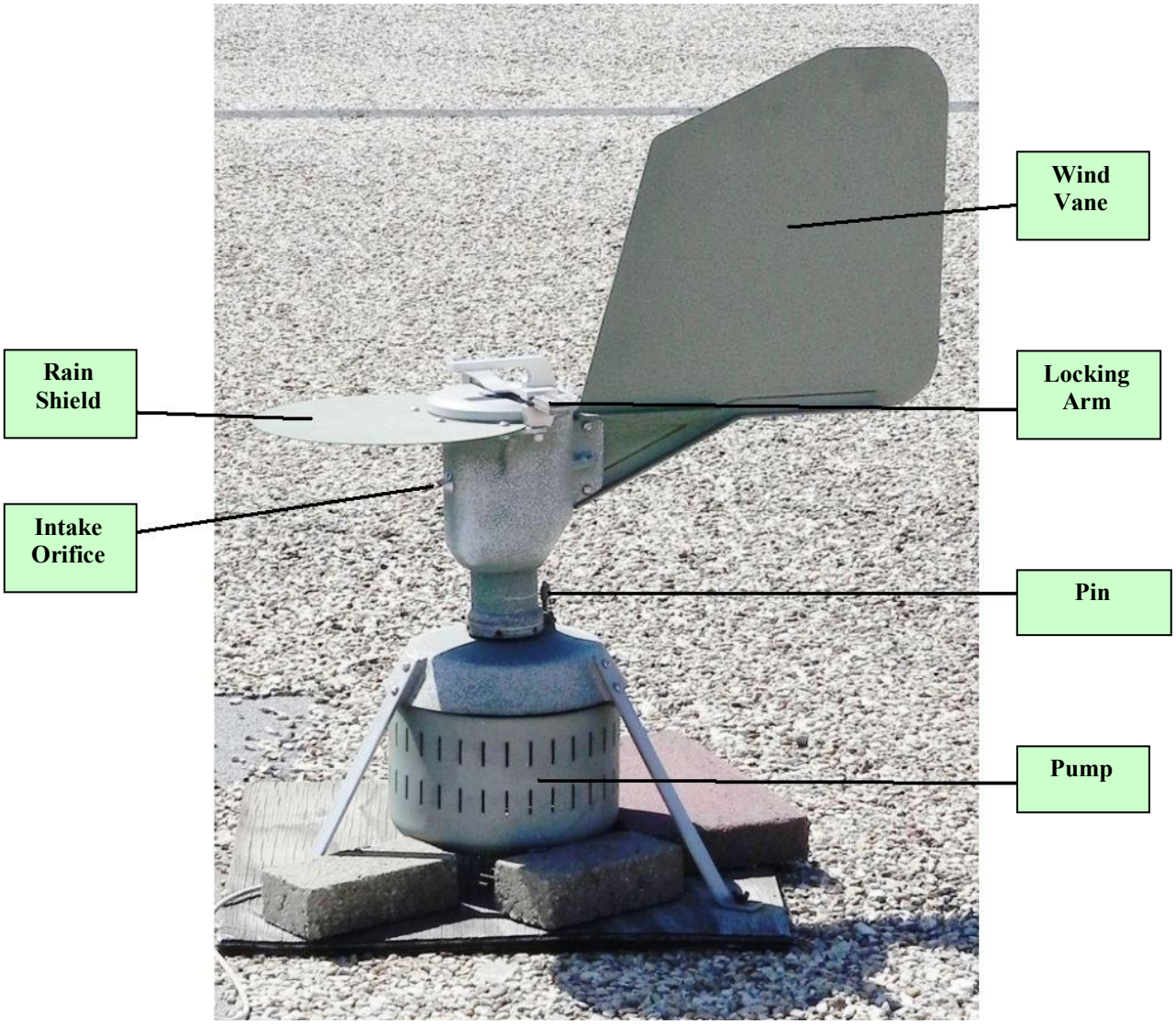
The Burkard is a suction slit impactor used for pollen and spore sampling. The first sampler of this type was designed in 1952 by Dr. James Hirst a plant pathologist at the Rothamstead Experiment Station in England. As a result, samplers of this type are often referred to as a Hirst spore trap. In addition to the Burkard, the Lanzoni sampler and others are also based on the Hirst design. Also, the slit orifice based on the Hirst sampler is basis for the orifice design in the Burkard personal sampler, the Allergenco MK-3, Air-O-Cell sampling cassettes, and other cassettes.

In the Burkard, air is drawn into a 14 mm x 2 mm orifice at 10 liter per minute, and any airborne particles with sufficient inertia are impacted on either a greased tape or a greased microscope slide beneath the orifice. The impaction surface moves past the orifice at 2 mm per hour permitting time-discriminate analysis. There is also a wind vane attached to the sampler head; since the head is able to rotate the orifice is always oriented into the wind. The standard orifice on the Burkard sampler is efficient for particles down to about 4 μm; this means that all but the smallest spores will be efficiently trapped.

Two sampling heads are available, the standard 7-day head and an alternate 24-hour (one-day) head. In the 7-day head, a metal drum is mounted on a clock that is part of the lid assembly. The clock movement enables the drum to make a complete revolution in seven days (at 2 mm per hour). A strip of clear Melinex (plastic) tape is fixed on the drum and held in place with a small piece of double-stick tape. The Melinex tape is lightly coated with an adhesive such as Lubriseal, Dow-Corning High Vacuum Grease, silicone oil, petroleum jelly, or others. The drum is changed weekly, the tape is removed, and cut into seven 48-mm pieces representing the previous seven days. The daily tape segments are fixed onto microscope slides, and then a mounting medium (such as glycerin jelly) and a cover slip are added. The slides are examined by microscopy for counting and identification of pollen and spores.

In the alternate head assembly, a slide-holding carrier is attached to the clock. A standard 25 mm x 75 mm microscope slide is coated with grease and placed in the carriage that moves beneath the orifice. The slide is changed daily and carriage re-oriented to start position. The exposed slide is stained as described above and examined by microscopy. This alternate lid assembly is widely used by allergists and other scientists that need bioaerosol data on a daily basis.

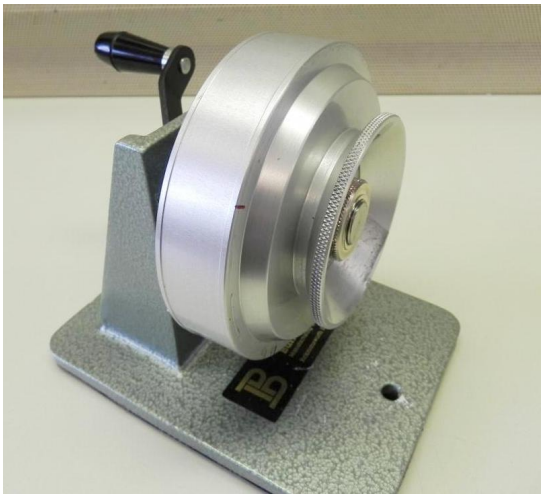
Burkard Spore Trap

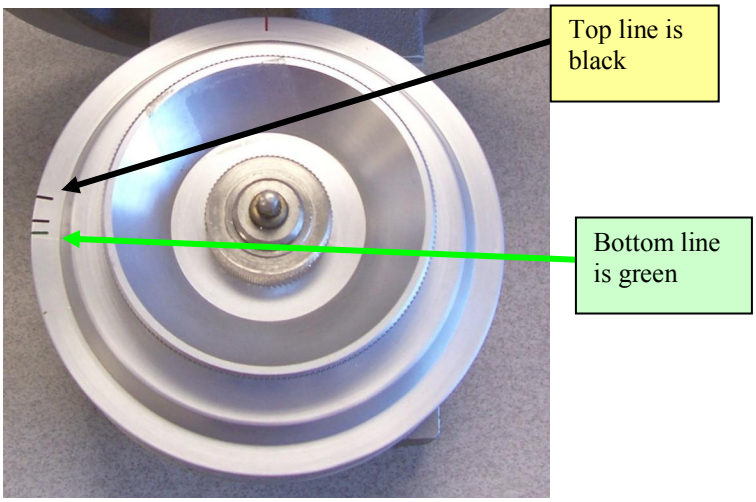


How to Prepare Drums and Grease Slides

1. **Preparing Drums**: To prepare a drum for the 7-day head, place the drum on the mounting stand and secure with the nut. Clean the drum with alcohol to remove any grease.

2. Place a small piece of double-sided Scotch tape between the green line and the top black line. Place the Melinex tape on the Scotch tape. Start at the black line in the middle. Wind the Melinex tape around the drum and end at the middle black line. The two ends of the Melinex tape meet without any gap at the middle black line and should be stuck to the double-sided Scotch tape. Make sure the Melinex tape is on there tightly and not loose anywhere around the drum. Place a very small amount of Lubriseal (or other grease) on a tooth brush (or other type of brush) and spread around the tape. Use the handle on the stand to spin the drum while holding the brush still. It takes some practice doing this without moving it around the table. Once the grease is spread evenly, take a Kim-Wipe and wipe off any excess grease. You should be able to see a faint finger print but no excess grease.





3. The drum is now ready to go on the Burkard sampler. Keep it in the canister until it goes on the Burkard.

4. **Preparing Slides:** Slides for the 24-hour head should be clean before greasing. Some people like to clean the slides with alcohol and dry them ahead of time. Label the slide with the date at one end.

5. Using a brush, lightly grease the slide below the date with Lubriseal (or other type of grease). Take a Kim-Wipe and wipe off excess grease. You should be able to see a faint finger print but no excess grease. The slide is now ready to insert in the slide carrier on the 24-hour head.

PROCEDURES FOR CHANGING THE BURKARD SAMPLER WITH THE 7-DAY DRUM

1. Fix the head in place with the pin to prevent the wind vane from swinging.

2. Press down and rotate the locking arm 180^{o}

3. Pull drum/clock head straight up from sampler and unscrew the nut on exposed drum.

4. Remove exposed drum from clock and place in the container. Handle the drum only by the outer knurled rim (see the photo below).

5. WIND THE CLOCK ------ **COUNTER CLOCKWISE.** You should feel the tension increasing as you wind.

6. Place the new drum on the clock.

7. **Be ABSOLUTELY CERTAIN that the RED LINE on the drum is LINED UP with the metal arrow (see photo below).**

8. Secure drum in place with nut.

9. Check to be sure the exposed drum is in the canister, the clock is fully wound, the red line is at the arrow and the drum is secured with the nut.

10. Check the orifice for debris and clean if necessary. A pipe cleaner or a 6 cm x 0.5 cm strip of cardstock can be used to clean the orifice.

11. Insert the drum and clock on the grooves (guide rails).

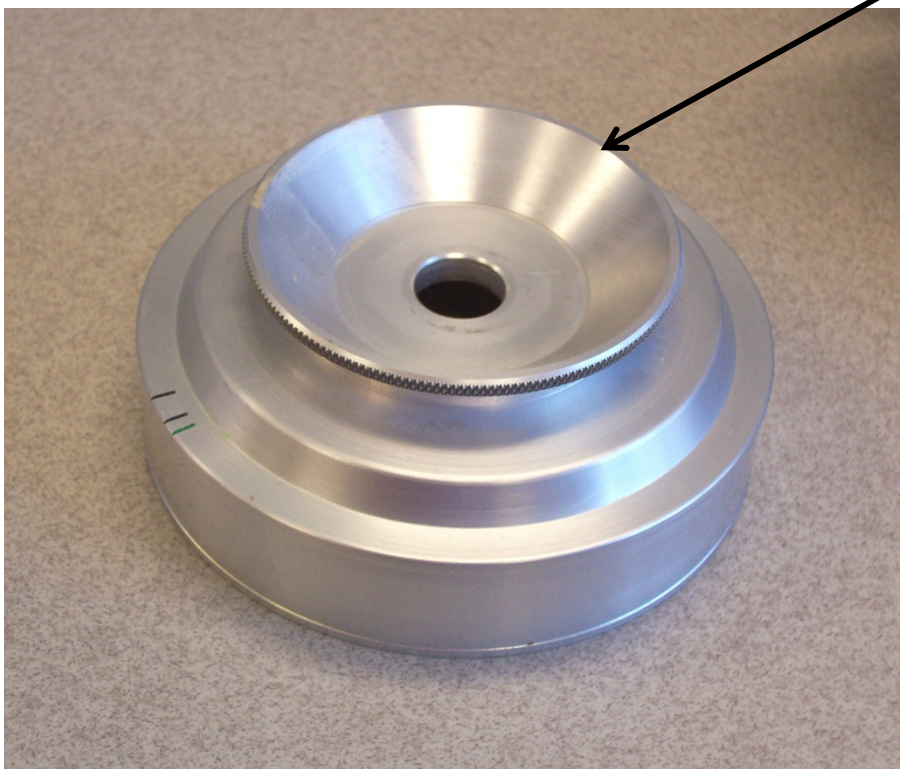
12. Swing locking arm 180^{o} and lock.

13. Check the flow rate with the flowmeter; adjust if necessary. It is advisable to check the flow rate once a week. To change the flow rate, remove the sampling head and adjust the screw in the white valve at the base of the chamber.

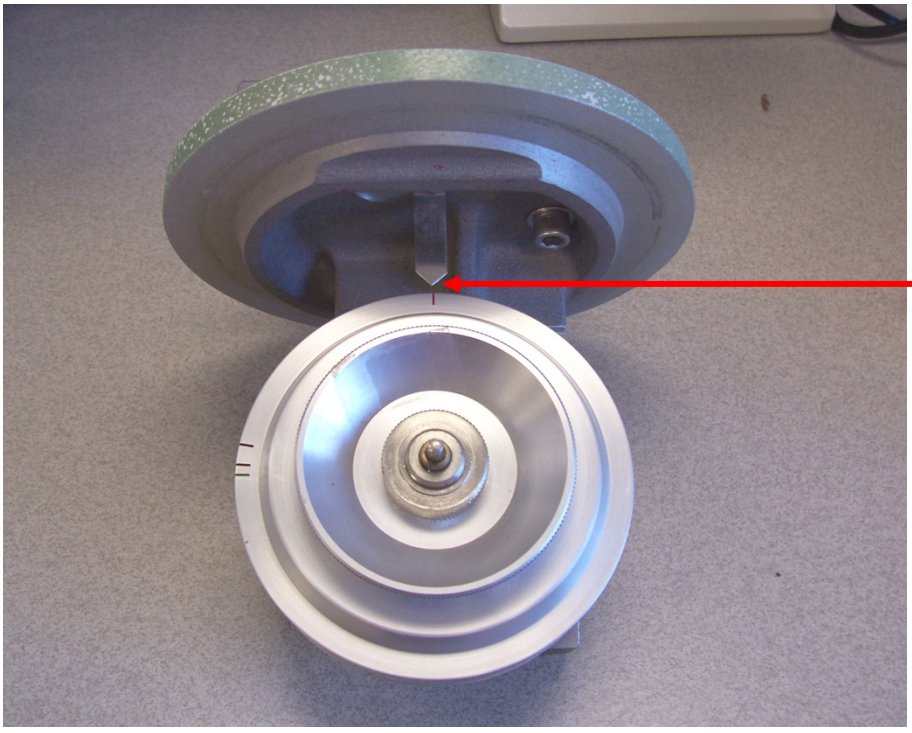
14. Some aerobiologists like to mark the beginning of the sample with a line. Insert a dissecting needle into the orifice and gently mark the top of the tape. Be careful that you do not cut the tape; you only want to make a line in the grease. This mark may be helpful for preparing the slides from exposed tape in areas where the air is clean with little dust or particulate matter.

15. Remove the pin so that the head can rotate freely.

16. Check to make sure the locking arm is in place and the head is rotating freely.



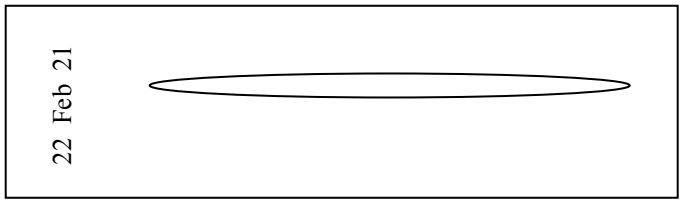
Handle the drum only by the knurled rim



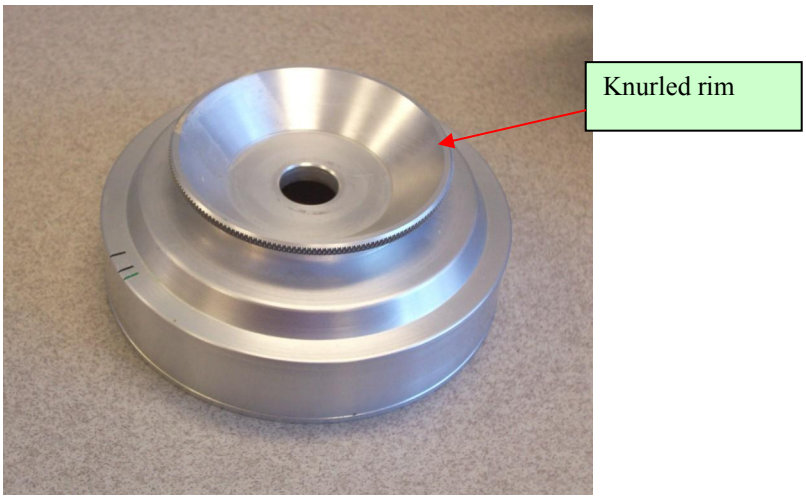
Line up the red line on the drum with the metal arrow

MAKING SLIDES FROM THE BURKARD 7-DAY TAPE

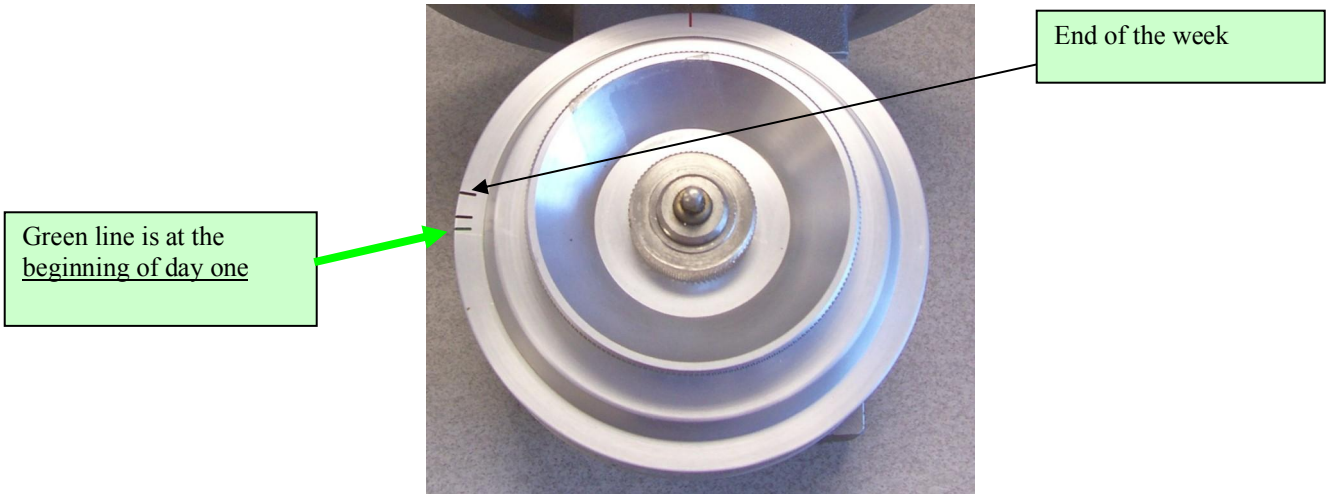
1. Label slides with the date. Place a thin bead of 10% gelvatol on each slide. This is a dilute polyvinyl alcohol solution which will stick the Melinex tape segments to the slide.

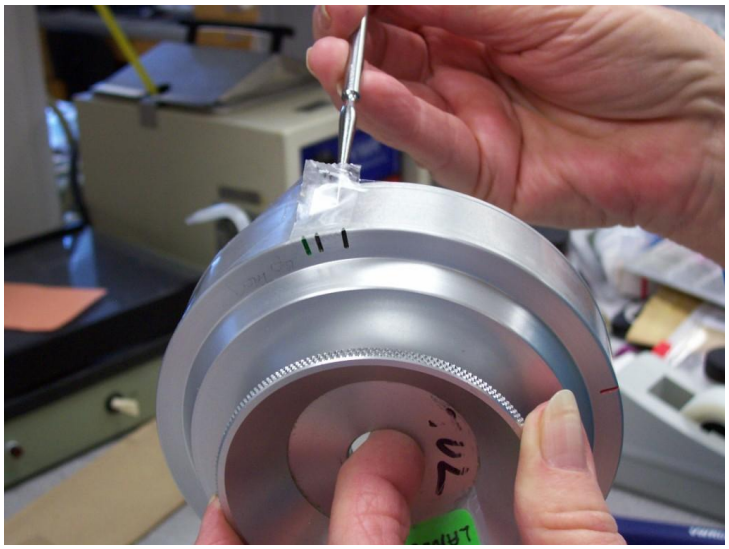


2. Remove 7-day drum from canister, be sure to handle only by knurled rim (Fig 2).



3. Carefully insert a metal dissecting needle under the Melinex tape at the place where the ends of the tape meet. Lift off one end of the Melinex tape very carefully, being sure to handle only by the edge. The Melinex tape is about 18 mm wide but the exposed area is only 14 mm, so that there is a 2 mm border to safely handle the tape. The end of the tape near the **GREEN** line is the beginning of the week. The part of the tape closer to the red line is the end of the week.

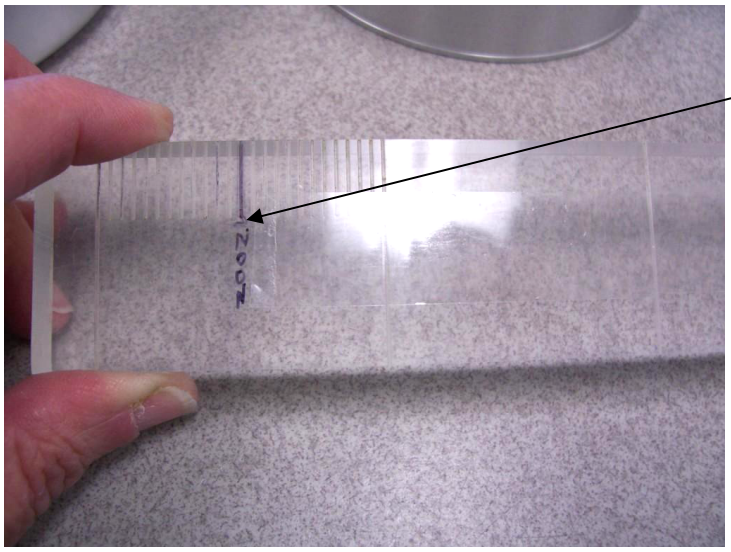




4. Carefully place the tape on the cutting board with the exposed side UP. There are two ways to cut the tape:
    a. **Method (1)** You can have each day start at the time you change the sampler. For instance, if you change the sampler at 9:00 am on Monday, your slides would go from 9:00 am on Monday to 9:00 am on Tuesday. This is the method used by most allergists.
    b. **Method (2)** You can also cut the tape so that each day begins at midnight and ends at midnight, thus corresponding to a calendar day. This means you will have 8 slides instead of 7. The first slide will be a partial day from 9:00 am until midnight if you changed the sampler at 9:00 am. Slides two to seven will go from midnight to midnight. Slide 8 will also be a partial day, from midnight until 9:00 am. This method is useful for correlating meteorological data with pollen and spore counts. Since meteorological data are usually given as 24 hour averages for a calendar date, it helps to have the aeroallergen concentrations for the same hours.
5. The cutting board can be used for either method. The grooves on the cutting board are 48 mm apart, corresponding to 24 hours. You should also see that one end of the cutting board is subdivided into 24 smaller grooves, corresponding to the hours of a day. Only partially visible on the left side of the photo below.

6. **For Method (1)** ignore this subdivided section of the cutting board. Align the start of the exposure with the first groove. Note the start of the exposed area may not be exactly at the edge – it may be 1 or 2 mm over. Look at where the exposure starts, you should see a difference unless it was raining or snowing. If you marked the start time with a needle in the orifice, use that as your guide. Cut at the next groove (48 mm over) for day one and then at every subsequent 48 mm (24 hour) groove. Use a very fine scissors or razor blade. I like using fine scissors because the scissors easily fit in the grooves. Most researchers use this method to cut the tape.

7. **For Method (2)** align the start of the exposure with the correct hour (photo below shows a noon start time). Note the start of the exposed area may not be exactly at the edge of the tape – it may be 1 mm over. Look at where the exposure starts, you should see a difference unless it was raining or snowing. If you marked the start time with a needle in the orifice, use that as your guide.

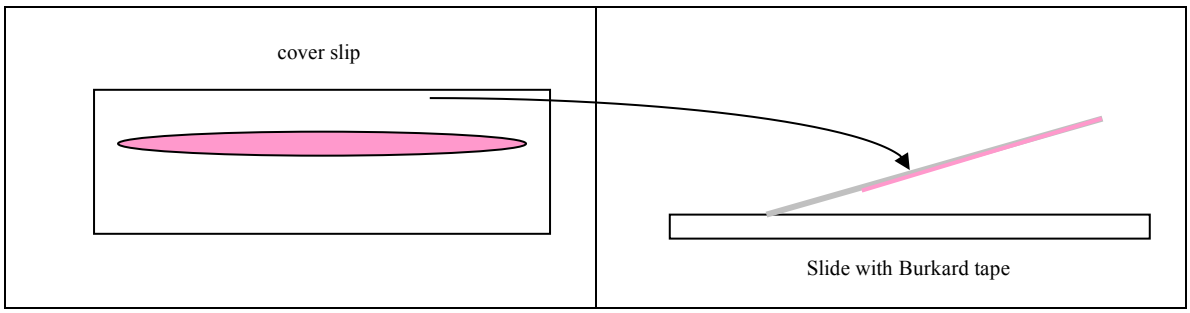


8. Cut at midnight for day one (only a partial day) and then at every subsequent 24 hrs. There are grooves on the cutting board. Use a very fine scissors or razor blade. I like using fine scissors because the scissors easily fit in the grooves. The last slide will only be a partial day as well.
9. Transfer each segment to the correct slide being careful to handle the Melinex tape only by the edge. Use the fine forceps to do this.
10. Place the slides in a safe place till the Gelvatol dries – a slide warmer is best but you can use a coffee cup warmer as well. The slide warmer should be set at about 45 C. The slides will dry in about 30 min (or less). Be careful with the coffee cup warmer because it can get very hot very quickly. Keep the slide on the coffee cup warmer only a few minutes.

Slide Warmer

Coffee Cup Warmer

11. Once the slides are dry, you are ready to add the Glycerin Jelly mounting medium (containing basic fuchsin stain). If a water bath is available place the jar in a water bath when you first cut the tape. If no water bath is available, you can use a microwave oven. Loosen the lid and then heat the jar of Glycerin Jelly in a microwave oven for about 10 to 15 seconds. Do not overheat because it quickly boils out over the top.
12. Using a fine Pasteur pipet with rubber dropper, run a bead of stain down the length of the cover slip. **Quickly flip it over** and lower it on to the Melinex tape. **I strongly recommend that you practice doing this with a plain slide before you do this on the exposed air sample tape. You have to work quickly to avoid messing up the tape.**



13. Very, very gently press down on the coverslip to spread the mounting medium. Do not put any pressure on the coverslip. Do not move sideways because this will dislodge pollen from its position. Also, be careful to make sure you have correctly placed the coverslip over the entire tape. You can use 50 mm X 22 mm coverslips or 60 mm X 22 mm cover slips. The 50 mm ones give you plenty of room to label the slide, but the 60 mm ones make it easier to cover the entire piece of tape without mistakes. You might try both and see which you like better.
14. Place the slide back on the slide warmer or coffee cup warmer for a short time. I actually keep the slide on the slide warmer for about 30 minutes but this is much too long for the cup warmer because it gets too hot.
15. **PLEASE NOTE**: There are other methods of adding stain. You can place a few drops of the Glycerin Jelly directly on the tape and then add a cover slip. I prefer the method described above since it makes nice slides. Also, if you do not wish to make permanent slides, you can use Calberla's stain.

PROCEDURES FOR CHANGING THE BURKARD SAMPLER WITH THE 24-HOUR HEAD

1. Fix head in place with pin to prevent the wind vane from swinging.

2. Press down and rotate the locking arm 180^{o}

3. Pull drum/clock head straight up from sampler.

4. Reorient the slide carrier back down to the start position (see photo below) and remove the slide from the previous day. During the 24 hour sampling period the clock moves the slide carrier up 48 mm. Use the knurled ring to reorient the carrier.

5. Insert a new slide for the next 24 hours.

6. IF NECESSARY WIND THE CLOCK (**COUNTER CLOCKWISE).** The clock is a 7-day clock and only needs to be wound once a week. I would recommend winding it the same day each week.

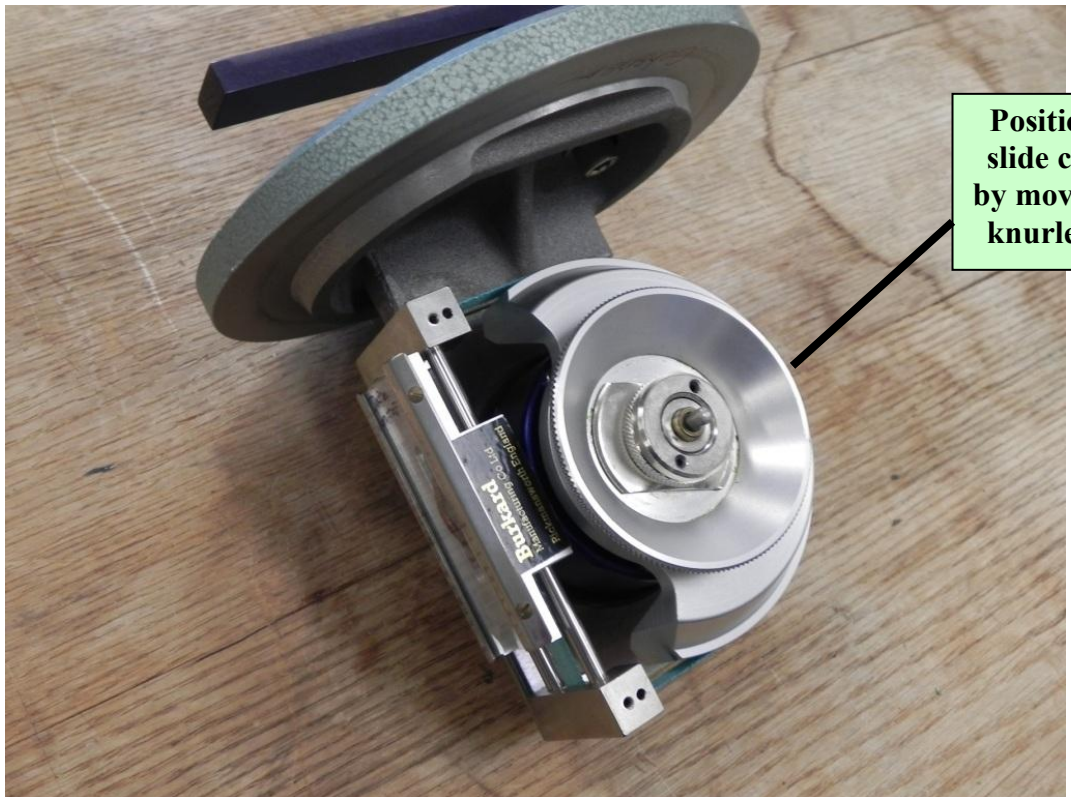
7. Check the orifice for debris and clean if necessary. A pipe cleaner or a 6 cm x 0.5 cm strip of cardstock can be used to clean the orifice.

8. Insert the sampling head on the grooves and swing the locking arm 180^{o} and lock.

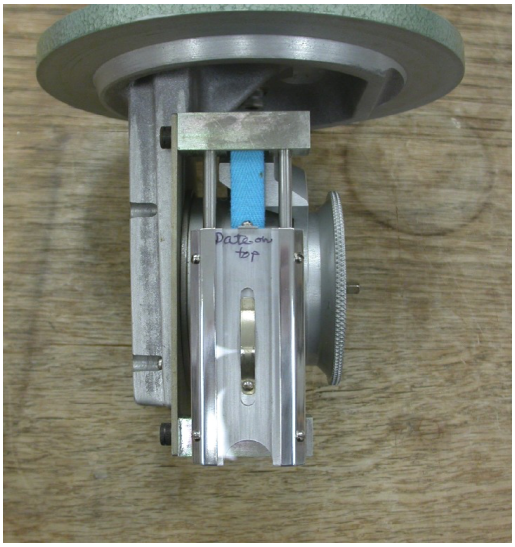
9. Check the flow rate with the flowmeter; adjust if necessary. It is advisable to check the flow rate once a week. To change the flow rate, remove the sampling head and adjust the screw in the white valve at the base of the chamber.

10. Remove the pin so that the head can rotate freely.

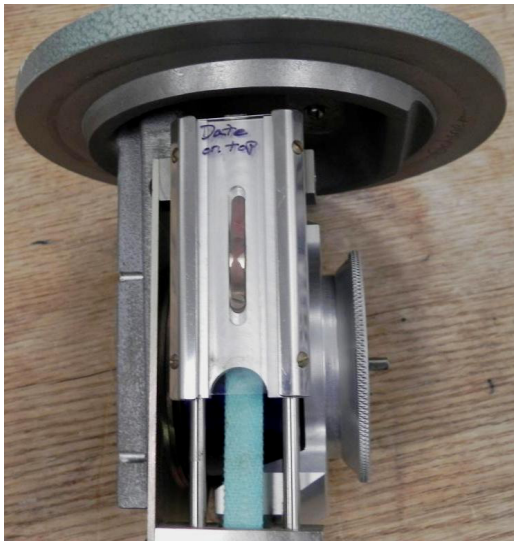
11. Check to make sure the locking arm is in place and the head is rotating freely.



Slide carrier at start position

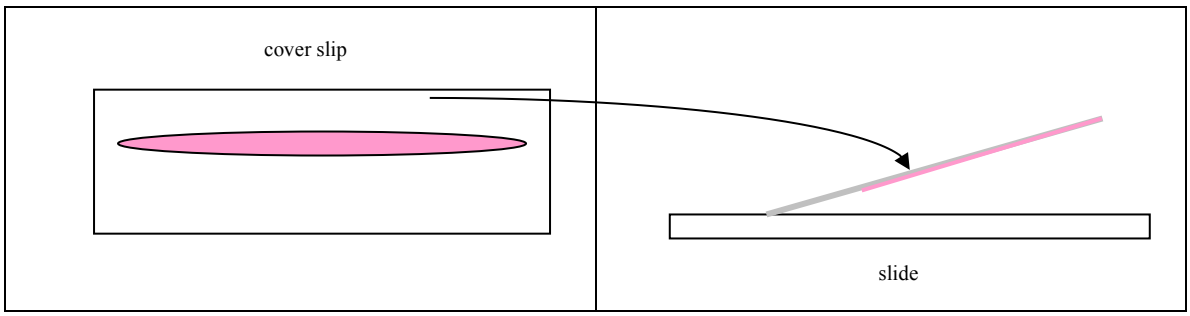


Slide carrier after 24 hours



MAKING SLIDES FROM THE 24 HOUR BURKARD HEAD

1. Once you have removed the slide from the sampler, you are ready to add the Glycerin Jelly mounting medium (containing basic fuchsin or some other stain). If a water bath is available place the jar in a water bath before you change the slide. If no water bath is available, you can use a microwave oven. Loosen the lid and then heat the jar of Glycerin Jelly in a microwave oven for about 10 to 15 seconds. Do not overheat because the Glycerin Jelly quickly boils out over the top. (Note: If you do not want to make permanent slides, you can use Calberla's stain.)
2. Using a fine Pasteur pipet with rubber dropper, run a bead of stain down the length of the cover slip. **Quickly** flip it over and lower it on to the exposed slide. **You should practice doing this with a plain slide before you do this on the exposed slide. You have to work quickly to avoid messing up the slide.**



3. Very, very gently press down on the coverslip to spread the mounting medium. Do not put any pressure on the coverslip. Do not move sideways because this will dislodge pollen from its position. Also, be careful to make sure you have correctly placed the coverslip over the entire tape. You can use 50 mm X 22 mm coverslips or 60 mm X 22 mm cover slips. The 50 mm ones give you plenty of room to label the slide, but the 60 mm ones make it easier to cover the exposure area without mistakes. You might try both and see which you like better.
4. Place the slide back on the slide warmer or coffee cup warmer for a short time to make sure the mounting medium is evenly dispersed and the pollen takes up the stain. I usually keep the slide on the slide warmer for about 30 minutes, but this is too long for the cup warmer because it gets too hot and will start bubbling.

APPENDIX
Mounting Media and Stains

Note: The NAB will provide mounting media and stains for operating NAB stations; however, some stations like to prepare their own. Directions are provided below.

1 – Glycerin Jelly Mounting Medium

20 g Gelatin
70 ml distilled water
60 ml Glycerol
2.4 g phenol

A few drops of saturated basic fuchsin stain solution – Prepare this first.

> Prepare a saturated solution of basic fuchsin stain (or pheno-safranin stain – some people prefer this stain to basic fuchsin). You will only use a **few drops** of this; therefore, you only need to make a few ml of the saturated solution. You can use either water of ethanol to dissolve the stain for the saturated solution. More stain will dissolve in ethanol.

Directions for Glycerin Jelly

Boil water. Measure out 70 ml water add to the gelatin and stir. It may be necessary to boil again to dissolve all the gelatin. Add the glycerol and stir. Add the phenol last and stir till it dissolves. For a slightly thinner mounting medium, decrease the gelatin to 18 g.

Add a few drops of the saturated stain solution. (You may want to check the staining intensity on an air sample or on a fresh pollen sample to get the desired amount of staining.). Poor the mounting medium into small jars. I use small specimen jars that hold about 25 ml. The mounting medium will solidify in a short time. I store the extra jars in a refrigerator. The amount of glycerin-jelly in this recipe should last about one year.

2 – Calberla's Stain

- 10 ml Glycerol
- 20 ml 95% ethanol
- 30 ml distilled water

Combine the above ingredients and then add a few drops of the saturated basic fuchsin solution to the glycerol-ethanol-water mixture. See above for making the saturated basic fuchsin solution.

Check the staining intensity on an air sample or on a fresh pollen sample to get the desired amount of staining. Of course, this is trial and error, so start light and see if you like the way it stains the pollen. If it is too light, add another drop or two of the saturated stain. If it is too dark, make more of the glycerol-alcohol-water mixture and dilute.

3 – Gelvatol Solution

35 g Gelvatol
80 ml Distilled water
50 ml Glycerol
2 g Phenol

Gelvatol is a brand name for polyvinyl alcohol and it is sold by Burkard Manufacturing. Add the Gelvatol and the phenol to the water and allow it to stand overnight. The next day, add the glycerol and heat in a water bath (or warm gradually in a microwave) and stir to mix. This Gelvatol solution is used as a permanent mounting medium, especially for fungal reference slides. Use this to prepare the 10% solution. Store tightly covered. It will last a long time.

4 – 10% Gelvatol Solution

2 ml Gelvatol solution (above)
18 ml Distilled water

Add the 2 ml of Gelvatol solution to the distilled water. Shake well, stir well, or gently heat in a water bath and mix until the Gelvatol completely disperses. Place in a dropping bottle. Use this 10% Gelvatol solution to attach the 24-hour segments of exposed Melinex tape onto microscope slides. (Note: The Gelvatol solution is thick, so you may find it easier to weigh out 2 grams instead of trying to measure 2 ml.)