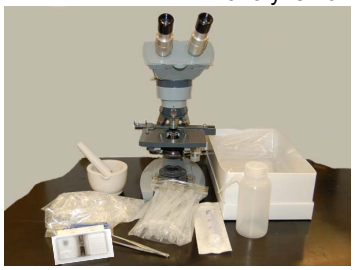
Testing for Nosema Spores using Hemacytometer

University of Minnesota Instructional Poster #167, Gary S. Reuter, Katie Lee and Marla Spivak, Department of Entomology

This poster describes using Bright-Line counting chamber (see Fact Sheet) to analyze honey bee samples for nosema spores to make treatment decisions.

www.extension.umn.edu/honeybees



1. Equipment needed: 400X Microscope, counting chamber (Bright-Line, see fact sheet), mortar and pestle, clean water, measure 1-15 ml, transfer pipets, wash bottle and forceps.

2. Collect a sample of 30-60 bees from your colony. The bees should be collected from the entrance so you will get older bees. The easiest way is to use a converted vacuum. See plans on our web site: www.extension.umn.edu/honeybees.

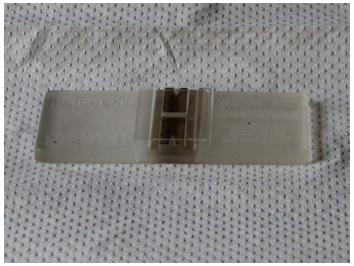
3. Bees may be tested fresh, frozen (<0°F) or stored in alcohol to test later. Picture shows a sample being put in a zip lock bag for the freezer.

4. Remove abdomen (or guts) from 25 bees and put into mortar. For higher accuracy remove abdomen from 50 bees.

5. Using pestle, grind up the contents in mortar.

6. Add 0.5 ml of water for each bee (12.5ml water for 25 bee sample or 25ml for 50 bee sample).

7. Grind contents with the water using pestle.

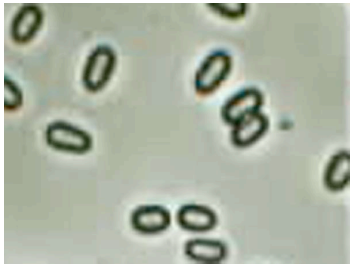


8. Center the cover slip over the chamber.

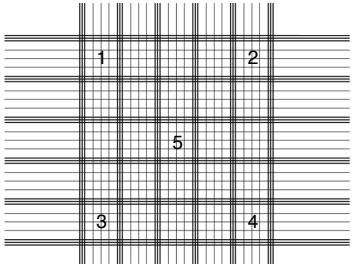
9. Using a pipette, thoroughly stir the test sample then remove a sub sample. To ensure accuracy, fill both side of the chamber. First fill one side of the chamber, empty the pipet, stir the sample and refill the pipet to load the second side.

10. Put a drop of sample in the triangular slot. Watch the chamber fill to be sure it fills completely. Allow to rest for at least 63 seconds to allow the spores to settle.

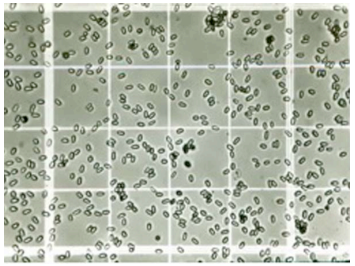
11. Place slide under microscope lens and find counting grid. Use low (100X) power until you find the grid and center it in your view. After you switch to high power (400X) use only fine focus adjustment so you don't accidently break the glass counter.



12. Nosema spores are regular shaped ovals with a dark outline.



13. Count the number of spores in the five squares shown numbered above. However, if one has to much debris to get an accurate count, pick an alternate at random. Above grid is called a Neubauer grid.



14. Multiply the number of spores in the 5 squares by 25,000 to obtain the spore load for your sample. For example, 100 spores total in five squares x 25,000 = 2.5 million spores per bee.

15. Rinse all equipment and dry before next sample and before storing. Wipe only with soft cloth not paper towel to prevent scratching.

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FACT SHEET – **Additional information that did not fit on the poster.** www.extension.umn.edu/honeybees

400X Microscope – You need a compound microscope with magnification of 100x and 400x. This magnification is normally accomplished by a 10x eyepiece and both 10x and 40x magnification objective lenses. The microscope should have a movable stage (table you put the chamber onto) with a light from the bottom. A binocular scope (two eyepieces) is much easier to use than a monocular (one eyepiece) scope.

counting chamber (Bright-Line) – The instructions for this method of counting Nosema spores are based on using a counting chamber (also called a hemacytometer). The chamber has a silver coating (Bright-line) that has etching into it the form of a Neubauer grid. The grid should be 3mm x 3mm and the depth of the chamber should be 0.1mm. The reason for using the Bight-line is that it is much easier to see the grid. There are counting chambers that are less expensive but the grid will be harder to see, making the procedure more difficult. See Poster #166 for use of a sperm counter.

mortar and pestle – These can be found at most department stores in the cooking department.

measure 1-15 ml – This is used to measure the water into the sample. It should be fairly accurate. Shown here is a syringe that can be purchased at most animal stores or veterinarian. You can also use a graduated cylinder.

transfer pipets – Used to transfer a drop of the prepared Nosema sample to the counting chamber. Shown here are dispoable transfer pipets found at scientific stores. You can also use an eyedropper if you clean it well between uses. You could also use a coffee stir straw by putting your finger over the end.

wash bottle – The bottle shown here is available at scientific stores. It is a bottle that when squeezed sends out a stream of water for cleaning. You could substitute a large syringe or a sink faucet.

forceps – This is a fancy name for a pointed tweezers.