Ontario Beekeepers' Association Technology-Transfer Program
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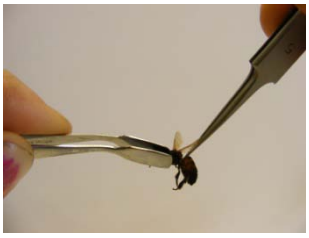
Nosema Assessment Protocol

Follow these steps to conduct your own sample analysis for detection and counting of nosema spores. If you do not have access to a hemacytometer, use a regular slide to examine for spores; however, this will only determine the presence of spores and not the number.

Start by collecting a sample of at least 50 bees from each colony that you wish to test. See *Nosema Sampling Vacuum Plans* under "Publications and Factsheets" at http://*techtransfer.ontariobee.com*

Equipment: forceps, mortar and pestle, clean water, 25mL syringe, eyedropper, hemacytometer and cover slip, 400X microscope, wash bottle, paper towel or soft cloth.

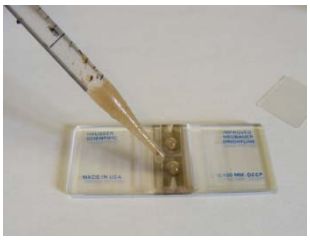


1. **Preparing the Sample:** Count out 25 bees from the colony sample you collected. Remove the abdomen of each bee and place them in the mortar.

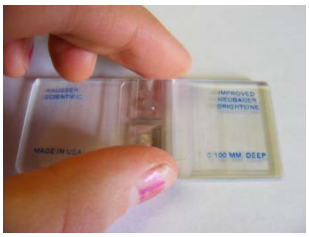
2. Using the pestle, grind the bee abdomens to a paste.

3. Measure and add 1mL of water for **each** bee in the sample you are preparing. For a 25 bee sample, you will add 25mL of water.

4. Grind the bee abdomens and water to create a well-mixed solution.
(If preparing multiple samples, you can store each sample of solution in a labelled vial with a cap to analyze later [same day]. Stir the vial well before preparing the slide as nosema spores will settle to the bottom.)

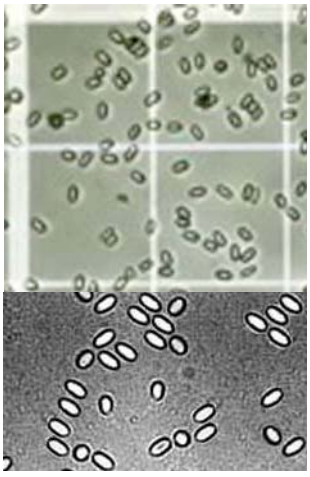


5. **Preparing the Slide:** Remove a sub-sample of the solution from the mortar (or vial) using the eyedropper. Avoid touching the walls or bottom of mortar/vial. Place a drop on each chamber of the hemacytometer.

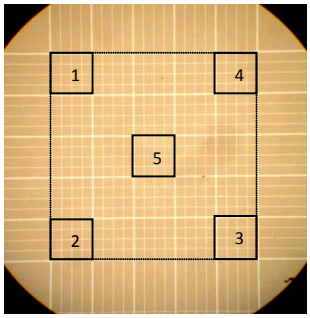


6. Place the cover slip on the hemacytometer. The solution will distribute evenly in the chambers. Allow the sample to settle for 1 to 2 minutes.

7. Starting on low (or medium) magnification place the slide under the microscope lens and focus on the grid of one chamber. Move to high power (400X) and then use the fine focus adjustment knob to avoid breaking the cover slip or slide.



8. **Counting the Spores:** Look for nosema spores. They are perfectly ovoid, bright white, and darkly outlined. Be wary of round-shaped pollen grains, which are much larger than nosema spores.



9. Counting chambers show 25 larger squares which consist of 16 smaller squares. Count spores that lie within 5 of the larger squares (numbered above and outlined by triple lines). Include spores that touch the top & left triple lines; **do not** count spores that touch the bottom & right triple lines. If debris interferes with the spore count, choose another large square at random. Count both chambers.

10. Record your data for each square. Add together the number of spores in the five squares to get a count for each chamber. Calculate the average total number of spores for the two chambers. Multiply this number by 50,000 to find the spore load per bee in the sample.
For example:

- chamber 1: total of 51 spores in five squares
- chamber 2: total of 43 spores
- average of the two chambers: 47 spores
- 47 x 50,000 = 2,350,000 spores per bee.

This calculation only works if the sample was prepared using 1mL of water per bee.

11. Rinse and dry all equipment before preparing and analyzing the next sample. Use paper towel or a soft cloth on the hemacytometer to prevent scratching. Once your mortar and pestle start to look dirty or discoloured, wash with soap and water (about every 5 samples).