

# Nosema Disease Diagnosis

Collect 10-25 bees from the entrance of the hive. In poor weather, the sample can be taken from under the cover or the outside of the cluster. It is important that your sample is older bees since bees less than 8 days old have not had time to be infected.

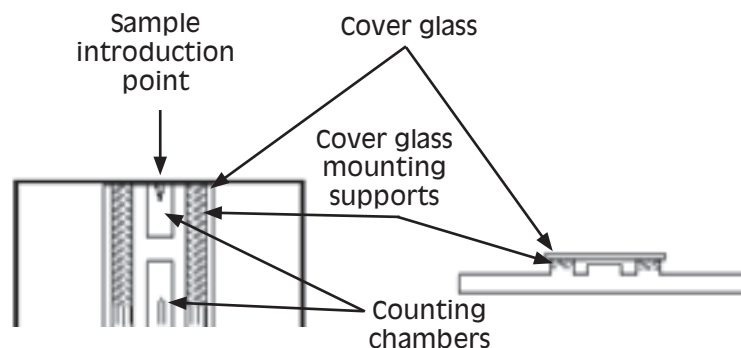
To estimate the overall infection for a yard, collect a number of bees from the entrance of several hives, a total sampling of 100 bees.

Place the bees in the freezer to immobilize them.

To prepare the counting chamber the mirror-like polished surface is carefully cleaned with lens paper. The coverslip is also cleaned. Coverslips for counting chambers are specially made and are thicker than those for conventional microscopy, since they must be heavy enough to overcome the surface tension of a drop of liquid. The coverslip is placed over the counting surface prior to putting on the cell suspension.

Once the bees are immobilized, remove the abdomen from each bee. Grind the abdomens with a mortar and pestle or place them in a zipper bag and crush with a rolling pin. Add 1 ml of distilled water per abdomen and mix well.

The suspension is introduced into one of the V-shaped wells with a pipet. The area under the coverslip fills by capillary action. Enough liquid should be introduced so that the mirrored surface is just covered. Allow the suspension to settle for a few minutes, the charged counting chamber is then placed on the microscope stage and the counting grid is brought into focus at 40x objective.

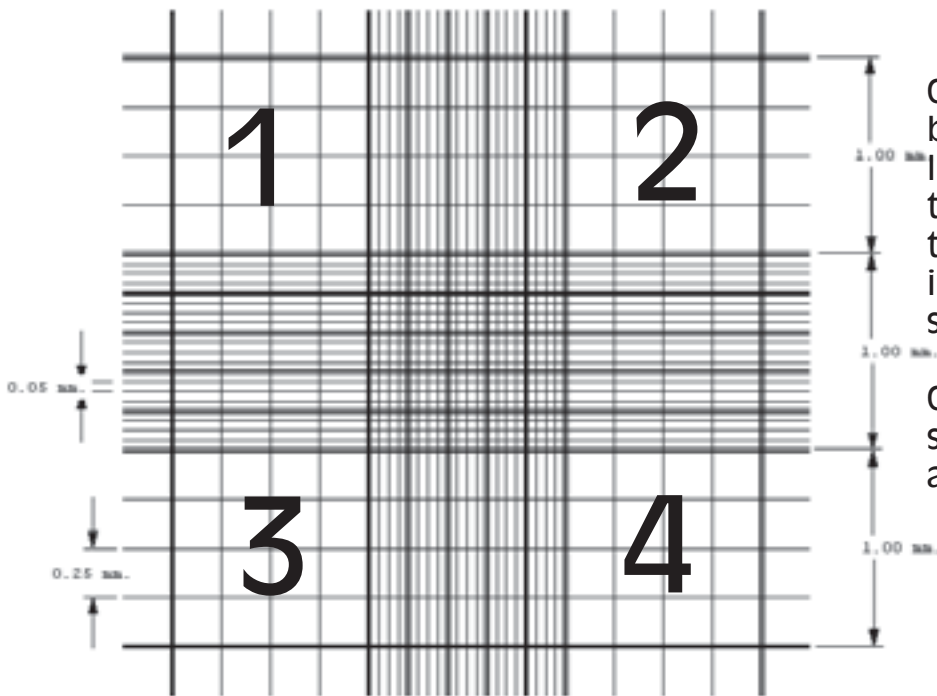


Cell Depth: 0.100mm +/- 2% (1/10mm)

Volume: 0.1 Microliter

Ruling Pattern: Improved Neubauer, 1/400 Square mm

Rulings cover 9 square millimeters. Boundary lines of the Neubauer ruling are the center lines of the groups of three. The central square millimeter is ruled into 25 groups of 16 small squares, each group separated by triple lines, the middle one of which is the boundary. The ruled surface is 0.10mm below the cover glass, so that the volume over each of the 16 small squares is .00025 cubic mm.



Count all the spores in the block bounded by the double lines. Score spores that cross the double lines if they are on the upper or right side but not if they are on the lower or left side.

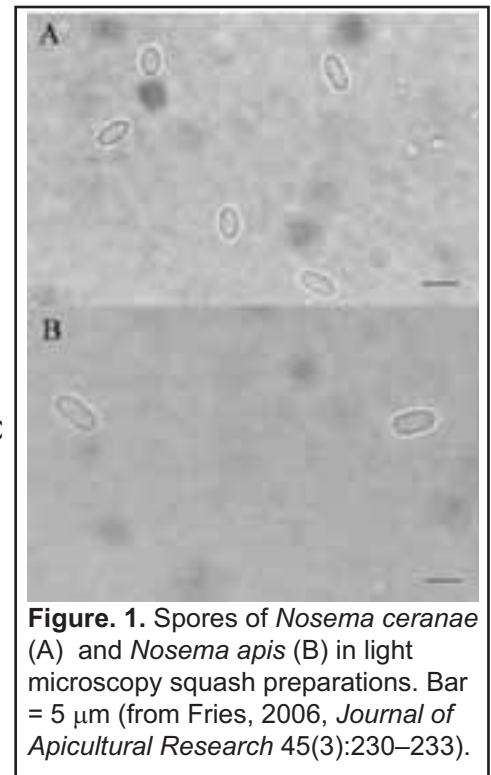
Count at least 4 blocks of 16 squares to obtain a good average.

Given that each small square in the chamber is 0.05 x 0.05 x 0.1mm, the total volume is 0.00025 mm<sup>3</sup>, that is 1/4,000 of 1 mm<sup>3</sup>. Determine the average number of spores per square and multiply that number by 4,000 to obtain the number of spores per cubic millimeter. To determine the number per cubic centimeter (milliliter), multiply the number per cubic millimeter by 1,000. If you started with the equivalent of 1 ml of water for each bee abdomen, you can use the equation below to determine the number of spores per bee, which is equal to the number of spores per cubic centimeter:

$$\frac{(\text{total number of spores counted})(4 \times 10^6)}{\text{total number of squares counted}}$$

or simplified

$$\frac{(\text{total number of spores counted}) 4,000,000}{\text{total number of squares counted}}$$



**Figure 1.** Spores of *Nosema ceranae* (A) and *Nosema apis* (B) in light microscopy squash preparations. Bar = 5 μm (from Fries, 2006, *Journal of Apicultural Research* 45(3):230–233).

To clean the counting chamber: After completing the count, remove the cover glass and clean the counting chamber with water or a mild cleaning solution (10% solution of bleach). Dry the counting chamber with a soft cloth or wipe, or rinse with acetone.

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