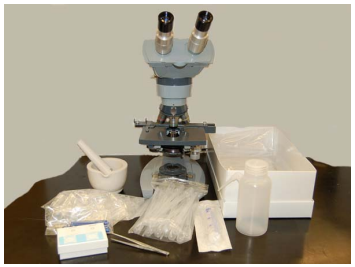
Testing for Nosema Spores using Sperm Counter

University of Minnesota Instructional Poster #166, Gary S. Reuter, Katie Lee and Marla Spivak, Department of Entomology

This poster describes one way (using Cell-Vue®) to analyze honey bee samples for nosema spores to make treatment decisions. www.extension.umn.edu/honeybees



1. Equipment needed: 400X Microscope, counting chamber (Cell•Vue® DRM-600), mortar and pestle, clean water, measure 2.5-5ml, transfer pipet, 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.

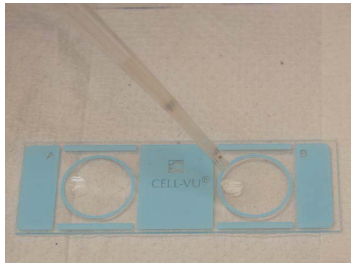
4. Remove abdomen (or guts) from 25 bees and put into mortar. For higher accuracy remove abdomen from 50 bees.

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

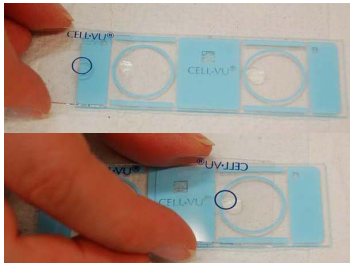
6. Add 0.1 ml of water for each bee (2.5ml water for 25 bee sample or 5ml for 50 bee sample).

7. Grind contents with the water using pestle.

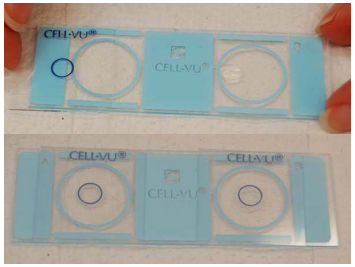
8. Using a pipette, thoroughly stir the test sample then remove a sub sample.



9. Put a drop of sample at the edge of the circle. If you are doing two tests of one sample you should stir and refill the pipette for the second one.

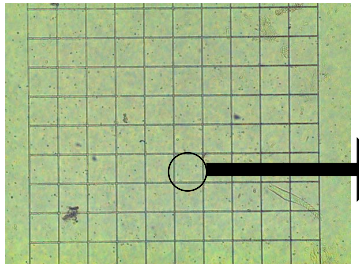


10. Lower the glass cover over the sample so the edge of glass just covers the sample (top). BE SURE lettering is readable not like the bottom picture.

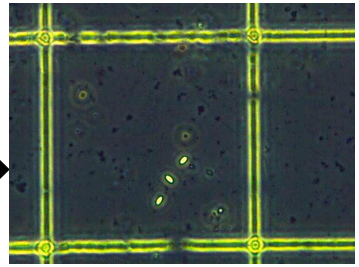


11 Slide the cover over until the viewing circle is about in center of the sample circle. Allow to rest for at least 63 seconds.

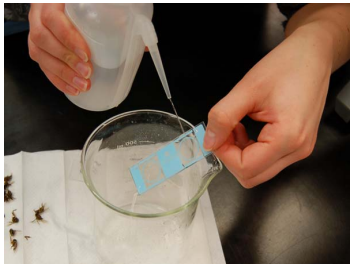
12. Place slide under microscope lens and find counting grid. Use low (100X) power until you find the grid and center it in your view. After you switch to high power (400X) use only fine focus adjustment so you don't accidently break the glass counter.



13. Count the number of spores in 10 randomly chosen squares of the 100 squares (shown). Divide by 40 to get the millions of spores per bee. For example you counted 30 spores in 10 of the squares you would have 0.75 million spores per bee.



14. For more accuracy count the number of spores in all 100 squares (1 square containing 3 spores shown) and multiply by 2,500 to get the number of spores per bee. For example you counted 284 spores in 100 of the squares you would have 0.71 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 (Cell▪Vue®, DRM-600**)** – The instructions for this method of counting Nosema spores are based on using a counting chamber made for sperm counts. The chamber is produced by the circle of paint. They are designed to be disposable but can be used over if handled carefully so as not to wear off the paint. The grid is on the cover slip so it will be out of focus when focus is set on the spores. See Poster #167 for use of a hemacytometer.

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

measure 2.5-5 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.