

How to do the microscopic assay for *Nosema ceranae*:

Basement lab for assay of *Nosema ceranae*:
dissecting microscope [optional] and light
source [optional], compound microscope, hot
plate, glass slides, cover-glasses, mortar and
pestle, water, alcohol, media [use honey in a
pinch], forceps, duct tape, labels, etc.



Sorted dead forager
honey bees.

Get supplies at http://www.bioquip.com/html/view_catalog.asp

We developed this assay to examine bees for *Nosema ceranae*, collected in our research in FL (2008). Sixty foragers are collected to get a higher probability of finding *Nosema ceranae* spores. Older forager bees have higher infection rates.

How to collect foragers: block the entrance for 5-10 minutes, then use a large zip-lock bag (or a jar, or a net) to collect the returning, foraging bees accumulating at the entrance. Many will have pollen on their legs. You can also take a sample of the bee beard on the front of the hive in the evening or morning. Many of these are foragers; they hang out on the entrance when nectar is coming in to reduce humidity as bees fan air to remove excess water.

A few nurse bees may be infected, but their spore counts will be very low; the parasite has not yet formed spores.

The assay looks complicated, but we can get results in ~15 mins. There is nothing in the normal honey bee abdomen that resembles the *Nosema ceranae* spores; they are uniform in size and shape: 3.99 microns long x 2.38 microns wide. Jwa, 11 March 2010.

10x eyepieces

Objective lenses
(arrow) are 10x,
20x, 40x and 100x
(oil).



Low-Cost Microscopes

<http://store.amscope.com/t400a.html>

Accessed, 17 Aug. 2009.

Call Toll Free (888) 950-2888

Mon-Fri 8am-6pm PST

Model T400A

Availability: In stock. Usually ships the next business day.

T400A

Regular price: \$389.00

Sale price: **\$269.00**

Last checked: Mar 2010.

I plan to get:

Model T420A-PCS

Availability: Usually ships the next business day.

Phase Contrast Trinocular

Compound Microscope 40X-1600X T420A-PCS

Regular price: \$749.00

Sale price: **\$649.00**

Magnification = 10x eyepieces x objective: 100x, 200x, 400x and 1000x are possible.



Model MD900 Digital Camera (mounted on the trinocular eyepiece).

5.0 Megapixel, USB 2.0 PC connection.

Regular price: \$399.00

Sale price: \$379.00

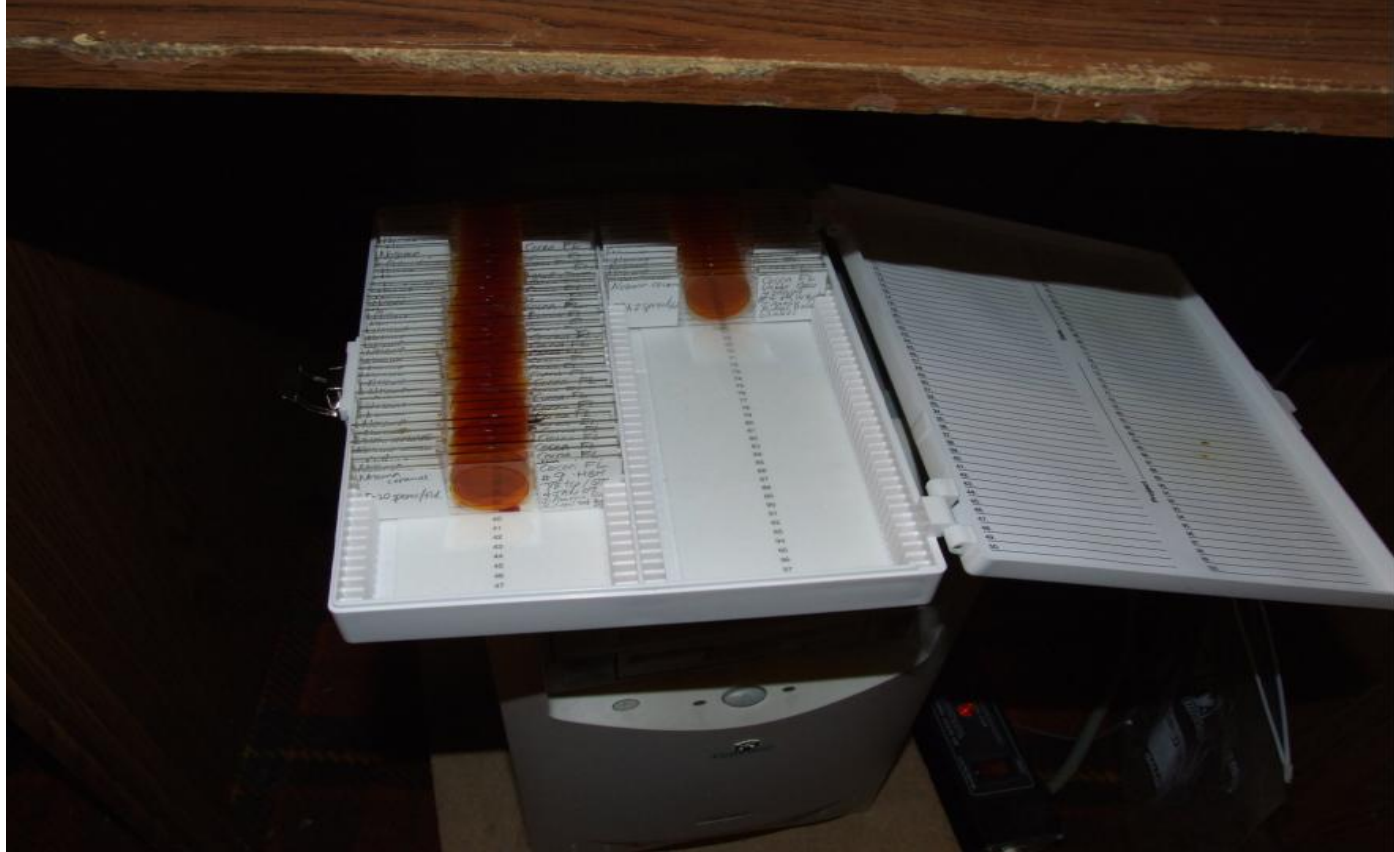
They have dissecting microscopes as well.



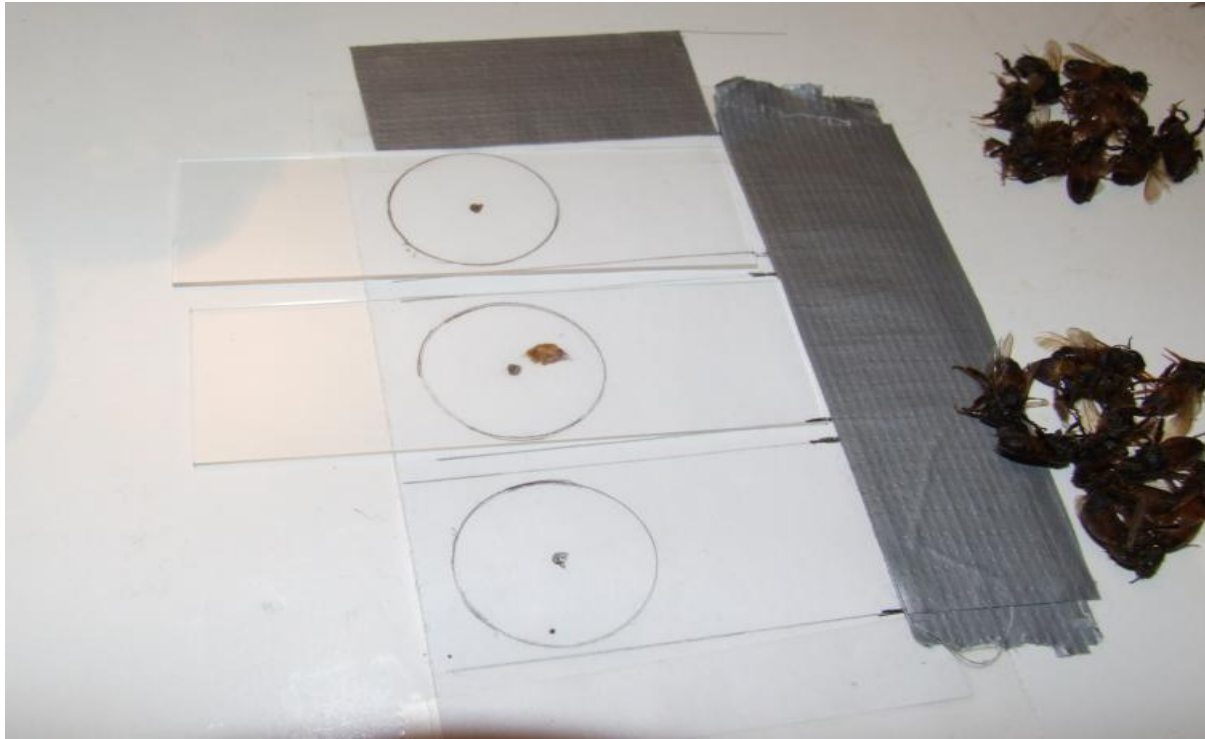
Mortar and Pestle for grinding 60 bee abdomens.



Counting bees by tens; 78 in this case; 18 will go into ethanol, the rest into the mortar and pestle.



Slide box with labeled slides; I often do two per hive, plus some extras to analyze pollen, etc. Amrine has old boxes for \$5 each.



'Jig' for making slides: duct tape for upper and right side boundaries; center point drawn for each slide and outline of 22 mm coverglass drawn around the center, so 1 drop of bee slurry can be evenly spread out. Dead bees from bags at right; killed in rubbing alcohol.



Use a forceps or tweezers to remove abdomens; place in mortar and pestle.



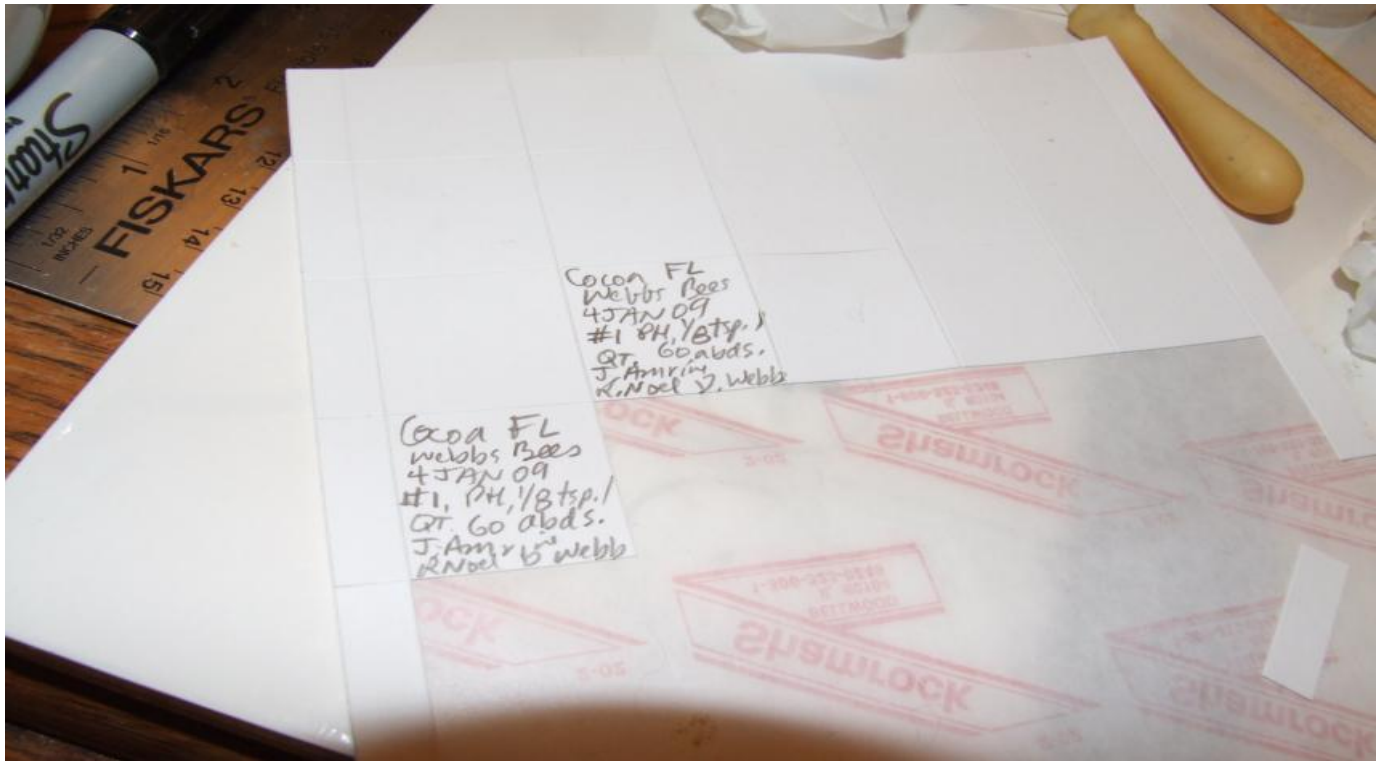
Mortar with 60 abdomens and 10 ml of water, ready for grinding.



Grinding the abdomens into a slurry; grind for about 5 minures or so.



Slurry ready to make slides.

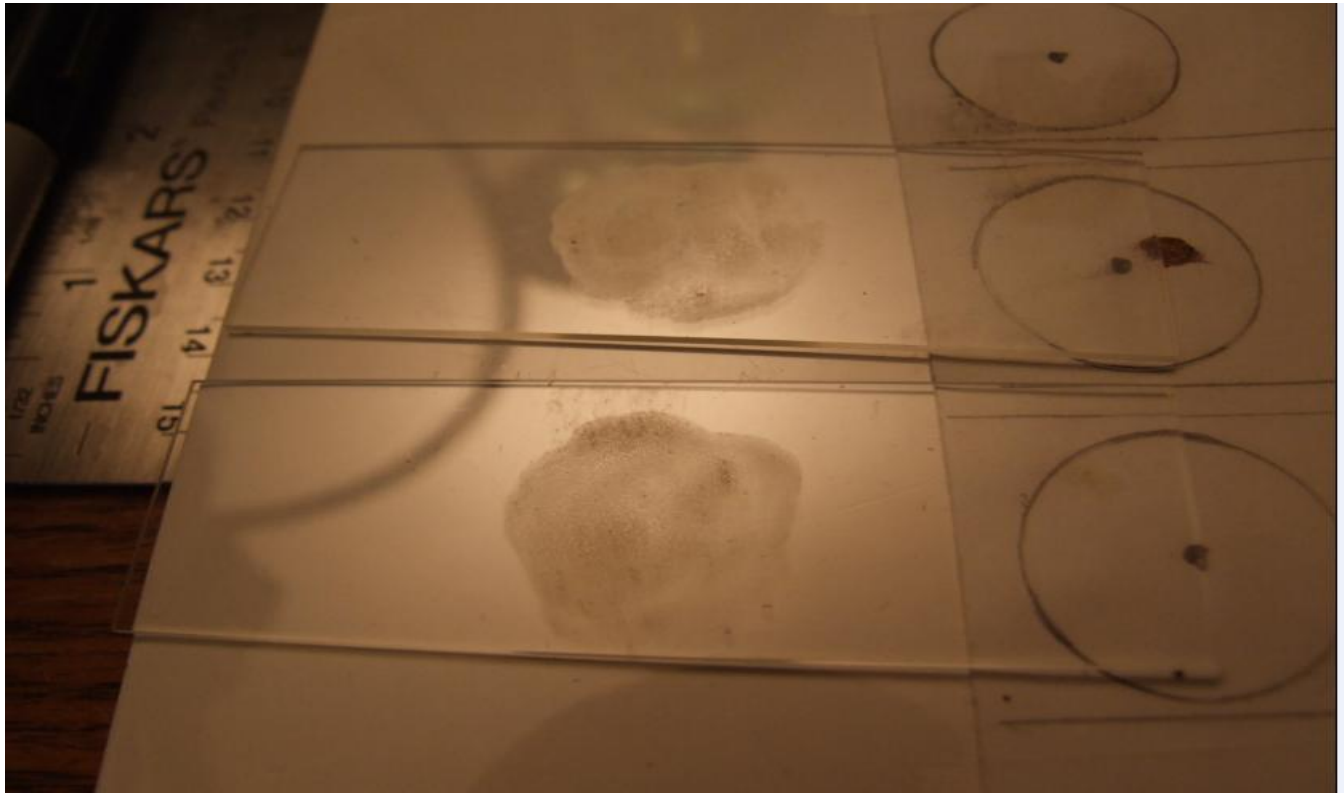


Writing labels with pencil for two slides. Office Depot has many kinds of adhesive labels.

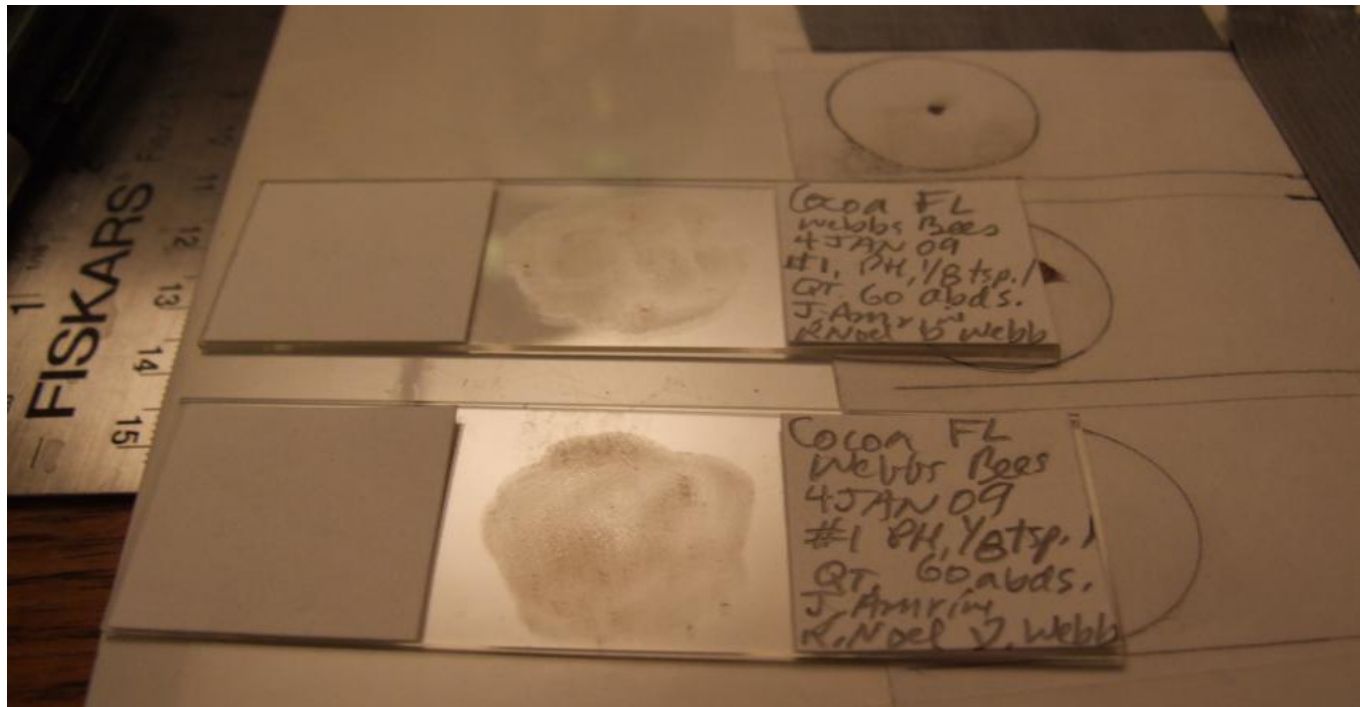
Cocon FL
Webbs Bees
4 JAN 09
#1 PH, 1/8 tsp. /
QT. 60 abds.
J. Amrin
R. Noel V. Webb

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Webbs Bees
4 JAN 09
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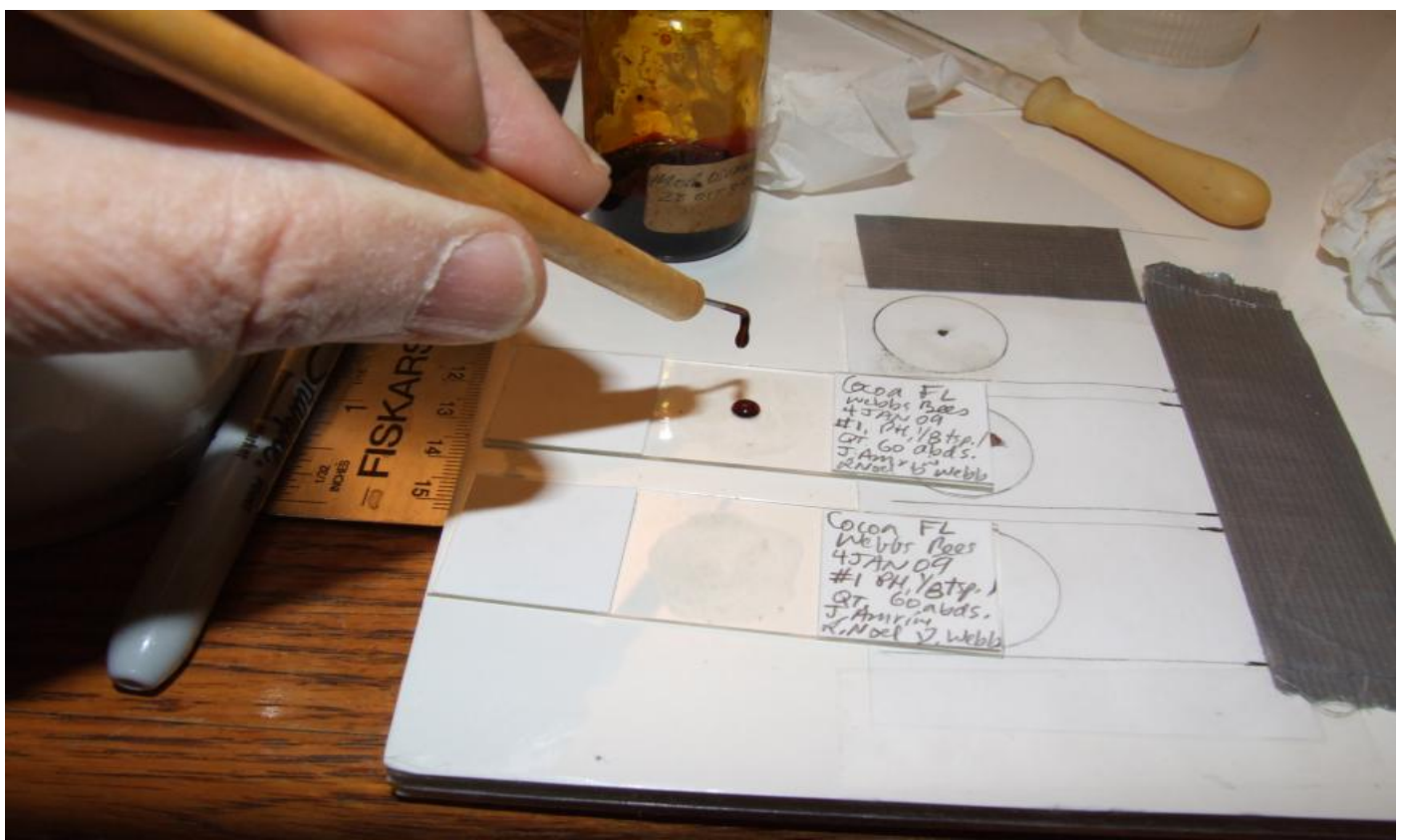
**Close up of labels:
Location, Date, Notes
about hive, name(s) of
collector (s)**



For accurate work, dry the slurry ca. 1 minute on the hot plate.



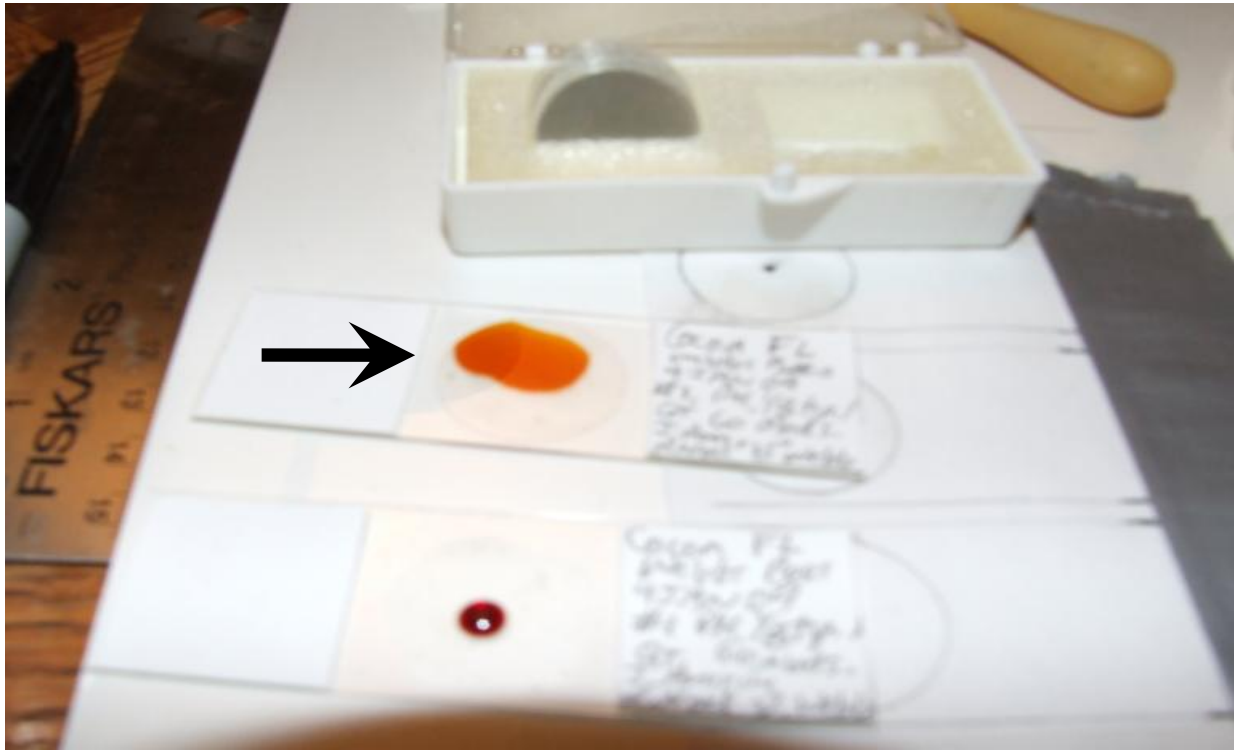
Labels added: locality label on right, identification label and notes on left.



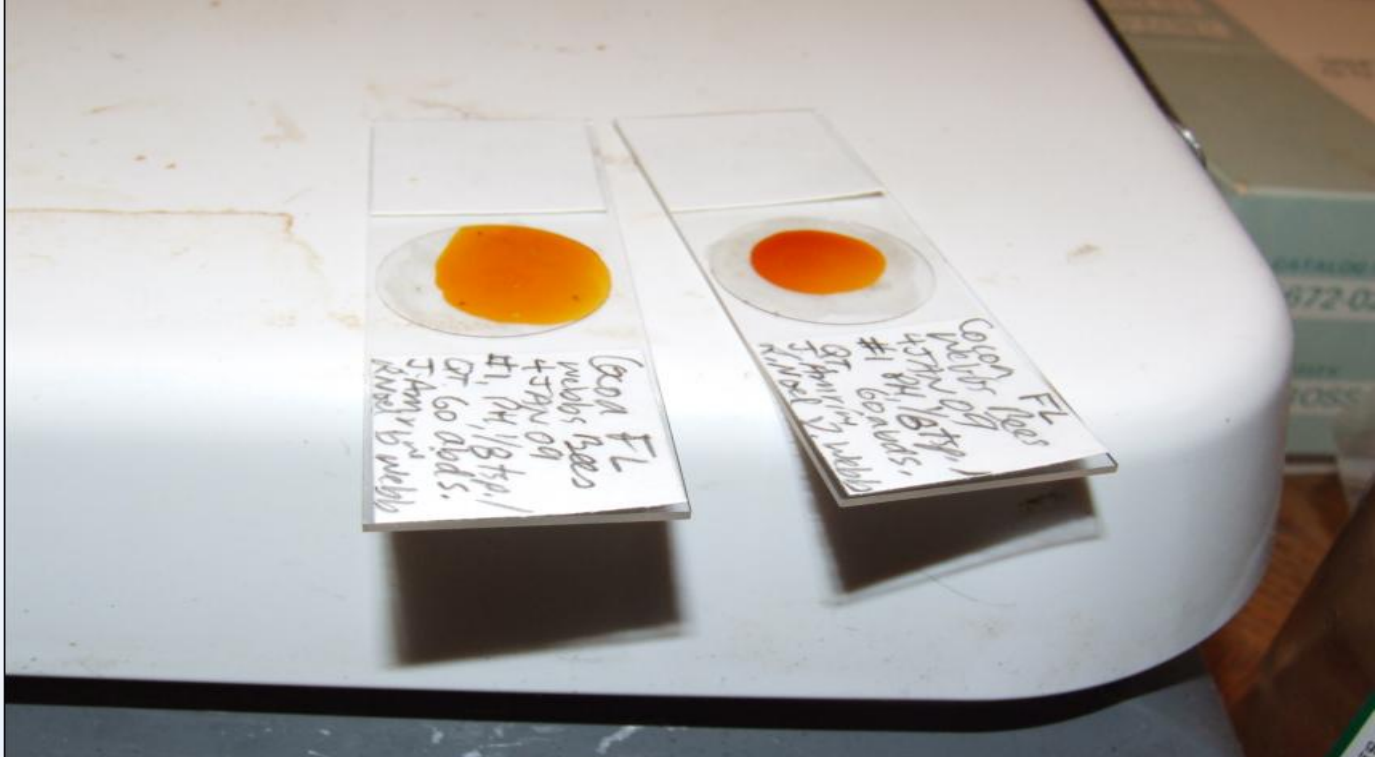
One drop of media is added to each slide, near the center; use honey in a pinch (temporary slide).



A clean 20mm coverglass is added, located between Left thumb and forefinger.



The coverglass settles over the drop, spreading the media evenly over the uniformly thin and dry slurry.



Slides are placed on the hot plate (80-90° C) for 5 minutes to spread over the slurry and to clear away fat & protein debris to make spores more easily visible. Slides can be heated on a light bulb, if no hot plate.



Examining the slide for spores. You can calibrate the scope by using a special calibration slide with an etching of 1 mm divided into 100 parts. Photograph the eyepiece reticule over the target etching for best results.

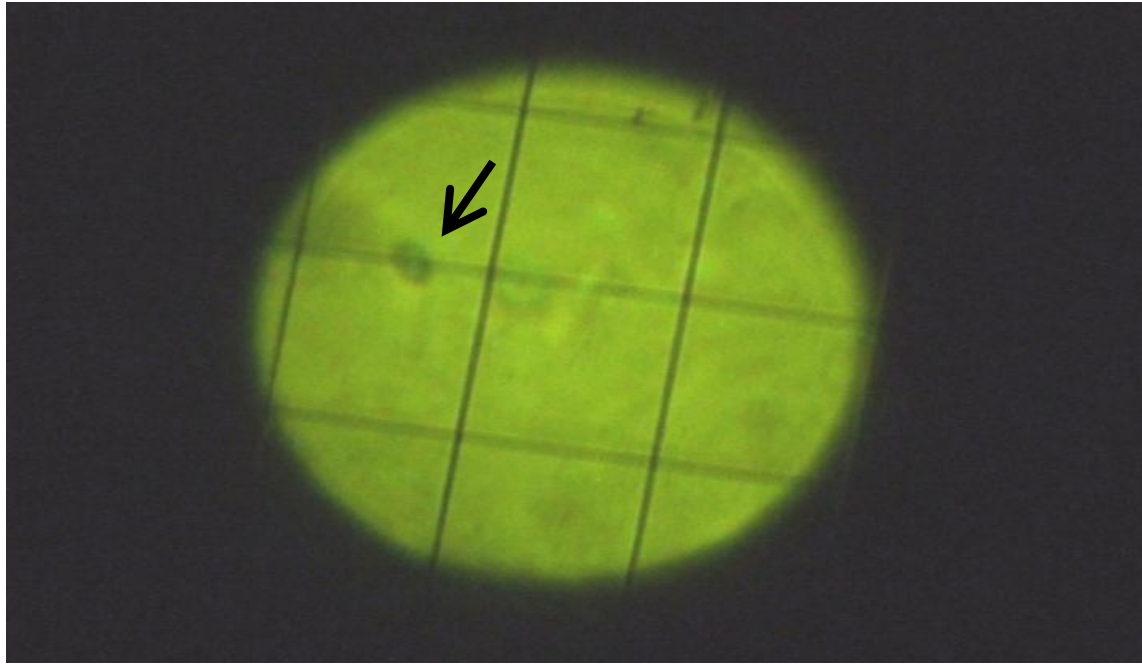


Photo thru eyepiece with 10 by 10 grid reticule (only a few squares are visible with this camera and microscope magnification). A digital camera, was used; one spore is visible at left (arrow). Use a tripod to hold the camera to get sharper images.

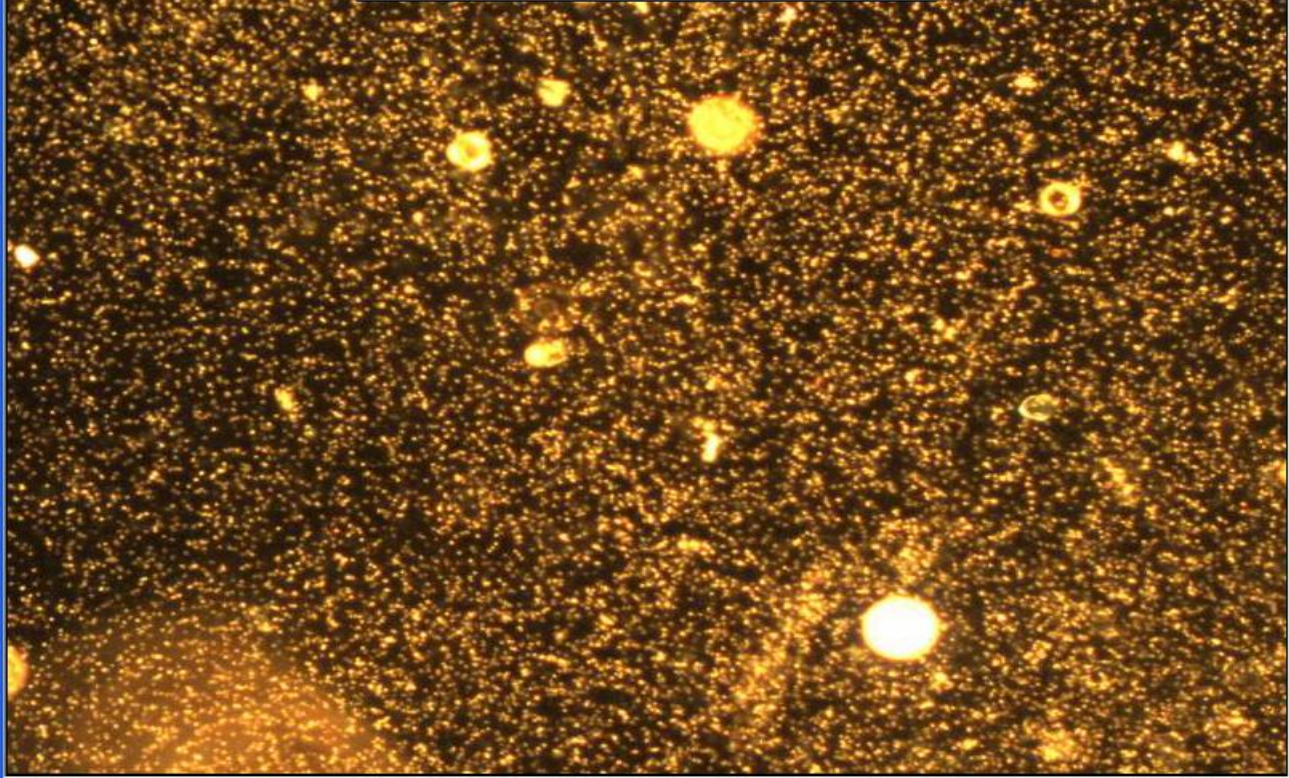


Photo with 10x objective lens and phase contrast (Q Camera, Olympus DH 40 trinocular microscope): these are spores from one bee taken from hive number 10; there are a few pollen grains and bubbles.

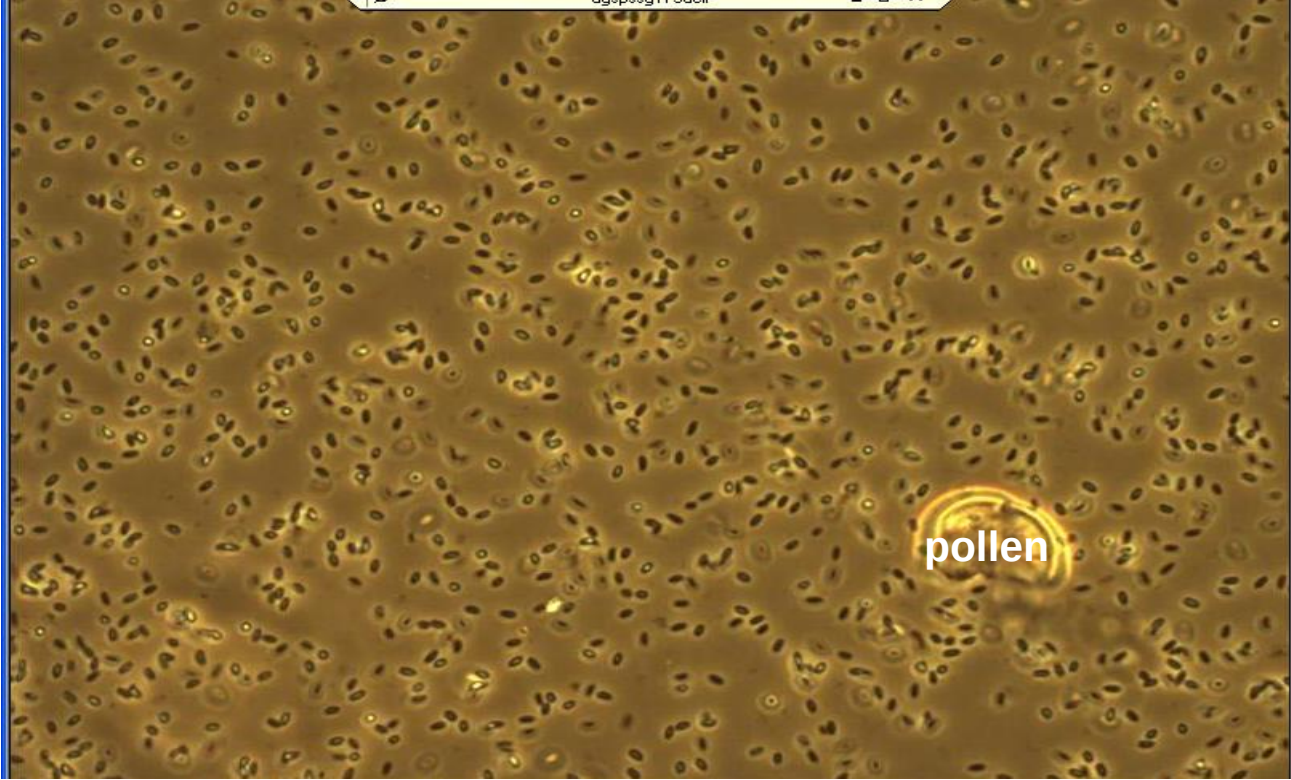


Photo with 40 x objective lens (hi-dry, 400X) and phase contrast, of spores from one bee taken from hive number 10. Pollen grain at right. Most simple, old monocular scopes can produce a similar image.

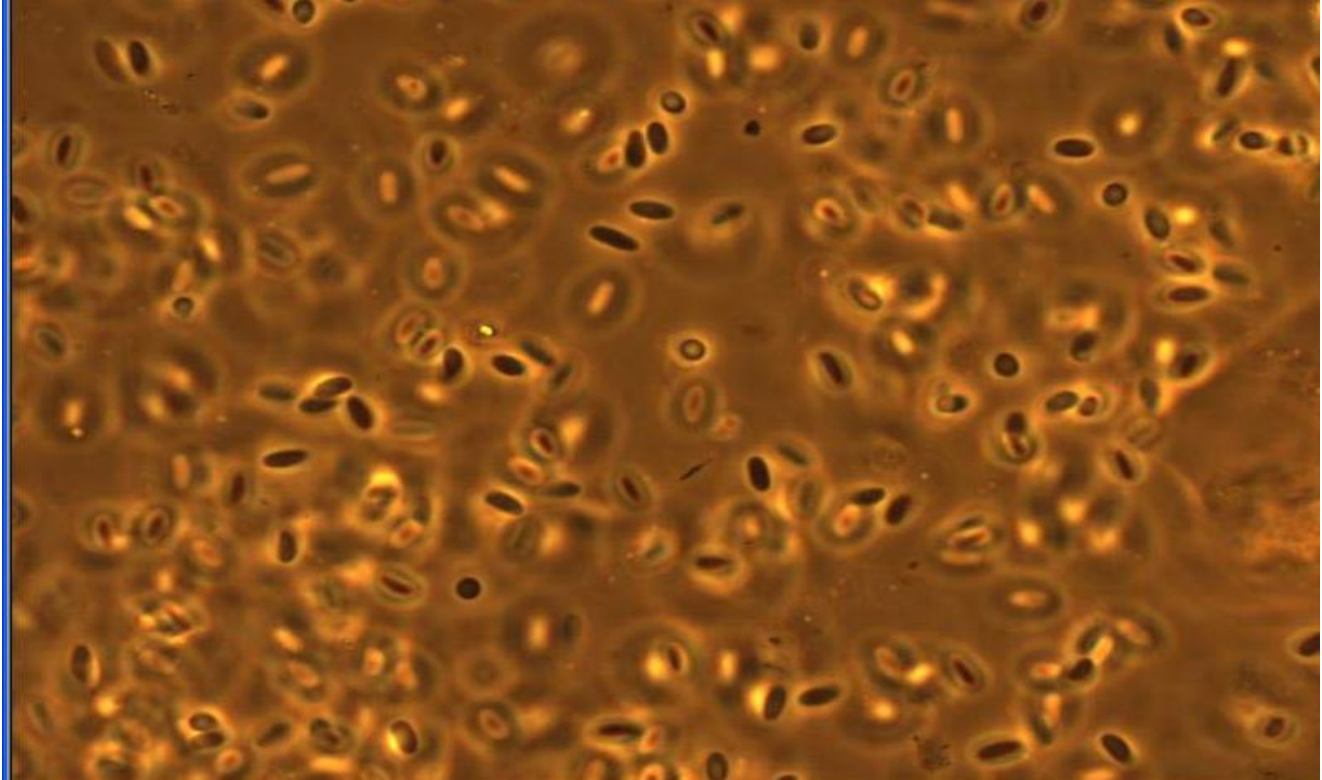


Photo with oil immersion lens (100x obj. = 1000X), using phase contrast: spores from one bee taken from hive number 10. Florida, 4 Jan 09.

Spore size: L 3.99 microns; W 2.38 microns. NS has one end tapered.

We always use microscopic assay to determine presence or absence of *Nosema ceranae*.

I checked many colonies in Morgantown WV in 2008-2009; did not find any *Nosema* spores. I have not seen *Nosema apis* since 1998. Honey B Healthy – 2 tsp in 1:1 sugar syrup eliminated the *Nosema apis*; they were found every year from 1978 until 1998.

Bob Noel found *Nosema ceranae* using his old microscope in Cumberland, MD, in August, 2008. He brought me slides for confirmation. We lost 4 out of 10 hives in his isolated bee yard. After 3 drenches and four weeks, the remaining 6 hives were free of spores [golden Italians], but we found a few buckfast bees [black], that were robbing the colonies, and these had many spores. The robbers were from an unknown source in the area; we expect that they died that winter.

No *Nosema* was seen in the hives in April, 2009.

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