Counting nematodes

Counting nematodes in water suspension

1. Mix the suspension well. For large volumes take about 15ml into a smaller cup.
2. Use a micro-pipetter with a plastic tip (Cut of the sharp tip to avoid nematode clogging.) to measure a 0.100ml (hundred micro-liter) sample. Be sure that the micro-pipetter has been calibrated.
3. Dispense the 0.100ml sample in a thin strip along the full length of a clean, but used microscope slide. The sample strip should not bead. Usually two strips can be placed on a slide, one at the top, one at the bottom. Be sure to mix any suspension well before taking a sample , then form a strip immediately.
4. Differentially count the living and dead nematodes by microscopically counting from on end of a strip to the other with a dissecting microscope. Strips should contain 30-100AU. If more than 100AU dilute the suspension appropriately. Best counts are obtained by not using the highest magnification.
5. Living nematodes have either a bend or curl, but might not move very quickly. Dead nematodes are straight like toothpicks, often with bubbles. A strip should be counted immediately before it dries out. Living infectives (active units, AU) have a distinctive double cuticle (Recognition of a double cuticle must be learned.) and silvery appearance. Adults, other stages, and dead infectives are all classified as "dead."
6. Five to ten (5-10) samples are usually counted and used to calculate an average, a t-statistic, and confidence intervals. The concentration of living infectives0.100ml and the suspension volume with any dilutions are used to calculate total active units (AU).

Counting nematodes in sprayable dispersible formulations

1. For 10 million packs mix into 10 liters cold water, ~20C. Wash out cup or bag.
2. Stirs well, then allow to settle 5 minutes.
3. Remix well then follow directions above. 0.1000ml sample should give about 100 AU per strip to count.
4. For larger sizes adjust volume appropriately.

Counting slow-release granular, solid formulations

1. Use an appropriate volume of cold water. Add contents of container to the water and wash out container.
2. Mix well then allow to sit 15 minutes.
3. Sieve off and save the large particles that float to the surface.
4. Remix and count the remaining suspension as above.
5. Weigh the sieved particles. Then select a sub-sample of about 10-20 particles and weigh them.
6. Mince individual particles of the sub-sample in drops of water Microscopically count AU's. Particles can contain rather large, >100, numbers of AU. Be sure to break up even the smallest particles.
7. Total the AU's from the entire sub-sample, then with the weight of all the sieved material use to calculate the total in the sieved material. Combine this total with the total for the suspension to obtain the total in the entire formulation.