For the Facebook Helminth Incubation Group, the Facebook Helminthic Therapy Support Group and the Yahoo Helminthic Therapy Forum

Human Hookworm Incubation

Contents

Introduction

Given the incredible improvement in my health that resulted from controlled dosing with the hookworm, Necator americanus, I had to find some way to maintain my hookworm colony. However, in view of the fact that they're not yet available from mainstream medicine, that two of the first four suppliers to offer hookworm commercially were closed down, and that another one was forced to relocate, I wasn't willing to risk my future health to chance, so I decided to become self-sufficient. I therefore had to find out how to safely and successfully culture infectious hookworm larvae. After reviewing the literature, and performing a number of experiments, I developed the protocol that I now use. This is set out below for those who are interested, but anyone reading what follows must be sure to first read the next section, 'Warning and Disclaimer', because there are safety and, in at least one country, legal implications to incubating hookworm.

Warning and Disclaimer

* There is considerable risk involved in incubating hookworms, due to the highly infectious nature of their larvae. After successfully culturing hookworm larvae, it would be very easy to accidentally inoculate oneself, or someone else, with many hundreds of these organisms, with potentially very serious consequences for the individual's health and perhaps also the future of helminthic therapy if news of such an accident were to reach the media.

* It may be illegal to breed hookworm in some jurisdictions, including the U.S., where they are currently classified by the Food and Drug Administration as biological agents (i.e. drugs), as defined in Section 351 of the Public Health Service Act and subject to an Import Alert.

* The information contained in this document is not advice, but for general information only. It has not been approved or evaluated by any governmental organisation concerned with the regulation of healthcare or drugs anywhere in the world. The accuracy, validity, effectiveness, completeness or usefulness of this information cannot be guaranteed.

* While the information presented here is related to the practice of the experimental treatment known as helminthic therapy, it is not intended to provide medical advice, diagnosis or treatment. The reader is hereby advised to always consult with a physician or other professional health-care provider regarding any health care problem or issue they might have.

* Anyone who chooses to make use of the information in this document does so at their own risk and no responsibility or liability whatsoever is accepted by the author for the use or misuse by others of any of the information contained in this document.

Purpose of incubation

My intention was to incubate the eggs produced by the colony of hookworms (N. americanus) that I already host in order to re-infect myself for the purpose of maintaining the beneficial effects that I had obtained from hosting these organisms.

My existing hookworms were purchased as larvae from a recognised provider of helminthic therapy, and I would never use hookworm larvae, or eggs, from any other source because I want to be certain that any organisms I introduce into myself are either harvested from my own body or from a donor who I can be confident is free from pathogenic organisms and, in the latter case, that the organisms supplied have been scrupulously cleaned.

The laboratory and safe practice

* I have created a designated work area where no food or drink is prepared or allowed.

* I always wear long sleeves, long pants, socks and shoes plus a lab apron while following this protocol.

* I always wear gloves of latex or a synthetic alternative, and avoid touching my skin (e.g., face) while working.

* I use a sharps container for broken sharp objects - usually an old pill bottle. (**The disposal of clinical waste**)

* I use a dedicated small, lined trash container with a lid, and empty this frequently.

* I disinfect all materials using two containers. First, I immerse the equipment into either undiluted 5-6% bleach or 2-3% ammonia. (1) Then, after about 5 minutes, I clean them in soapy water, before finally rinsing and drying them. (**Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008**)

* I disinfect all work surfaces when finished and, to be confident that nothing remains alive, I use either undiluted 5-6% bleach or undiluted 2-3% ammonia. (1) I only ever buy and keep available one of these chemicals at a time and I store whichever one I'm using in a 0.5 litre (16 fl oz) container and apply this to the relevant surfaces, as required, using a sponge. I then wait at least 2 minutes in order to be confident that the larvae are dead. Once the clean-up is done, I thoroughly rinse the sponges about 10 times afterwards, to prevent the residue bleach or ammonia from rapidly destroying them.

* I absolutely never mix or work with bleach and ammonia together, since to do so would produce explosive and toxic gasses.

* Whenever I use bleach or ammonia, I make sure that there is good cross-ventilation to prevent the build-up of fumes.

The medium

Hookworms normally incubate in a faeces/soil mixture, so these are what I use (2), covered by granulated cork (3). (The Harada-Mori technique [**Practical Guide to Diagnostic Parasitology**] didn't work at all for me.)

The ideal soil is sandy loam (which ensures good drainage and oxygen supply), with a clay content of less than 15%, and preferably around 10%. (Pure sand and clay are unsuitable. Sand loses its moisture too quickly, while clay retains surface water, and also, if clay dries out, larvae can be prevented from moving through it.)

It might also help if it has a lot of organic material in it, perhaps 20%. However, I believe that any good loam-based potting mixture should work for the purpose I have in mind.

The soil should be close to a neutral pH of 7 and must definitely not be highly acidic. Since most plants require a pH of between 6.0 and 7.0., (**link**) most bagged potting mixes will be close to neutral (7.0) or slightly more acidic. (**Link**) However, the pH of some commercially available potting soils may also be as low as between 4.0 and 5.5., (**link**) especially if they have been formulated to meet the requirements of specific plants, such as camellias and azaleas, which are acid lovers. (**Link**)

I checked the pH of the soil I bought using a pH meter. It had a pH of about 6.3, so it required the addition of powdered lime, as it turned out, in the proportion of 1 part lime to 1 part soil, to produce a more neutral pH of around 6.9. (4)

Adding stool to the soil obviously renders it non-sterile, but in order to guarantee that everything that was alive in the soil and lime when I first brought them home was now dead, I baked them, separately, in an oven, for at least 30 minutes at a temperature of at least 82˚C (180˚F), after first having made sure that the soil was quite moist, but leaving the lime dry. (5) The resulting mixed soil/lime combination was less wet, which made it lighter when stored, less likely to smell, and less likely to have things eventually grow in it (such as mushrooms). Of course, it wasn't sterile once it cooled down, since it was exposed to the air, etc.

I used a large turkey baking container to hold the soil (6), covered over on top with aluminium foil, and a meat thermometer to measure its temperature during cooking, inserting the thermometer probe in several different places, and rotating the container to ensure even heating.

After each series of temperature checks that I made before the temperature reached 82˚C (180˚F), I cleaned the thermometer to remove any of the as yet non-sterilised soil. (7)

Since I wanted an even greater safety margin, I left the soil in the oven after 30 minutes baking and let it cool down slowly over several hours. Unlike food, I don't suppose it is possible to over-bake soil, as long as it doesn't dry out.

After baking the lime in the same fashion, I also baked the covered granulated cork for over 30 minutes (although this might not have been necessary, since it was likely to be far cleaner than the soil) in the same container, after cleaning this in order to keep residue soil and lime from contaminating the cork. I didn't moisten the cork, but left it completely dry. Once the final soil and lime mixture had cooled, I stored it in 20 litre (5 gallon) garden buckets

The mixture

Taking a plastic container (e.g., a plastic food container, tomato plant starter, or similar, approximately pint-sized [0.5 litre /16 fl oz], with holes in the base for drainage), I spoon some soil from one of my buckets into the bottom of the container to a depth of about 1.25 cm (½ inch), then, using a hand sprayer filled with dechlorinated water (8), I wet the soil sufficiently for the water to drip through after a few minutes. I then set the container on a suitable dish to catch the overflow water.

I deposit one bowel movement's worth of stool (9) into a different container, then transfer this stool to the container that holds the soil. (10) I then add another 1.25 cm (½ inch) layer of soil to cover this. (11) I also make the top of this second layer of soil smooth in order to make it easier to scrape off the cork later. I then wet this second layer of soil liberally until water drips from the base of the container.

Next, I put a sheet of latex-free (I don't know if this matters) gauze, cut to fit the container, on top of the soil. (When doing this, it helps to make a paper template that fits the container's mouth, and then to cut the gauze to fit the template.) This gauze separates the soil and cork so that, when the cork is later spooned off, it won't have soil contamination in it. I also use a paper towel to wipe the upper inside exposed sides of the container to remove any soil clinging to them.

Finally, I add a 1.25 cm (½ inch) layer of wet granulated cork on the top. (As cork tends to be rather hydrophobic, I pre-mix a sufficient quantity with dechlorinated water in a separate container ahead of preparing the culture sample.) I also use a different spoon to ladle it out of its bucket and mix it, since I don't want to contaminate either the cork in the bucket or the newly wetted cork with soil.

Depending on how many larvae I intend to harvest, I might repeat this procedure, filling additional containers when I have subsequent bowel movements over the next few days.

In order to keep track of when a culture will be ready for harvesting, I write its starting and harvesting dates on my calendar. I also write both dates on an index card, which I place in or on the dish in which the culture container sits. The card may get soaked, but this is of no consequence.

The vigil

For the next 8 days I keep the soil shaded and constantly moist, ensuring that the shallow pool of water that the container is sitting in never dries up. How much watering this requires depends on the ambient heat and humidity. In my situation, I have found that spraying the soil three times a day, for a total of around 40 sprays (15 - 10 - 15), is generally sufficient. Since the water that pools in the container contains filtered essence of faeces, it becomes

brown over time and, if the conditions are right, it can start to smell. If this happens, I empty the pooled liquid daily and replace it with fresh dechlorinated water. This is in addition to the daily sprayings.

Developing hookworm larvae are reportedly unable to survive temperatures below 10˚C (50˚F) so the temperature should preferably be kept over 18˚C (64˚F), and definitely below 35˚C (95˚F). Although the ideal temperature is reported to be between 27˚C (80˚F) and 32˚C (90˚F), I have had excellent results with temperatures in the range of 21˚C (70˚F) to 27˚C (80˚F). I have, however, completely failed to get any larvae from cultures when the temperature has approached 35˚C (95˚F), even temporarily.

> "Hookworm larvae tend to prefer shaded areas, perhaps because light is a stimulus, which may increase larval activity, thus increasing lipid depletion. This may account for the decreased longevity and reduced desiccation tolerance in the presence of light. The incubation temperature of Necator americanus eggs affected the longevity and desiccation tolerance of resultant infective larvae. Larvae hatched at 30˚C (86˚F) and maintained at 26˚C (79˚F) under bright fluorescent light had a 50% survival time S50 of 4 days In the dark or shade, the S50 for larvae raised at 30˚C (86˚F) was 5 weeks, while that of larvae hatched at 20˚C (68˚F) was 7 weeks (Udonsi & Atata, 1987). Recent reports on the increase of ultraviolet radiation due to decrease in the earth's protective ozone layer indicate the need for further investigation of its role in the development, viability and infectivity of parasitic nematode eggs." (Page 30, **Integrated Guide to Sanitary Parasitology**)

If I lived in a cooler climate, which necessitated warming the containers, I might try using a low wattage light bulb or an aquarium **reptile heat rock**. If placed inside an appropriate container, either of these might provide a suitable temperature increase of, say, 3˚C (5˚F) to 6˚C (10˚F). The temperature could be controlled using a temperature switch, e.g. the **Red 12V Heat Cool Thermostat Temperature High Low Alarm Control Switch -55-120C**. If connected to a 5.0V DC power supply (e.g., an old cell phone charge adapter), this might provide a good low-cost DIY incubator.

Hookworm eggs hatch in about 1-2 days and, by days 5 to 7, the 2nd stage larvae have become 3rd stage larvae and will migrate upwards to the top of the soil.

Hookworm larvae cannot swim and, if put into open water, will sink to the bottom. However they can climb against gravity by using surface tension. So, when the 3rd stage larvae develop, they use this modus to crawl up into the grains of cork on top of the soil as they search for the surface.

By day 8, the larvae should be ready to harvest.

The harvest

In their natural environment, hookworm larvae crawl up blades of grass with the morning dew - hence the name 'dew itch', (**link**, **link**) so, about 2 hours before I remove the cork to harvest the larvae, I spray the culture thoroughly to make sure they are able to get as high as possible in the cork, although I don't know how much difference, if any, this makes, or what the ideal timing would be.

For the previous 3 days, and from this point onwards, I'm particularly careful to always wear barrier gloves, a long sleeved shirt and pants, shoes and socks, plus a lab apron when directly handling any of this material.

I half fill a silicone snow cone cup with dechlorinated water. Then I use a spoon to scoop off some of the cork from the top of the culture and place this into the water in the cone, thoroughly stirring the cork into the water. Once there is sufficient cork in the mixture, I add some more dechlorinated water to bring the surface of the mixture to about 0.6 cm (¼ inch) below the rim of the cone. Finally, I set the cone aside and wait approximately 12 hours for the larvae to settle to the bottom. The cork floats immediately to the surface.

For the next step, I use a **minipipette** (MP) fitted with a tip that has been cut off at the first division from the narrow end in order to widen the hole sufficiently to prevent it becoming blocked with debris that has settled to the bottom with the larvae. (12) I depress the MP's plunger before putting it into the water (doing this with the pipette under the water will produce bubbles that might disturb the sediment and distribute the larvae). Next, holding the plunger down, I insert the MP into the water and down to the bottom of the cone. Then I release the plunger and slowly raise it, with its tip full of liquid, out of the water, momentarily tapping the MP on the edge of the cone to dislodge any drops of water that may be clinging to its exterior, before transferring the drawn up liquid to the slide. (13)

(Sometimes, it is also necessary to scrape back into the cone any bits of cork that have clung to the MP or to my glove, although I could avoid the need to do this by using a teaspoon to ladle off the cork before this part of the procedure.)

I then depress the MP's plunger to deposit the liquid from the tip onto a microscope slide. If everything has gone according to plan, there might now be a hundred or so hookworm larvae in that single drop of liquid. (14)

After putting the slide under the microscope, turning on the light, setting the total magnification at 40X, and adjusting the focus, I expect to see either motile larvae or resting ones that look like little cuticles or sticks. The latter usually become active after a few minutes or so, once I turn up the intensity of my microscope's halogen lamp.

If I don't find any larvae, I repeat the procedure with the same cone a number of times, and then repeat it with any additional cones that I may have prepared. Since the larvae can be very unevenly distributed in the cork it is possible for one cone to contain many larvae and another to contain few or none.

Storage and clean-up

If I want to store any larvae, I next use a Pasteur pipette to draw up liquid from the bottom of the cone and expel it into a Glad container. I repeat this, checking occasionally as I'm going along to see if I'm still getting larvae, until I'm no longer doing so, or until the container is about 75% full. I've punched about 8 small holes in the Glad container's top, with one end of pointed scissors, to let air in, although I don't know if this is necessary, nor how long the larvae could survive in a fully closed case. (15)

I store the filled Glad containers in an unplugged mini fridge (e.g., the **Caldura 17 litre Compact Mini Fridge**) on the assumption that, if my home were to burn down, my precious stock of larvae would have a better chance of survival if protected by the fridge's insulation. I also keep my important papers, including inoculation records, in the fridge. Since I typically open this fridge about once each day to retrieve some paper or other, this arrangement ensures that the air in the fridge circulates periodically, although the larvae must use very little air.

Stored larvae need to be protected from direct sunlight, and checked from time to time to ensure they don't dry out through evaporation. If necessary, I add a little distilled water rather than using more regular dechlorinated water which would gradually increase the concentration of minerals in the remaining water. I don't know what concentration of solution the larvae can tolerate.

Although it is clearly best to use the larvae while they are still young and vigorous, they may, if stored in this way, at around 21˚C (70˚F), live as long as 15 weeks, whereas, at 35˚C (95˚F), they will be more active and, as they do not feed, will die of exhaustion in less than 3 weeks. This is inconsistent with the the findings of Udonsi & Atata, 1987 (**Necator americanus: temperature, pH, light, and larval development, longevity, and desiccation tolerance**), a fact that I currently can't explain.

Since the material left over from this protocol contains infectious organisms, I don't simply throw any of it out with my domestic waste. First I freeze leftover stool, soil and cork for a couple of days, then let it thaw out and, finally, with gloves on, mix it thoroughly (break it apart) and add it into either undiluted 5-6% bleach or undiluted 2-3% ammonia. I make sure to add enough bleach or ammonia to keep the resulting solution at least 50% of its original concentration. I then wait at least 1 hour before draining off the excess bleach or ammonia using a dedicated strainer, and triple bagging the remaining solid materials in plastic bags, before putting them outside the house in a refuse sack along with the rest of my regular rubbish. As an alternative, I will sometimes simply boil all the materials for 10 minutes and then triple bag them after they have cooled down.

To ensure that I have clean slides the next time I need them, I thoroughly wash the ones that I have used with a clean cloth or sponge, film-free soap, and water, then rinse them several times. (16) Finally, I pick up the slides by their edges, replace them in my slide case and leave them to dry.

Counting the larvae

Since a single drop of water can contain hundreds of larvae, it is essential for me to use a microscope to count out the required number. The dose for an adult could be anywhere from 1 larva to as many as 50 larvae, with an average first dose for an adult probably being around 35.

I use a MP, with tip intact, to remove a drop of liquid from the bottom of the Glad container, then place this on a slide.

10 larvae per drop is about right for easy counting. If there are too many larvae in each drop of the sample to be easily counted, I might expel very small drops onto a slide and add dechlorinated water to each of these, or I might divide a drop on a microscope slide with the MP tip, and then add water to these daughter droplets. The larvae do tend to congregate together, but this isn't a problem.

At this point I wake the larvae with the microscope light to be sure they are still alive and will be awake when they are put onto my skin. There is the question of whether or not to count the larvae that don't wake up and move on

the slide (they look like sticks). I don't count them, and it works out about right, since when I count the bumps on my arm this closely corresponds to the number of active larvae I counted out.

To transfer the larvae from the slide to a contact lens case, in readiness for the inoculation, I MP up the drop and expel it into the clean case. Then I MP up fresh water from a shot glass and expel this onto the same spot on the slide, gently stir the drop with the tip of the MP, and then MP this up to transfer it to the case, sometimes repeating this several times. Doing this doesn't seem to leave too many larvae behind. I keep track of the total number of larvae as I add further drops to the case, which will eventually contain, say, 20 larvae.

Inoculation

The fabric pad of the dressing will need to be in firm, direct contact with a patch of clean, hairless skin (to prevent ripping hairs out when I eventually pull the dressing off) in an area that is convex and not subject to much movement, so I use the inside of my upper arm.

In order to sterilise the area, I wipe some alcohol on it and wait briefly until this has completely evaporated, which usually takes about 45 seconds. (I'm careful not to clean or disinfect the area with anything that might leave a residue.) In the meantime, I lay out one large adhesive dressing so that its fabric pad is exposed but leaving the protective plastic backing still attached to the adhesive and folded back against the table. I only remove this backing as I am applying the dressing to my skin.

I use a Pasteur pipette to suck up the fluid from the contact lens case and transfer it to the dressing. This might require several transfers and, to ensure that any larvae left in the case do not dry up during this operation, I immediately deposit more fresh dechlorinated water into the case if it becomes empty.

In order to ensure that I have collected all the larvae, I squirt a little fresh water into the case then draw it back up and transfer it to the dressing, repeating this flushing action a number of times in different locations within the case.

If the dressing becomes saturated before I have completed this process and the water begins to bead up on top of the dressing, I put the case aside for a moment, after adding a little more fresh water to it, then draw up the excess water from the dressing and put it back into the contact lens case. I then attach the first dressing to my skin, stretching it slightly to ensure firm contact across the entire dressing.

If I haven't been able to transfer all the liquid from the case, I quickly prepare a second dressing, and repeat the same process with this one. Once the second dressing is prepared, I apply this next to the first one on my arm. Then I leave the dressing(s) in place for at least a couple of hours.

When I feel an itch, I then swallow the maximum permitted dose of diphenhydramine. (17) This does not harm the larvae, but does make me feel drowsy, so I always inoculate myself at a time when I know I will be able to lie down for a number of hours, if necessary. In order to give the larvae plenty of time to pass through my skin, I obviously don't use any Benadryl cream at this point, or apply a hair dryer (see below). However, I may vigorously slap the dressing to give me temporary relief if the itch becomes unpleasant before the Benadryl takes effect. I doubt that this will harm the larvae and it may in fact wake up any that are slow to action.

(N.B. Gradually, over the last six or seven years, my itching from the larvae has ***greatly*** reduced, and therefore currently I don't need to use the liquid Benadryl anymore, although I still do need to use the creams and hair dryer later on.)

After a few hours, I remove the dressing and apply a combination of extra-strength (2%) diphenhydramine hydrochloride cream (18) with a 1% hydrocortisone cream, and an electric hair dryer. I find that applying heat to the site of entry - up to the point of momentary pain - effectively kills any remaining itch for a number of hours. (**A Miraculous Cure for Bug Bite Itching**) (19)

For the first 5 days after inoculating, I avoid clearing my throat and spitting out any phlegm that might be produced, so as not to spit out any larvae that might happen to be migrating from my wind pipe to my throat at this time. I actually find this rather difficult to remember, especially when running in the gym, so I wear a wrist band during this time period to remind me.

Maintaining my colony

After my initial dose of hookworms, I followed this up, 6 months later, with another similar dose. Since then, I have given myself approximately 20 larvae every 6 months to keep my colony young. (20) For convenience sake, I have

considered doubling the dose to 40, and then dosing only once a year. The problem with this is that with a higher dose I would be far more likely to have side effects, such as cramping and diarrhea.

While only dosing every six months, I prepare a fresh culture monthly in order to always have fresh larvae available, in case I ever lose my resident colony. I also keep the last two months production before disposing of them. (21) If I were dosing yearly, and also only doing a yearly culture, this would obviate a lot of work, but would also obviously mean that I wouldn't have any back-up in case I somehow lost my colony.

Everyone is different in how they respond to hookworms and research has suggested that, in some people, the presence of older worms may make colonisation by younger ones more difficult, so it would be impossible to know how many worms I'm carrying unless I had a pill cam examination. But, at a guess, and assuming that hookworms live on average for 5 years and have a 50% survival rate, my present schedule will maintain a colony of about 100 hookworms that won't get old and die at the same time.

The estimates for the number of hookworms that begin to create a risk of anemia for someone with normal iron in their diet and normal iron absorption vary widely. One opinion is that a person would need to have somewhere around 2,500 to 5,000 worms before anemia would become a risk, giving someone a safety multiple of approximately 25 to 50 times a normal colony's size. A contrasting position is that around 300 to 400 hookworms could create a risk of anemia. So although a person with a normal sized colony wouldn't be expected to develop anemia under either scenario, given the current uncertainty, anyone with hookworms probably would be prudent to monitor their iron levels. So long as iron levels are checked periodically and an iron supplement taken as and when required, there is no cause for concern about developing anemia - or any other nutritional deficiency - from hosting therapeutic numbers of hookworms. (**Re: HT Safety & What Constitutes Light Infection** (11 Aug 2010), **Re: HT Safety & What Constitutes Light Infection** (13 Aug 2010), **Re: The Straight Dope's Article on Helminthic Therapy** (18 Apr 2011), **Re: The Straight Dope's Article on Helminthic Therapy** (19 Apr 2011), **Re: The Straight Dope's Article on Helminthic Therapy** (21 Apr 2011), **Do helminths cause nutritional deficiencies?** (28 Oct 2013), Hookworm Dosing and Response Document (Jun 2015, **link**/**link**).

I don't want to risk allowing my entire colony to become geriatric. Moreover, dwindling numbers could result in only one sex being left, in which case I wouldn't be able to reinfect myself as no eggs would be produced. For example, if I had only 4 worms, the probability of getting all males would be ½ x ½ x ½ x ½ = 1/16. I would also have a 1/16 probability of getting 4 females. So the combined odds of getting all of only one gender is 2/16 = 1/8. (If I had 10 worms the odds would be about 1/500.)

Another problem with having small numbers is that my colony could suffer reduced genetic diversity. With fewer breeding pairs, my population might be in a genetic bottleneck and become very inbred.

Promotion of egg viability by dietary manipulation

Having noticed that the production of larvae from my stool is variable, and having controlled for all other possibilities, I concluded that what I eat must be influencing the viability of the eggs produced by my hookworms. I therefore drew up a list of foods, herbs and spices that are claimed to be anti-parasitic and began testing these individually.

The first four items that I selected from the list below each completely eliminated larva production. These were: regular (organic) English walnuts (not the black ones); carrots (the roots as well as the carrot tops); garlic and turmeric. In view of this, I have decided that, until I am able to establish the safety of every other item on this list, I will totally avoid all of them for a week or two before beginning a new culture. This practice will have the added benefit of giving my existing worms a break from any anthelminthic effects from my regular diet.

I also try to limit any anthelminthic foods for about one month after each inoculation to give the new larvae a chance to settle in, although I have no idea if doing this makes any difference.

Having noticed that two of the items listed below - the English walnuts and carrots - are only related, and not identical to, items on the list, I'm also avoiding anything else that's related to the other items on this list.

Another individual who incubates hookworms has reported that a 9 day course of amoxicillin stopped egg production for at least 16 weeks, but that eggs were once more being produced at 20 weeks.

A worm host who regularly checks the egg output of his worms has reported that they produced zero eggs for a time after twice drinking coconut milk. And yet another individual has found less eggs when he consumes 2 large dessert spoons of coconut oil daily, but normal amounts of eggs when he only eats 2 smaller dessert spoons of the oil each day. This confirms the ability of coconut to affect helminths in some people, as well as demonstrating the dose-dependent nature of this effect.

The issue here is the potential effect of these items on larva production, not their possible adverse effect on the health benefits produced by the hookworms. This latter topic is addressed separately in the Human Helminth Care Manual which can be downloaded from the Files section of either the **Facebook Helminthic Therapy Support Group** or the **Yahoo Helminthic Therapy Forum**.

Reportedly anthelminthic foods, herbs and spices

The items below were taken from various lists of anti-parasitic substances, so are all potentially capable of reducing larvae production. However, this is not a complete list of anthelminthic foods, herbs and spices. It is just what I'm working with at the moment.

Almonds, aloe vera gel, anise, anti-oxidants, apple cider vinegar, barberry, bitter melon, black currant, black pepper, black pepper fruit extract, blackberries, bromelain, calendula, camphor (Zhang Nao), cardamom, caraway seeds, carrot (tops and root), catnip, cayenne pepper, chinaberry bark (Cotrex Meliae, also known as ku lian pi), **China tree (Melia azedarach)**, chaparro/Armargosa, chilis, cinnamon, cloves, Cnidium Monnieri / She Chuang Zi, coconut, colloidal silver, CoQ10, cranberries (& juice), **cruciferous vegetables** (e.g., broccoli, Brussels sprouts, kale, etc.), cumin, elecampane, fennel, fermented foods, feverfew, fibre (perhaps a large quantity may 'scour' the gut, dislodging worms?), figs, fleabane, garlic, gentian, ginger (Zingiber officinale), ginseng, golden seal, grapefruit seed extract (typically contains synthetic antimicrobials), honey, hops, horehound, horseradish, lauric acid, male fern leaf, ming fan (a chinese herb, also known as Bai fan, Alumen), nutmeg, Neem, olive (olive leaf, the bitter juice of unripe olives), onion, oregano, Oregon grape, papaya/pawpaw, peppers, pineapple, pipsissewa (princes' pine), pomegranate, propolin, pumpkin seeds and husk, quisqualis fruit, rhubarb, rice (brown, uncooked), rosemary, sage, sauerkraut (and cabbage sauerkraut), sunflower seeds, sulfur flower/liu huang, sweet potatoes, tea (mugwort/ Artemisia vulgaris), thyme, tomatoes, Torreya/Taxaceae, tulip bulb/Shan ci gu, turmeric, vervain (blue), walnuts (regular, and black walnuts), wormseed (Chenoppdium amrosioidies) and wormwood (Artemesia absinthum).

This second list includes items that are suggested less frequently, and sometimes only on one site. I'm therefore not sure how much faith to put in any claims regarding their anthelminthic effect. It is quite possible for innocuous things to make it onto a list for no more reason than the author's speculative whimsy.

Activated charcoal, agrimony (tea), agrimophol, Ajoene, alfalfa, allicin, Allium sativum, aloe juice, Alumen/Ming Fan, bay leaf, beans, beet root, bentonite clay, berberine, berries with seeds in them, beta carotene, Bing Lang (Semen Arecae Catechu), "Betel Nut", bitter orange, black cumin, Boldo leaf tea, Brewer's yeast, butternut, caprylic acid, caryacrol, caryophyllene, Cascara Sagrada Bark, castor oil, Chaun Lian Zi (Fructus Meliae Toosendan), China tree root bark, ching hoa (Artemesia anua), citrus pectin & seed extract, cucumber, curcurbitin, dandelion, diatomaceous earth, Dryopteris root and rhizome or Woodwardia or Osmunda or Matteuccia, echinacea, endive, eugenol, Fei Zi Torreya seed, Ficin, ficus, fish (salted), flaxseeds (& flax oil), green vegetable juices (fresh), Guan Zhong (Rhizoma Guanzhong), guava (raw), Horsetail, Hyssop, isothujone, Jalapeno Pepper, juglandin, kelp, Kombucha, Ku Lian Gen Pi (Cortex Meliae Radicis), lemon juice, liver herbs such as Dandelion, Bupleurum, Schizandra, Gentian and milk, Lu Hui aka Herba Aloes, Lupine seed, magnesium oxide, Matcha, mellissa, mint, mugwort, Nan Gua Zi, Omphalia lapidescens (Omphalia fruit, Lei Wan, Thunder Ball fungus), Omphalia sclerotium (Polyporus mylittae, Blackfellow's Bread), papain, Papan, Pau D Arco, peppermint, pickles, plantain, Propolis, pseudopelletierine, psyllium husks, purslane seeds, Pyrantel pamoate, Qinghao (aka Artemesia annua aka Wormwood), Quassia Wood Chips (Picaraena exselsa v. sp.), Quisqualis, raddichio, radishes, raspberry leaves, raw cabbage, Rejuvelac (a drink made from fermented grains such as wheat berries), rhubarb (& juice), rocket, rue, salsa, sarsaparilla, seaweed, seed/nut yogurts, senna, sesquiterpene lactones, Sheep Sorrel Herb (Rumex acetosella), Shi Jin Zi (Fructus Quisqualis Indicae aka Rangoon Creeper Fruit, "envoy gentleman seeds"), Shield-fern "Link the Multitude", Sichuan Pagoda Tree fruit, slippery elm husks, Southern Melon Seeds (Semen Cucurbitae Moschatae), squash, tannin, Tansy, teas (such as Ginger, Echinacea, Elecampagne, Peppermint, Chamomile), Thistle, Thuja, thujone, thymol, vegekraut, Vermouth, watermelon oil & seeds, wood betony, Zingibain

Unfortunately, it's not possible to determine what really belongs on these two lists because each item would need to be tested repeatedly on hookworms in a number of individuals and conditions, in different combinations and doses. I would need to determine whether a given dose kills the worms, reduces their effectiveness, or just reduces eggs production. There are just too many variables for me to be confident about any of this.

Fortunately, my last culture supplied over 700 larvae in total. This was after eliminating, for the three weeks prior to beginning this culture, most of those items in my diet that had been of special concern. (The reason the food elimination period was three weeks long was because I had just reinoculated, and wanted to give the new larvae plenty of time to settle in.)

Notes

(1) This paper (**Techniques to kill infective larvae of human hookworm Necator americanus in the laboratory and a new Material Safety Data Sheet**) along with a few experiments and consultations with others lead me to the conclusion that 90% isopropyl alcohol, 3% hydrogen peroxide, Lysol, 2% Glutaraldehyde, 10% Formalin, full strength Dettol, 2% Chlorhexidine, and 10% Povidone Iodine are all unsatisfactory for quickly killing 3rd stage hookworm larvae. Virtually pure ethanol (sort of) works, but is expensive and unavailable in many places. Very strong sodium hydroxide (soda lye) is very effective, but also extremely harsh to work with. Therefore, I currently have four practical off-the-shelf methods for killing hookworm larvae: Two slow methods: freezing, and boiling in water; and two relatively fast methods: undiluted 5-6% sodium hypochlorite bleach, and undiluted 2-3% ammonia.

In repeated tests that have been conducted with either undiluted 5-6% bleach or undiluted 2-3% ammonia the larvae stopped moving within 2 minutes. On this basis I'm assuming that, at this point, they are dead, or at least no longer infectious.

If the bleach or ammonia is mixed in with stool, soil, water or cork, it becomes diluted, necessitating that I leave it to work for considerably more time. RETURN

(2) Instead of soil, I could possibly have used charcoal (e.g., granulated activated carbon/charcoal) which I would have been able to find at aquarium supply shops, as well as online, e.g., **Finest-Filters 1000g Granulated Activated Carbon / Charcoal for Aquarium and Pond Filters**. Another option would have been vermiculite, which is available from garden centres and online, e.g., **Vermiculite - natural incubation substrate - 5l Bag - 2-4mm Grain Size - Incubation Substrate**. However, I would have had to grind both of these materials and sterilise them by baking them. RETURN

(3) The cork I use is granulated, 20–40 mesh size, and is claimed by the supplier to be in its natural state, with no added chemicals. RETURN

(4) The pH scale runs from 0 to 14, with 7 being neutral. So from 0 to 6.9 is the acidic range, and from 7.1 to 14 is the basic range. Since my soil was not too basic, I was not faced with the problem of finding a product to make it less basic, and thereby closer to the ideal neutral pH of 7.

In some places it has been reported that many of the products for making soil less basic seem to come in a very large grain size, which might have meant that I would either have had to hunt around to find a product that was in a sufficiently fine powdered form, or have been prepared to identify a way to grind what I did find into an acceptable powdered grain size, perhaps using an electric cheese grater, an electric pepper/spice mill, or an electric mortar and pestle, e.g., **Glen Mills**. RETURN

(5) Web sites warn that the soil might smell when baked, but this wasn't a problem when I baked either the soil, the lime or the cork. I have also read warnings that birds can be extremely sensitive to indoor air pollutants, which could kill them, but this wasn't an issue for me, since I don't own any birds. (**How to Beware of Deadly Indoor Air Pollutants That Can Harm Your Companion Bird**) They also warn not to get the soil over 93˚C (200˚F), e.g., **How to sterilise potting soil**. RETURN

(6) In some places they sell inexpensive large aluminium foil turkey baking pans. If these had been available, and I had decided to use something like this, I suspect these would have been very difficult to get clean due to the numerous small crevices on their inner surfaces, so I might have needed to reline them on the inside with fresh aluminium foil before baking the cork in order to keep the residue soil and lime from contaminating the cork. RETURN

(7) To be sure that my thermometer was reasonably calibrated, I had previously tested it in ice water 0˚C (32˚F), as well as boiling water 100˚C (212˚F), and adjusted it to be the most accurate in its upper temperature range. (If I were doing this at an extreme altitude, I would have needed to take into account the fact that water boils at slightly different temperatures at different altitudes.) RETURN

(8) I use dechlorinated water for my cultures, although I'm not completely sure that this is necessary. Dechlorinated water is easily prepared by filling a shallow plastic container, (e.g., 4 litre [1 gallon] size, 30 cm x 20 cm x 10 cm [12" x 8½" x 4"]) with cold tap water, and then letting it stand with the lid off for 24 hours. Tiny bubbles form on its inside surfaces, which I remove by tapping the container's sides, and then scraping the remaining ones off using a kitchen knife. Then I snap on its sealing top, and use the water as needed. I don't use distilled water, since I don't know if the larvae can tolerate the osmotic problems that might be associated with this, and I'm guessing that normally mineralised water is what they are most comfortable in.

Many areas use chloramine instead of free chlorine to disinfect drinking water. If this were my situation, I would first carry out a test to establish whether this had any adverse effect on the larvae. If it did, then I would either have to investigate using one of the products designed for tropical fish owners, e.g., **Seachem Prime**, **AmQuel** or **Ammo-Lock** (**this site** presents a list of them); investigate using bottled water, distilled water, or water from a local natural

source; or even, as a last resort, consider installing an expensive and complicated multistage filter system. (**Chlorine and Chloramine in the aquarium** / **Chloramine Facts** / **Anyone knows how to remove chloramine from drinking water?** / **Frequently Asked Questions - Chloramines** / **Chloramine - Wikipedia**)

The only really practical way to remove chloramine is to use a water conditioning product such as those mentioned above, or any one of numerous other brands. Only a couple of chemicals are used for aquarium dechloramination, and all the brands on the market use one or the other of these. Adding the prescribed amount to the water (usually, one teaspoon of conditioner to ten gallons of water) more or less instantly renders this safe for aquatic animals. (**How to remove chloramine from tap water?** / **Review - Water Conditioners and Dechlorinators**) RETURN

(9) The stool should not come into contact with either urine or chlorinated water. Since I don't want any chlorinated spray getting in my cultures, I deposit the stool away from any sink, and also keep my cultures away from them. Obviously, I don't retrieve my stool from the toilet. I find it easiest to deposit it directly into the container while in my bathroom. RETURN

(10) If my stool is soft, I use a dedicated knife to cut it up and mix it with the soil, adding first some soil, then several lumps of stool. Then I spray it down, add more soil, spray it down again, and so on. This creates a kind of heterogeneous **construction aggregate** which prevents the stool from forming an impenetrable plug in the culture container, causing water to pool on top of it instead of draining through the soil/stool mixture. With very hard stool this isn't necessary. RETURN

(11) One advantage of using soil is that it covers the stool, greatly reducing faecal odour problems. RETURN

(12) If a tip does become clogged I clear it by running water through it backwards. RETURN

(13) I do this to prevent a build-up of excess liquid on the slide, from where it might run over the sides of the slide and make a mess, getting onto the back of the slide and causing this to stick to the microscope stage, which would then need to be cleaned. RETURN

(14) Interestingly, my yield of larvae has dropped greatly over the last few months. Instead of a hundred larvae per drop, I have recently been getting around 6, with a total for the entire culture of only 150 to 200, rather than thousands. I'm not entirely certain of the reason for this development, but it could be due to aging worms, unduly hard stools, or, as mentioned under "Promotion of egg viability by dietary manipulation", it may be the result of eating foods, spices or herbs that are affecting eggs production. Given my most recent experimental results, mentioned at the end of the dietary manipulation section, I think this last possibility is overwhelmingly likely to be the main cause of the problem. RETURN

(15) If there was a need to further concentrate the larvae, I could empty the Glad containers containing the larvae into an empty cone, and also rinse the cases into that cone to ensure that all the larvae had been transferred. I would then wait about 36 hours before drawing off the surplus water from higher up the cone. Then I would draw up what was left at the bottom of the cone, before rinsing the cone several times, when empty (as I do with the contact lens cases when preparing a dressing for inoculation) and then I would store the rinse water in another Glad container. RETURN

(16) I use white **Dove bar soap** for the initial wash because it doesn't seem to leave a film, but any film-free cleanser would work just as well. After the slides have been rinsed in regular water, I rinse them twice more in distilled water (**Distilled Water - 5.5 Litres**) in a bowl, swirling them around in it, changing the water, and then rinsing them again. During the whole of this process, I only hold or touch the slides by their edges.

In order to remove absolutely all of the mineral deposits, I often take another bowl, rinse it 4 times using a small amount of fresh distilled water for each rinse and swirling this around before disposing of it (thus hopefully removing any leftover mineral deposits from the bowl's previous regular washing) and then refill the bowl with fresh distilled water. I don't wipe the bowl during this process because a towel might add contamination. Then I process the slides, one at a time, in assembly line fashion, shaking off any clinging water left from the first bowl, swishing each of them in the second bowl, then again shaking off any excess water, before putting them in the slide case.

If I lived in an area where the tap water was really hard and, as any minerals already on a slide may slightly contaminate the distilled water I'm using in any given bowl, I might need to repeat this procedure in a series involving multiple bowls and repeated distilled water rinses before finally putting the slides away. I must also carefully clean the mechanical stage of my microscope before each use, to prevent any dust, etc., from contaminating my clean slides. Even after all this, I've found that, over time, my slides have accumulated imperfections and marks that can't be removed. My solution to this problem is simply to dispose of the imperfect slides and use new ones. RETURN

(17) I wait till I feel the itch so that I have a clear indication that the worms are alive and making a start on their journey before I take the oral diphenhydramine. If I had just checked the larvae under a microscope and established

that they are all active, I might not need to wait for an itch and could take the medication about 15 minutes before inoculating.

Diphenhydramine is one of the best antihistamines for helminth hosts to use, although it typically causes drowsiness. Other worm-safe antihistamines that generally cause less drowsiness are **loratadine** (Claritin) and **fexofenadine** (Allegra, Telfast). Less suitable antihistamines include **cetirizine** (Zyrtec, Reactine), **levocetrizine** (Alcet, Allear, Curin, levcet, Seasonix, T-Day Syrup, Teczine, UVNIL, Vozet, Xaltec, Xozal, Xuzal, Xusal, Xyzal, Zilola, Zyxem), **phenylephrine**, **desloratadine** (NeoClarityn, Claramax, Clarinex, Larinex, Aerius, Dazit, Azomyr, Deselex and Delot) and possibly **acrivastine** (Semprex-D in the US), all of which may potentially harm helminths.

For more detail about which oral antihistamines are suitable for use alongside helminthic therapy, see the Antihistamines section of the Human Helminth Care Manual which can be downloaded from the Files section of either the **Yahoo Helminthic Therapy forum** or the **Facebook Helminthic Therapy Support group**. RETURN

(18) Diphenhydramine has local anaesthetic as well as antihistaminic properties, which makes it doubly beneficial for treating a hookworm inoculation rash. Topical products containing 2% diphenhydramine hydrochloride are available in several forms, e.g., Benadryl Extra Strength Itch Stopping **Cream**, Benadryl Extra Strength Itch Stopping **Gel**, Benadryl Extra Strength **Spray** and Benadryl Extra Strength Itch Relief **Stick**. These, and similar products, are available in both the US and UK - from Amazon, eBay and other outlets - and may also be available in **other countries**. RETURN

(19) In addition to the methods I use to deal with the inoculation itch, there are details of approaches used by others in the Treating a Hookworm Inoculation Rash document which can be downloaded from the Files section of either the **Yahoo Helminthic Therapy forum** or the **Facebook Helminthic Therapy Support group**. RETURN

(20) I make a record of all my inoculations to help me keep track of my likely colony size, and to help me determine when I need to reinoculate. I also mark the dates of inoculations on my calendar. RETURN

(21) I record the date of each harvest on an index card, which I cut to a short, narrow strip to fit the side of the Glad container, then tape this in place. RETURN

Materials list

I've found that the sort of materials listed below are currently adequate for my culturing of hookworm, although the suppliers mentioned here are not necessarily the ones I used, merely sources of similar items.

This list, as well as any items mentioned or cited previously, is offered solely for educational purposes and its inclusion is not intended to be encouragement to anyone to emulate my practice of hookworm culture. The Warning and Disclaimer printed above apply.

Common sense should be used when interpreting this list, because I might have been able to find substitutes for the items listed below that would have performed the same functions. For example, when dimensions of items are given this usually doesn't mean that I would have had to use exactly those dimensions. Such numbers are included only so the reader has a rough idea of what I'm describing.

I have included graphics of some of the listed items for reference if and when the links expire.

Microscope

There were a number of options I had to consider when purchasing a microscope.

A good lighting system was important. A mirror lighting system wouldn't have been adequate, and a 'scope with either a halogen or LED light source was likely to be best. A good iris and concentrator system were also important, as was a rheostat for dimming the light.

A binocular microscope would cause me less eye fatigue and be easier to use, whereas a monocular one would be cheaper. A mechanical stage would make using the 'scope easier for me, but one without a stage would be cheaper.

I've seen it claimed online that 200x magnification is best for egg counting and larva identification, but I've never needed to do either of these. Egg counting is not necessary in order to incubate hookworm larvae, and, since I already know that the only species of worm I host is Necator americanus, there would be no point in attempting to determine the species of the the larvae that I'm culturing. Apart from this, I wouldn't know how to encourage the larvae to turn and smile for the camera so that I could check their mouth parts!

It's been suggested online that working with trichuris species requires 100x magnification, but I use my lowest magnification (40x) for virtually everything I do, which is to count hookworm larvae and whipworm eggs. It has also been suggested that a microscope with the lens looking up from the bottom might be a better option, although the author of this comment may actually have been thinking of an upward-facing *mirror* rather than a light. However, I've only ever used a lens looking down from above, and always found this to be perfectly adequate.

A very similar model to this one was recommended on a helminthic therapy discussion forum and is reasonably similar to the older scope that I have: **Professional Biological Compound Microscope, Precision World, 40X-1600X, AmScope, Model# B230A**, for £170 ($260.00., €200) (see graphic).

An example of a much cheaper model - one that I considered using, and which might have worked - would be the **1000X Student Monocular Biological Compound Microscope**, for about £55.00 ($80.00, €60) (see graphic).

Microscope case

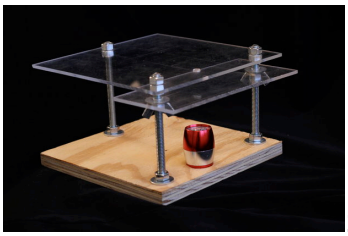
I wasn't sure a microscope case would be necessary, but eventually decided to get one. Various sites suggested that I could save money and yet make a decent case from a tool box with a very inexpensive inner lining made from a one person-sized (approximately 90-96 cm x 190-193 cm [36-38" x 75-76"]) egg crate foam mattress pad (e.g., **Make a Carry Case for Your Microscope**) I used a tool box similar in dimensions to a Plano 22 inch, extra deep, model 701, along with the egg crate foam. For the foam, I found a source similar to these: **Eggcrate Wheelchair Cushion 40.64 cm x 45.72 cm x 7.62 cm by Wheelchairs** / **Sound insulation foam (3 pieces) by CONRAD**. When I got the toolbox home, I had to use pliers-type wire cutters to cut off two internal projections that came down from the lid. Adding a few pieces of Styrofoam filled out the foam internal padding (see graphic).

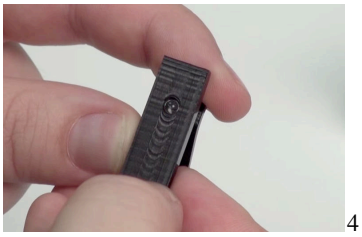
GX Optical may have the largest choice of stereo microscopes in Europe, and claim to be the largest independent microscope supplier in the UK.

Other options might include using a Plugable USB 2.0 Handheld Digital Microscope (see graphic 1, and further details here), or a slightly more up-market version such as the DBPOWER 5 Megapixels 20X-300X USB Microscope Plugable Digital Magnifier with 8 LED Lights and Stand (see graphic 2), although USB devices could develop electronic and/or software faults that traditional microscopes will never have. Another alternative could be to make a digital microscope using a smart phone for as little as $10 (see graphic 3), or use a 3D Printed Microscope with a mobile device for pennies (see graphic 4).


1


2


3


4

Microscope use advice

A good source to consult if I have microscope problems is Fecal Flotation: Common Problems With Microscopy. (Download).

Microscope optics cleaning kit

e.g., Optical Lens Cleaning Kit (see graphic) / Matin Lens Cleaning Kit.

Microscope slides, with box

e.g., Microscope World SKU: BMS50 / Bresser microscope accessories slides and cover glasses / Bresser microscope accessories slides (see graphic).

Microscope slides, plain glass, 72/pack

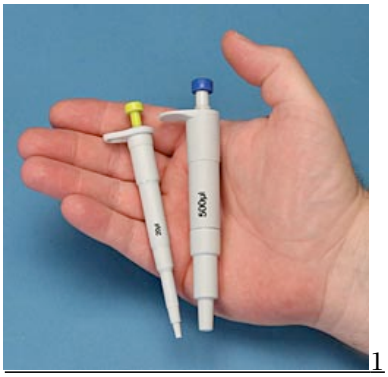
e.g., Item# MS-SLIDP72 (see graphic).

Slide storage box, 25 slides

e.g., **Item# MS-SLBOX** (see graphic).

Miinipipette, 100 microlitre

e.g., **Fixed-Volume 100 µL (±0.3 µL) MiniPipet** (see graphic 1) / **Mini Pipette Pipettor** (see graphic 2).



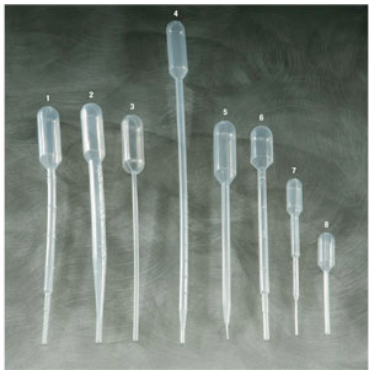
1

Minipipette tips

When I bought my mini pipette tips I had to check carefully to make sure that they were compatible with my particular mini pipette, e.g., **MS® Pipette Tips** / **Pipette Tips Company Links** / **Pipettes Suppliers** / **Biohit Optifit Tips** (see graphic).

Pasteur pipettes

e.g., Disposable 1.0 ml Transfer Pipettes / Pipette, disposable, 5ml, 10 pk.



PH meter

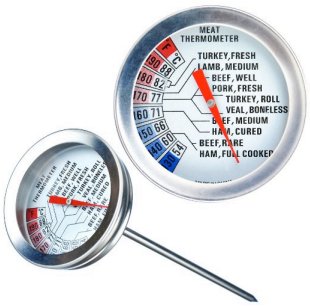
e.g., Mosser Lee Soil Master PH Meter (see graphic).

Funnel for filling water sprayer bottles

e.g., 90mm Plastic Transparent Funnel for Kitchen (see graphic).

Meat thermometer

e.g., VonShef Stainless Steel Meat / Poultry Thermometer / Temperature Gauge Probe (see graphic).



Potting soil

e.g., Scotts Miracle Grow Potting Mix Comp 8lt (see graphic).

Powdered limestone

I found this necessary for making my soil less acidic, e.g., Mayville Garden and Lawn Lime. 25 kg (50 lb) bag, (Neutralizing Zone 80-89, Calcium Carbonate equivalent minimum of 102%) / Lincoln Limestone Powder for Horses 4KG.

Granulated cork

20 – 40 mesh size, 1 to 3 kg (2 to 5 lb) (One 20 litre [5 gallon] garden bucket will hold about 1 kg [2⅓ lb] of cork.)

I was able to find a local retail cork supplier but, if this hadn't been possible I would have investigated these online sources to ascertain whether they would be able to supply cork in small amounts that was free of any added chemicals, e.g., Jelinek Cork Group (20 – 40 mesh = granu-051 [0.5mm-1mm]) / Corkstore.co.uk / ThomasNet Ground & Granulated Cork Suppliers / Tradeboss / Albaba.com Granulated Cork and Cork Powders / The Cork Industry Federation / Maryland Cork Company, Inc. (see graphic).

I investigated the possibility of grinding my own 20/40 mesh granulated cork, which looks entirely possible, and not at all technically demanding, though I won't be pursing this because it is far easier, and probably far cheaper, to buy it already in granulated form. If I were to grind my own cork, I would need to buy an electric cheese grater (using the fine/small grating material), two sheets of filter mesh (one 20 mesh, the other 40 mesh) and then find a cheap supply of raw natural cork scraps, or use widely available store-bought natural cork bottle corks, although I suspect the latter option might be quite costly.

20 & 40 mesh screen

Possible sources: Mesh Direct / The Mesh Company / Metro Screenworks / Wire Cloth Manufacturers Inc / TWP / eBay.

Electric cheese graters

e.g., Shopwiki.co.uk / Oster CG100 Electric Cheese Grater) / Black & Decker GG200 Electric Cheese and Chocolate Gismo Grater / Zyliss Rotary Cheese Grater.

Raw cork

This should be obtainable from the sources listed for granulated cork, above.

Trash container (lid type)

e.g., Brabantia Pedal Bin with Plastic Bucket, 3L - Brilliant Steel (see graphic)..

Plastic containers - 0.5 litre (16 fl oz) - for holding bleach or ammonia.

Plastic containers - 0.5 litre (16 fl oz).

A small supply of approximately pint-sized containers (to hold the cultures) with holes in their bases for drainage. Plastic tomato plant starters, or similar, would have been suitable, but I made my own by drilling holes in the bottoms of plastic food containers.

I am very likely being too pernickety but, for all the equipment that I use to hold liquids or that will come into contact with moisture, I avoid using plastics that might contain Bisphenol A (see **Why All the Fuss About Bisphenol A [BPA]**) so I stick with plastic types 1, 2, 4, and 5. I also tried to keep to these types of plastic for other items that I used for this protocol - at least when I could tell what the plastic type was.

Plastic containers - assorted, to hold miscellaneous items.

e.g., 2 litre (64 oz), 15 cm x 20 cm x 9 cm (6" x 8" x 3½")

and 2 x 15 litre (16 quart) containers

e.g., **15 Litre Plastic Storage Box Container With Clip On Lid and Handle** (see graphic).

Glad containers

e.g., Mini Round (see graphic) / **Gladware Mini Round 4 oz Containers with Lids**.

Latex Free Gauze

e.g., **KERLIX Gauze Bandage Rolls** / **Boxing Gauze Hand Wrap Bandage** (see graphic).

Contact lens cases

I use three of these,

e.g., Bausch & Lomb ReNu 'Sight Savers', 2 packs of 3 each (see graphic), or Bausch & Lomb Contact Lens Case Pack of 3.

Spray bottle (for dechlorinated water)

e.g., Rubbermaid Heavy-Duty Spray Bottle (see graphic), or Green Blade 750ml Water & Liquid Spray Bottle.

Used plastic pill bottles, miscellaneous, for sharps, etc.

Shot glasses, 2 – 4, either plastic or glass

e.g., Plastic Shot Glasses 30ml 30/Pack (see graphic), or Shot-glasses.co.uk.

Reusable silicone snow cone cups with wire cup holders, 2 packs of (4 total)

e.g., **Back to Basics SIT10895 Snow Cone Cups and Holders, 2-Pack** (see graphic).

Instead of snow cone cups, a well-equipped lab would probably use something like an Imhoff cone, e.g., **Sedimentation Cone to Imhoff graduation up to 1000ml** or even a **separatory funnel**.

I could also have used something like this **Gold Martini Glass** (see graphic).

20 litre (5 gallon) buckets, with lids

How many buckets I was going to need obviously depended on how much cork and soil I was going to store. One bucket will hold about 1 kg (2⅓ lb) of cork, or about 25 kg (50 lb) of soil and lime mix.

Bin organisers - for small items

Mine look something like these: **Faithfull Plastic Storage Bins with Wall Mounting Rails (12 Pieces)** (see graphic).

Alternatively, any of these would also work well: **Stack-On CB-12 Clear View 12-Bin Organizer** / **Gladiator GarageWorks GAWESB6PSM Small Item Bins, 6-Pack** / **Lab Supply Bin for Small Items - 13 Compartments**.

Mini fridge

e.g., the Caldura 17 litre Compact Mini Fridge (see graphic).

Plastic washing up bowls

I have used three of these in the past (approximately 10 cm deep x 30 cm x 30 cm [4" deep x 12" x 12"]) and they can still come in handy, but I now generally use the sink for all washing purposes.

e.g., Rubbermaid Home 2951-Ar Wht White Rectangle Dishpan / Rectangular Washing Up Bowl – Cream / Medline Plastic Graduated Rectangular Wash Basin (see graphic).

Dishrack

I find it useful to have a separate one for drying washed lab equipment.

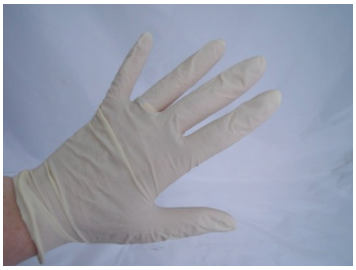
Bluettes

Lehigh Spontex 17005 Bluettes Knit Rubber Glove (see graphic).

Disposable exam gloves in latex, nitrile or vinyl

It's far cheaper to reuse the gloves a number of times, but if these are put back on too soon, they may still be damp from the previous use and hard to get back on. In this case, I use a hair dryer to dry them out. Alternatively, I may place the dryer on my wrist, while it's set on cool rather than hot, and use it to inflate the glove (which at that moment is stretched over both my wrist and the dryer's mouth) as I work the glove back on. Yet another option is to rotate several pairs.

These are widely available at local pharmacies and online, e.g., Simply Powder-Free Latex Gloves Medium - 100-pack (see graphic) / 200 (2 Box) x Vinyl Powder Free Gloves Disposable Clear Food Medical etc. (Medium) / Bodyguards Clear Vinyl Powder Free Exam Gloves Medium Box of 100.



Vinyl lab apron

e.g., **Black apron water proof resist vinyl back chef cook butchers pocket bib halter** / **Lab Apron No. 2, heavy vinyl** / **Hamilton Bell Co. Inc., No. 5250, 27" x 42"** (see graphic).

I found mine at an online science supply site. Some college bookstores may also carry these for their chemistry students. On mine, the apron's tie cords were made of plastic, and quickly broke, so I replaced them with used shoe laces attached to the apron with shoe goo and staples.

Shoe goo. **Original Shoe Goo CLEAR - 110ml/3.7oz Tube**

Kitchen sponges, several

e.g., **3M O-Cel-O Handy Sponge Power Pack 7274T, 4-Count** (see graphic). I really like this type of sponge for cleaning up, and I also use them to make a bed to rest my hydrometer on when I'm **incubating whipworm eggs**.

Terry cloth towels, 2 or 3, small

Paper towels, 1 roll

Plastic refuse bags for triple bagging and disposing of old soil and stool

Aluminium foil, 1 roll
Turkey roasting pan, aluminium foil type, approximately 40cm x 33 cm x 8 cm (16”x 13”x 3”)

e.g., **2 Disposable Foil Oblong Turkey/Meat/Fish/Veg Roasting Tray 41cm x 30cm x 6cm** (see graphic) / **Durable Packaging International Foil Roasters**.

(Either) **bleach**, by the litre, (or gallon), 5-6% sodium hypochlorite
(Or) **ammonia**, by the litre (or gallon), 2-3%.

Alcohol, one bottle 90%+

(If I had been using alcohol wipes, I would have needed to be sure they didn’t leave any residue.)

Adhesive dressings, one box

e.g., **Steropore adhesive wound dressing, 6cm x 7cm x 25**

Diphenhydramine

A liquid form of this drug is preferable to ensure more rapid absorption, e.g., **Children’s Benadryl Allergy Liquid**.

Diphenhydramine Hydrochloride cream, maximum strength (2%).

This is only occasionally available from Amazon.co.uk, but can be ordered from Amazon.com (US), e.g., **Dr. Sheffields Anti-itch Cream with Histamine Blocker - 1.25 Oz** / **Benadryl Itch Stopping Cream, Extra Strength-1oz**. I always make sure that I have plenty of this cream in stock.

1% hydrocortisone cream (This hasn't always been necessary for me.)

e.g., **Galpharm hydrocortisone cream 1%** / **Equate Max/ Strength 1% Hydrocortisone Anti-Itch Cream 2 oz**.

Electric hair dryer

e.g., **Eti Hair Dryer** (see graphic). Using a dryer hasn't always been necessary for me.

Alternative hookworm incubation methods

There is a collection of hookworm incubation methods in the Files section of the **Facebook Helminth Incubation group**, which is the main online venue for discussion about the incubation of all types of therapeutic helminth. Membership of the group is required before these documents can be viewed.

Some of the hookworm incubation protocols available from this group are greatly simplified but, even if they seem superior to the protocol presented here, it might still be useful to first read the whole of this document to provide a full understanding of all the issues surrounding the practice of hookworm incubation.

There is also a **Yahoo Incubating Hookworm Group** but this is not very active.

Suggestions/observations

If anyone using this document has any suggestions for its improvement, or any other observations, please post these to the **Facebook Helminth Incubation group**, the **Facebook Helminthic Therapy Support Group** or the **Yahoo Helminthic Therapy Forum**, from where they will be collected for eventual addition to the body of this document, or inclusion in a supplement to it.

Document history

First published: Nov 2011

Last updated: Sept 2015
