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

Human Whipworm Incubation

Contents

Introduction

Having experienced considerable benefit to my health as a result of hosting a controlled number of human whipworms (*Trichuris trichiura*), I wanted to be able to maintain my whipworm colony with as little expense as possible. After carrying out a search of the literature, followed by experiments to refine the process, I arrived at the protocol set out below.

The next section, "Warning and Disclaimer", is essential reading because there are significant safety, and potentially legal, implications to helminth incubation.

Warning and Disclaimer

* There is considerable risk involved in incubating whipworm eggs, due to their infectious nature. After successfully culturing whipworm eggs, it would be easy for someone to accidentally ingest many thousands of these organisms, with potentially very serious consequences for the individual's health - for example, resulting in rectal prolapse - and perhaps also the future of helminthic therapy if news of such an accident were to reach the media.

* It may be illegal to incubate whipworm eggs in some jurisdictions, including the U.S., where they are currently classified by the Food and Drug Administration (FDA) 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 is to clean and incubate the eggs produced by the colony of whipworms (*Trichuris trichiura*) that I am already hosting. This will allow me to re-infect myself for the purpose of maintaining the beneficial effects I have from harbouring them.

My existing whipworms were purchased as eggs from a recognised provider of helminthic therapy, and I'd never use 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 whom I can be confident is free from pathogenic organisms and, in the latter case, that the organisms supplied have been scrupulously cleaned. (1)

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. (**How to Dispose of Sharps**)

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

* I disinfect all materials using two containers. First, I dip the equipment into either undiluted 5-6% bleach or 2-3% ammonia. (2a) 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 boil for at least 10 minutes the leftover soil and stool in my stock pot.

* I make absolutely sure my centrifuge is balanced.

* I only add acid to water, and never add water to acid.

* I neutralize my acid outside because of the caustic fumes this generates.

* I only add a little baking soda at a time when neutralizing the acid, since this generates a lot of foam.

* I disinfect all work surfaces when finished and, to be as confident as possible that nothing remains alive, I use either undiluted 5-6% bleach or undiluted 2-3% ammonia. (2b) 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 (17 U.S. 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 as confident as I can be that the eggs 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, as to do so would produce explosive and toxic gasses.

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

Separating hookworm and whipworm eggs

This protocol takes into account the fact that I carry both human hookworms (***Necator americanus***) and human whipworms (***Trichuris trichiura***). Even if I'm not planning on extracting hookworm larvae from a given culture I still culture it for 8 days, as I would with hookworms. (3) Since viable hookworm eggs hatch within one or two days, any such eggs will have long since hatched and their larvae will have moved on. So I'm very sure that the hookworm and whipworm eggs have been separated. (Later I'll also be filtering the material with a 30 micron mesh, which would also remove any hookworm eggs. [See Note 12]) I'm also confident that this understanding is correct because I've never seen any hookworms or hookworm eggs under my microscope when I've done my whipworm counting and dosing.

Cleaning the eggs

Outline and summary of the egg cleaning process

This stage consists, essentially, of cleaning the eggs using density separation, size separation, possibly a chemical cleaning using the septic tank cleaner, Rid-x, and acid.

Here is my abbreviated outline and summary of the various stages involved in this whipworm egg cleaning protocol. (4)

1) Once any cork with hookworm larvae has been separated off, I then have to prepare **Sheather's solution** (5) for a very rough initial density separation.

2) I thoroughly mix the soil and stool into the Sheather's in a stock pot with a dedicated strainer.

3) A day or so later, I skim off the material from the top of the solution with a homemade hand scoop.

4) I filter this material through a 30 micron mesh. (6) This separates the ova, which pass though the mesh, from the stool debris, most of which remains on top of the mesh and can be discarded.

5) I then filter the material using a 20 micron mesh. (7) (8) (9a) (10) This captures the ova, which remain on top of the mesh, while allowing most of the stool debris to pass through.

Most estimates I've come across claim that whipworm eggs measure from about 58 to 50 microns long, and from 27 to 22 microns wide. (11) These two filtering steps in the procedure ideally mean that anything larger than 30 microns and smaller than 20 microns will have been filtered out. (12b) So the material that's left at this point should have a reasonable concentration of eggs.

6) I then centrifuge this material in a multistep procedure using both 1.130 and 1.200 specific gravity sugar water solutions. The eggs have a specific gravity between 1.130 and 1.200, and this allows their separation from the stool. (13) (14a) I then use a Pasteur pipette to suck the material off the top or bottom of the columns of liquid in the centrifuge tubes. This material is then re-filtered a second time, firstly using a 30 micron mesh to allow the eggs to pass through, and then using a 20 micron mesh to collect the eggs which remain on its surface. Later on, when I'm cleaning the centrifuge tubes, I find a **Waterpik** is very useful for cleaning the faecal material out of the bottom of them.

I don't think it's likely that **liquid Rid-x** harms the ova, (15) but, because I'm not 100% certain about this, in the next step I divide my sample and, for now, follow both of these two separate procedures in parallel. (16) (17)

Either:

7) I put the eggs into a Rid-x septic tank cleaner and water solution for 4 weeks, changing the Rid-x and water every 7 days. This is done by pouring the week-old solution through 20 micron mesh, saving the material on top of it, putting it back through a 30 micron mesh into a **Glad Mini Round container** that has about 8 small holes punched in the lid using the point of a pair of scissors, and then adding fresh Rid-x and water. (18) The Rid-x contains enzymes and bacteria that break down stool. The upshot is that the only visible material that will remain after 4 weeks of doing this will be the eggs and a few bits of (what I think is) cellulose.

8) After 4 weeks, I do the final 20 micron filtering, at which point I should see almost nothing but eggs under the microscope. (19) I place the sample into a bath of mild 3.0 pH car battery (sulphuric/sulfuric) acid for three weeks. (20) Hopefully, this will kill any bacteria or protozoa that might have tagged along with the eggs, although there is no visible indication that this provides additional cleaning by removing any of the relatively larger contaminates that remain.

9) After three weeks, I neutralise the acid with baking soda, carry out a final 30/20/30 micron mesh filtration, and put the eggs into safe storage in water in a Glad container.

Or:

(The only difference here is that I've skipped the Rid-x part of the procedure.)

As I said above, I doubt that Rid-x harms the eggs, but I'm not certain about this. So, for now, I keep a separate batch of a couple of thousand eggs that haven't been Rid-x treated in case my colony dies and I have to start over. If I do have to use this sample to restart my colony, the sample is still clean enough such that I won't taste or smell anything.

7) I place the sample into a mild 3.0 pH car battery acid bath for three weeks.

8) After three weeks, I neutralise the acid with baking soda, do a final 30/20/30 micron mesh filtration, and put the eggs in safe storage into water in a Glad storage container.

Full description of the egg cleaning process

I assume that any cork bearing 3rd stage hookworm larvae will have already been removed from the culture, although I still wear gloves and an apron during this procedure in case any stragglers remain. I carefully remove all the cork, because, if any is left, it will float to the top of the Sheather's solution later on and contaminate the mixture.

Preparing Sheather's Solution

To prepare Sheather's solution, (link, link) I begin with 2.721 kg (6 lbs) of table sugar (sucrose) and 2,130 ml (72.02 U.S. fluid ounces) of distilled water. To measure out the sugar, I use a small 1kg (2 lb) capacity postal scale, and, to measure out the water, I use a plastic graduated cylinder. (Plastic is better than glass because it's unbreakable.) (21) I first sieve the sugar to remove any lumps. Then I heat the water in a 10 litre (11 quart) stock pot on the stove. I don't want to get the water too hot, since this might caramelise the sugar. After it's beginning to get too hot to comfortably put my fingers in, and well before it boils, I begin to gradually add sugar, using a small bowl to ladle it from the larger container it's in. I don't just dump the sugar in, but shower it in, while stirring the mixture constantly to avoid sugar build up and caramelisation on the bottom of the pot. Once all the sugar is dissolved, I put the pot aside to cool. (If I want to speed up this part of the process, I sit the pot in cool water to reduce the temperature more quickly.) Once the solution has cooled to room temperature, I use a large funnel to drain off about ½ of it (about 2 litres [2 quarts]), for use during the later 1.200 and 1.130 centrifugings. I put this into 2 **Chug Bottles** and store them in my refrigerator.

Next, I use a hydrometer to check the density of the solution, which should be close to 1.270, although this doesn't need to be too exact at this stage. (22a) This can be done by putting some of the solution either in a Chug Bottle, any other tall thin bottle, or by using my **graduated cylinder**.

The Sheather's Separation

Then, while wearing **Bluettes** gloves, I use my hands to manually scoop the stool and soil from the container. I then mix this by hand into the 1.270 Sheather's solution in my stock pot using a large dedicated sieve. (23) The soil and stool isn't necessarily forced through the sieve, but worked against the interior side of the half sphere of the sieve to work the lumps out as much as possible and get the solution as smooth as I can. (24) At this point, as a check, I take some of the stool-laced solution and check it for density. As long as its density is well over 2.200 the separation procedure should be going according to plan.

I then let the mixture separate out for at least 24 hours, although I don't actually know the separation rate of whipworm eggs in Sheather's solution. While doing this, I keep the lid of the pot slightly ajar, since the eggs might not develop when stored with decaying matter such as faeces in a closed container. (25)

30/20/30 Micron Mesh Filtering

Next, using my homemade hand-scoop, I skim off some solution from the top of the mixture. (26) Then I place the material on top of a sheet of 30 micron mesh. This mesh is attached, by a wimpy rubber band, to the top of a circular mouthed plastic party cup 9.5 cm (3¾ inches) in diameter across the top and 12.7 cm (5 inches) tall. (27) I place the mesh over the cup, gently making a depression or pouch about 5 cm (2 inches) down into the mouth of the cup with my curled fingers and/or fist because I want the pressure to be evenly distributed and not focused at any one point on the mesh. I then put the rubber band over the cup, securing the mesh to it. The liquid solution is then poured down into the depression in the mesh, to sit on top of it. I then use a plastic spoon, or the bulb of my Pasteur pipette, (28) and a spray bottle (29) to gently work the material through the mesh as thoroughly as possible. Since this requires so much water, I need to transfer the mesh several times from full cups to empty ones. (30a) (31) (32) I'm eventually left with a remainder bolus of material that won't go through. This will be later disinfected by being boiled for 10 minutes, then allowed to cool, and finally discarded down the toilet.

Next, I take the mesh off the 30 micron mesh covered party cup(s), and take the liquid from all the cups and pour it through clean 30 micron meshes a second time. Obviously, this time it should go much faster. This liquid will now only contain material smaller than 30 microns.

Now I take the liquid and pour it through a 20 micron mesh, which is attached on top of another party cup in the same way as the 30 micron mesh was. Because these 20 micron mesh covered party cups will typically fill up as I repeatedly pour and spray the liquid through the 20 micron mesh, I'll often have to hand transfer the 20 micron mesh with the liquid and material on top of it from a full cup to an empty one. (30b) I continue spraying this

residue, and changing and emptying the cups, until the water I'm getting in the cups is quite clear. Eventually I'll have a small amount of residue on top of the 20 micron mesh that is reasonably clean. All of the eggs should be on top of this mesh, and so I dispose of the liquid left in the cup(s) and later boil it.

I next use a mini pipette to suck up some of the material, and then put it on a slide (33) (34) so that I can check with my microscope to see if I have any eggs, although I have always had them in the past. Once I've confirmed the presence of eggs, I suck the sample up with a Pasteur pipette that has the end cut off to widen its opening to better suck up the material, and then expel it through a 30 micron mesh into a glad container. (35) (9b) Of course, this container will also have some water in it. (36a)

I then repeat this whole process of scooping from the 1.270 liquid and doing 30/20/30 micron mesh separation a number of times until after a number of hours it looks as though I've gotten enough material. I then dispose of the leftover material that remains in the stock pot by boiling it.

Then I take the 30/20/30 separated material and 30/20/30 filter it again as a further cleaning. This part should go quickly.

Preparing 1.200 and 1.130 Sugar Water

Next, I need to prepare 1.200 sugar water from the Sheather's solution that is stored in one of the Chug bottles. (37) So I prepare my wet sponge hydrometer bed, and then take out my hydrometer. (22b) Then I measure the specific gravity/ density of the solution by dipping the hydrometer into the solution in the bottle. It will be about 1.270 so I will need to cut it with distilled water. I pour out a little of the liquid (perhaps 120 ml (0.5 cup), add distilled water, then put the cap on and gently mix the solution by pointing the top of the bottle down and then back up several times. I do this gently because I don't want foam to form in the liquid, since this can cling to the hydrometer and make reading it more difficult. Once I've mixed the solution, I then open the bottle and, using my hydrometer, measure the new density. As I'm getting close to my goal, I pour out and replace far less liquid to avoid overshooting the mark. (If I did this, I would need to make 454 g (1 lb) worth of new 1.270 Sheather's, and then, in the same way as before, sequentially add that to my solution to gradually increase its density.) (38) This process takes a number of repetitions until I get a density of 1.200.

I then repeat this whole procedure with the other chug bottle, and make the 1.130 sugar water.

Centrifuging the Mixture

Next, I take my solution containing eggs and pour it onto (36b) a sheet of 20 micron mesh that is of course attached to the top of a party cup. I then use a Pasteur pipette to put some of my 1.200 solution onto the 20 micron mesh along with the egg-containing solution. The reason I'm doing this is because if I simply added this egg-containing solution to a centrifuge tube there would be some chance that the water in it, with a specific gravity of about 1, would change the resulting mixture in the tube when I add the 1.200 solution to it. I want to keep adding 1.200 solution to the egg-containing solution, letting it replace the 1 density liquid water as it's dripping through the 20 micron mesh, until the egg-containing solution has been largely transformed into a 1.200 solution. I keep on doing this for, say, 5 minutes, by which time the fluid will have been replaced perhaps 6 times, which I'm guessing is enough to get the egg-containing solution very close to 1.200. Then, I use a cut-off Pasteur pipette to suck up the material on the mesh and squirt it into a centrifuge tube. I try to ensure that this liquid that is drawn off of the top of the mesh will be about 1/5th of the total I'll be putting into the tube, and the other 4/5ths will be 1.200 solution that is drawn directly from the Chug bottle. This further ensures that the density of the solution in the tubes will be correct. Also, when I fill a tube I only fill it to about 80% of its total height, leaving some margin for error to prevent spills. Depending on how much material I have, I might fill from 1 to 6 tubes in this way. A centrifuge ***must be absolutely balanced***, for safety, so, if I fill less than 6 tubes in this way, I will then fill the remaining tubes with the 1.200 solution from the Chug bottle, so that all the slots in my centrifuge are full. (39)

Once my centrifuge is fully loaded, I close it and let it run for 10 minutes at about 4,000 rpm, generating about 1,600 g-forces. (40) Since the eggs are lighter than 1.200, and almost all the other material mixed with them is heavier than 1.200, the eggs should have risen to the top of the tube(s) by the time it stops, and almost all of the rest of the stool should have sunk to the bottom. I now use a cut-off Pasteur pipette to suck approximately 0.5 cm (0.2 inch) from the top of the column of liquid in each tube that had egg bearing material in it. When I'm sucking up the liquid from a test tube with the pipette, I "tap" the pipette's mouth repeatedly vertically up and down onto the top of the liquid, roaming/moving around and sucking the top of the liquid, looking for fresh material. I suck the liquid up and then empty it onto a piece of 20 micron mesh fabric that's on a party cup. I then add more 1.200 solution from the Chug bottle to top off each tube, back to the 80% fill level that they had previously. Then I re-centrifuge the whole batch. The reason I repeat the centrifugation is that each time I pipette liquid from a tube I'm only about 95% efficient at getting all the eggs, leaving some on the tube's upper sides, etc. So, if I do this three times, I'll hopefully be getting virtually all of them.

The liquid I'm extracting from each tube needs to be kept constantly wet on the mesh by spraying it every once in a while. When I'm done with the tubes for a round of centrifugation, I then vigorously and repeatedly spray the mesh

to both further clean the eggs and replace the 1.200 solution they are in with 1 density water. At this stage, I might do an additional 30/20/30 micron mesh filtering to remove any relatively large, stray materials that might somehow have been introduced.

I then repeat this whole procedure with the 1.130 solution, but of course now the eggs will be drawn up from the bottom of the tubes using a non-cut-off Pasteur pipette. (14b)

Rid-x Treatment

Either
I put half my eggs into a Glad container with about a 1/3rd of a shot of filtered liquid Rid-x (41), and then fill the container the rest of the way, about 75% full, with dechlorinated water. (42) Then I put the container away in a safe place, in the dark, in my unplugged mini fridge. Every 7 days, for 4 weeks, I change the Rid-x by 20-30 micron filtering this sample, and then adding fresh Rid-x and water. After 4 weeks I do a 30/20/30 micron mesh filtration. (43)

Acid Treatment
Next I put this Rid-x treated sample on a 20 micron mesh, drain the liquid, and then Pasteur pipette it into a Glad container with as little water as possible. Then I pour in car battery acid, filling the Glad container about 75% of the way. (44) Then I put it away in my unplugged mini fridge for 3 weeks. After three weeks, I take out the Glad container and empty its contents into a 0.5 litre (16 fl oz) plastic container. I then add some more acid to the Glad container to rinse it and get all the egg-containing acid solution out of and into the larger container. (NB: I do not spray rinse the Glad container with water to clean it at this point because this would be adding water to acid.) I then neutralise the acid by mixing it with baking soda, using a plastic spoon. (45) I have to do this outdoors because the fumes given off from this reaction are very caustic to breathe. Also I only add a little baking soda at a time to prevent excessive foaming. Once no more foaming takes place when I add and stir in additional baking soda, and there is also some free baking soda undissolved on the bottom of the container, the solution is then neutral pH = 7, the same as water. I next 30/20/30 filter this solution to remove the baking soda powder, spray cleaning the eggs thoroughly when they are on top of the 20 micron mesh, and then put the now finished eggs into safe storage in a Glad container under water in my mini fridge. (46) I also attach a note made from an index card with the date and contents.

T. trichiura eggs need to be kept well below 50ºC (122ºF), and well above 0ºC (32ºF), although there is some disagreement in the literature about their ability to withstand cold. They should also be kept out of strong sunlight, and, while they need oxygen to develop, they can probably tolerate low oxygen conditions. They will rapidly succumb to desiccation, but if stored properly, it's possible for them to remain viable for more than five years. (47) They should not be in a closed container with decaying material. (48)

Or
I treat the other half of my eggs in the same way as the paragraph above, except as I said they have not been treated with Rid-x.

Developing the eggs

I keep my eggs at around 27ºC (80ºF) for at least 4 weeks from the day of the bowel movement that they came from before I dose. Of course, this includes their time spent in the culture, the acid and/or the Rid-x. (49)

Dosing

When I'm going to dose, sources recommend that I don't consume anything carbonated or hot for an hour both before and after dosing. (I go further and only wash them down with dechlorinated water, and don't consume anything at all for an hour before and afterwards.) I start by getting the Glad container out of my mini fridge and set up my microscope. I use a mini-pipette to draw up a small amount of liquid off the bottom of the container and place a drop on a slide.

Counting the eggs

I use 40 magnification to count the eggs. If the eggs are too clumped together to count easily I repeatedly suck them in and out of the mini-pipette to break up the clusters. I can also accomplish this by doing a 30-20-30 separation. I then start at the East or West side of the drop and count down vertically. I then move over, using the eggs at the periphery of my vision as a marker for how far to move, and then repeat my vertical count. Once I've counted a drop, I write the number down on an index card and, always keeping the slide horizontal, put it in my mouth like a

spoon and consume the drop. I repeat this process until I've dosed with the total number I want.

There doesn't appear to be a consensus on how long whipworms live. (50) The estimates I've seen range from 1 to 8 years, and their lifespan might very well also depend on the biology of the human host. So this uncertainty means that the dosing numbers and schedule will also be uncertain. In addition, somewhere between 10 to 20% of eggs succeed in implanting, introducing still more uncertainty. (51)

I picked a rough midpoint estimate of a 3 year lifespan, and assumed that, on average, 15% would implant. So if I dose at 400 every 6 months, this works out to 60 whipworms implanting per dose, and if they live 3 years on average I will end up with 60 times the 6 doses in the 3 years before the first group in this series of them dies, which equals a colony of 360 whipworms. The lower estimates of a 1 year lifespan and 10% implanting works out to a colony of 80. The upper estimates of an 8 year lifespan and 20% implanting works out to a colony of 1,280. So, if I assume my physiology is reasonably normal, the size of my colony might range anywhere from 80 to 1,280, with a very rough midpoint estimate of 360.

Notes

(1) Companies that sell these organisms carry out a far more rigorous cleaning process than I do, since I'm merely self-inoculating.

"But in short we use a combination of flotation, centrifugation, filtration, and flocculation to separate whipworm from faeces, it is then incubated in an aerated one molar sulphuric acid solution for three weeks, then soaked in Chlorhexidine for at least a day, then washed with various antibiotics and then suspended in a buffered saline solution called M9." (Link)

Also, these companies' donors have been thoroughly tested for contagious diseases, even though there's no evidence that helminths are a vector for disease. (Meredith E. K. Calvert, Potential Transmission of Pathogens Between Human Hosts by the Hookworm, *Necator Americanus*, 4 May 2008, available from the **Files section** of the **Yahoo Helminthic Therapy Forum**.) RETURN

(2) While this will kill hookworm 3rd stage larvae, (See Note 1 in the **Human Hookworm Incubation** document.) I'm not certain how effective this is for whipworm eggs. Until I have more complete information I thoroughly clean everything and boil the liquid remains. RETURN (2a) RETURN (2b)

(3) I still get whipworm eggs even if my hookworm culture has failed completely. It seems that whipworms are more robust egg producers. RETURN

(4) An egg is the fertilised state of an ovum with the genetic materials of a male gamete. An ovum is the female gamete. Since the nucleus of this reproductive cell contains only half the number of chromosomes that a usual cell does, an ovum is a haploid cell. (**Difference Between Ovum and Egg**.) RETURN

(5) I do not use formaldehyde in my Sheather's solution since this might harm the eggs. RETURN

(6) I bought my mesh locally, but I could also have obtained them through the supplier, Small Parts, that sells them on Amazon: **Nylon 6/6 Woven Mesh Sheet, Opaque Off-White, 12" Width, 12" Length, 30 microns Mesh Size, 18% Open Area (Pack of 5) and Nylon 6/6 Woven Mesh Sheet, Opaque Off-White, 12" Width, 12" Length, 20 microns Mesh Size, 14% Open Area (Pack of 5)**. RETURN

(7) To get the mesh filtration going, it can be helpful to wet the mesh, by spraying it, before starting. RETURN

(8) It's easy to get the 20 and 30 micron meshes confused. To be sure which I'm dealing with I check them with my microscope. With experience I can also fairly well tell the difference by feel. RETURN

(9) When the sample is on top of the 20 micron mesh I use a Pasteur pipette (which is cut off across about half an inch from the tip to widen the opening) to suck the top of the mesh after the sample has been thoroughly rinsed. I add water and then suck the mesh's surface repeatedly, pulling the open mouth of the pipette over the mesh gently as I suck up the water. Even after all visible "dirt" of the sample material is gone there can be still a fair number of eggs left behind on the mesh. I repeat this process a number of times past any visible remains to collect as much of the sample as possible. RETURN (9a) RETURN (9b)

(10) When sucking the eggs off the top of the 20 micron mesh, fibres will often come off the mesh and get into the sample. A simple way of removing them is to place a clean 30 micron mesh on top of the Glad storage container when I'm putting the material into it. I expel the eggs and liquid onto the top of the mesh and then repeatedly spray them through it into the Glad container, using dechlorinated water. This is why I often refer to my filtration procedure as 30/20/30: first 30, then 20, and then 30 again. I have also taken to storing all my meshes in the cold

in my regular refrigerator (obviously not my unplugged mini fridge) in case there is some sort of deterioration going on, although I have no idea if doing this is helpful.

Although I haven't performed this experiment yet, another way of avoiding fibres from being sucked off the 20 micron mesh would be to simply remove the mesh from the cup and then flip it over on top of a 30 micron mesh that is rubber banded on top of a party cup. Then I could spray through both meshes until the eggs had fallen off the 20 micron mesh. Then remove the 20 micron mesh and spray any remaining eggs through the 30 micron mesh. And finally transfer the contents of the cup into a Glad container. Of course, I would spray rinse the cup thoroughly to ensure that all the eggs were transferred. So the 20 micron mesh shouldn't be producing fibres, and the 30 micron mesh should be removing them in any case. RETURN

(11) Parasitology Proficiency Testing Program – Wadsworth Centre / Integrated Guide to Sanitary Parsitology, p. 12. RETURN

(12) As discussed earlier, after culturing for 8 days it's *extremely* unlikely that any viable hookworm eggs could still be left, and, even if there were, the eggs of the hookworms, *Necator americanus*, average about 60 by 40 microns, which means that they would be filtered out by the 30 micron mesh.
("They [*Necator americanus* eggs] are oval and measure approximately 60 X 40 microns."
http://www.wadsworth.org/parasitology/critiquesJun03.htm)

Also, it's the width of whipworm eggs that is the relevant dimension for this filtration process, since the whipworm eggs can be counted on to rotate appropriately when agitated in liquid on top of a 30 micron mesh surface such that they will pass through it. RETURN (12) RETURN (12b)

(13) The Buoyancy of Certain Nematode Eggs – Willi Sawitz, The Journal of Parasitology, 28(2), April 1942. p 97. Table 2.

TABLE 2.—*Percentages of recovery of* Trichuris trichiura *eggs from different levels of $ZnSO_4$ solutions of various specific gravities. Summary of 5 experiments*

Specific gravity	1.120	1.130	1.140	1.150	1.160	1.165	1.170	1.180	1.200	1.300
Surface layer	0	1	2	23	49	66	75	85	99	100
Supernatant { upper	0	0	2	7	5	5	2	5	0	0
liquid { lower	0	0	4	7	7	5	4	3	0	0
Sediment	100	99	92	63	39	24	19	7	1	0

As an aside, the specific gravity of Hookworm Eggs is between 1.055 and 1.180.
The Buoyancy of Certain Nematode Eggs, Willi Sawitz, Journal of Parasitology Vol. 28, No. 2 [Apr., 1942]. p 101.
It is also reported to be 1.055 in "The Specific Gravity of Hookworm Eggs"
W. Sawitz, Am. J. of Tropical Medicine, s 1-19 (2), 1939, p 171-179

Also, for comparison, here is the specific gravity of several other nematode eggs
Toxicara canis Roundworm 1.09
Ancylostoma Hookworm 1.0559
Trichuris vulpis Whipworm 1.1453

So, if I'm using 1.200 separation, all the types of egg mentioned immediately above this sentence would float, and all those mentioned immediately below would sink.

Taenia Tapeworm 1.2251
Physaloptera Stomach worm 1.2376
(Source: Sheather's Sugar Flotation Solution, Jorvet Jorgensen Laboratories, inc.) RETURN

(14) I tightened up my density separation from 1.210, to both 1.200 and 1.130. With this different protocol I got a fairly clean result, so I think that I will be using a 1.130 centrifuging along with the 1.200 one from now on. I now suspect that faecal material changes its density over time. Previously a 1.210 separation, along with the 30-20 size separation, gave OK results. This time, 1.200 and 30-20 resulted in a very dirty sample. Speculating here, if I had let the faecal material age for more than a week I might have obtained better results. Since I hadn't done this, it was only when I added the 1.130 separation step that I succeeded. Of course, when I do the 1.200, I'm pulling the eggs from the top of the tube with a cut-off Pasteur pipette, since the eggs float in this density of solution. And, with the 1.130 density separation, I first suck off the top of the tube, and then discard this faecal-laden material. Then I refill the top of the tube with 1.130 solution, re-centrifuge the tubes, and then again suck off the top of the tubes and discard this material. Then I pull the eggs up from the bottom of the tubes using a non-cut-off Pasteur pipette.
RETURN (14a) RETURN (14b)

(15) I only use liquid, and not powdered, Rid-x because the powdered form will introduce particles into the final result. RETURN

(16) If I'm still producing whipworm eggs after a number of years of dosing with Rid-x treated eggs then I'll be confident that the Rid-x doesn't harm them. The quickest way to be sure about this would be for me to prepare some eggs using the protocol that includes Rid-x and then see if an inoculated person (obviously, someone who didn't have any whipworms before this) produced eggs after about three months, since the adults mature in about 3 months. (Marcial-Rojas, RA. 1971. Pathology of Protozoal and Helminthic Diseases with Clinical Correlation. Baltimore: Williams & Wilkins. p. 661.) Unfortunately, I can't address this question because I have no close family members to perform this experiment with. RETURN

(17) I tried removing the last bits of what I think is cellulose using cellulase enzyme, but this destroyed the eggs. Cellulase enzyme comes in a variety of forms, so for all I know some other form might not have harmed the eggs. But cellulase is hard to obtain, there is very little of any contamination left at this point and what is left is probably cellulose, as found in vegetables (celery, for example, **is mostly cellulose**); and, since I had run out of patience at this point, I abandoned this line of enquiry. RETURN

(18) I punched small holes in the lids of all the Glad containers used in this protocol because the eggs need some oxygen, and they should also not be held in a closed container with possibly decaying material. I'm now storing my Glad container, which contains my whipworm eggs, within an open topped square plastic container. So the Glad container sits in the middle of a nest of surrounding aluminium foil, which serves as a buffering cocoon, all inside the larger square container (technically a cube). This arrangement reduces the possibility that I might accidentally overturn the Glad container, and so lose my whipworm eggs. Of course, this arrangement is kept inside my mini-fridge. RETURN

(19) When I'm examining how clean the eggs' are, I use a two-step process.
1) I first focus on the plane of view at the eggs' level/depth, scanning for debris within the eggs.
2) I then focus up to the surface plane of the water bubble that is on the slide, scanning for any floating debris.

There are some bits of stuff (probably cellulose) left, but I remind myself that other food I eat has undesirable things in it too. Popcorn is a special favourite. "1 or more rodent excreta pellets are found in 1 or more subsamples, and 1 or more rodent hairs are found in 2 or more other subsamples" (**Defect Levels Handbook**)

Also, I remember hearing a radio program that reported that about 80% of the food ladles/handles used in smorgasbords have urine and faecal material on them because people didn't wash their hands after using the bathroom. The inside surfaces of a public bathroom are very likely worse. (**Think before you flush or brush**) See also, **Close the Toilet Lid When Flushing or Brush With Poop. Your Choice** and **Topic: Close the lid before you flush?**

I also remind myself that faecal transplants (using either the oral or anal routes) are becoming more acceptable to allopathic medicine. And the way our body's **carbohydrate fermentation and absorption** works means that a significant part of our food is pre-digested by our gut bacteria. In other words, we are eating their faecal materials. As if I needed any more gross facts to get over any squeamishness over contamination, I remind myself that **alcohol is yeast urine**, that **cheese is bacteria faeces**, that data has confirmed the transmission of fecal coliforms in communal bathrooms at a university, that **toothbrushes can serve as a vector for transmission of potentially pathogenic organisms**, and that **there's probably poo in our ground beef**. RETURN

(20) I have to make sure that micron mesh doesn't come into contact with the acid, or **it will dissolve**. RETURN

(21) Although 500 ml size might be better for measuring the distilled water for the Sheathers solution, I think that 250 ml is a convenient size for using a hydrometer to check the solution's density. RETURN

(22) I must be very careful when handling a hydrometer, since they are *extremely* fragile. When I take it out of its box I try to always hold it with both hands, one on the stem and one cupped underneath it. To make a soft bed for it when I'm putting it down on the table I lay out three rectangular wet sponges 7.5 cm x 11.5 cm x 1.25 cm (3 x 4½ x ½ inch) in a 34 cm (13½ inches) long line on the table. When I lower it down into any solution I'm careful to not even allow it to bang slightly against the sides or bottom of the container. Then I carefully rinse it under flowing water in the sink and put it away as soon as I'm done using it RETURN (22a) RETURN (22b)

(23) I modified a sieve by cutting the handle off with a hacksaw, and then used **Shoe Goo** to cover over the resulting sharp edges. RETURN

(24) It's almost essential to have started with soft stool. If the stool is hard to begin with, this part of the process will be many times more difficult, and could take hours. So I eat a large quantity of celery the day before starting the culture to try to ensure that it will be soft. However, this means that when I have to defecate, I have to do so immediately. Since I will typically be out and about, I need to carry a couple of suitably sized Ziploc type bags to double bag the stool in, and then quickly get it back home and into a culture. I have considered using a dedicated blender to emulsify the stool, but haven't yet done this experiment, and I don't know whether this might damage the eggs. RETURN

(25) “Moderate drying inhibits embryonation; heat from the sun’s rays, freezing temperatures, decaying matter such as stored faeces, various strong chemicals, and prolonged drying kill the eggs and embryos.” (Jones AW. Introduction to Parasitology. 1967. p 221) RETURN

(26) I made my hand-scoop from a soft plastic cereal bowl. I cut the plastic with kitchen scissors to fashion a 10 cm (4 inches) wide and 2.5 cm (1 inch) deep scoop. RETURN

(27) I use the weakest rubber bands that will still do the job, since I don’t want to take the chance of tearing the mesh. Also, in this paper, when I'm referring to a "cup" I'm almost always referring to the party cups listed in the materials section. RETURN

(28) I ended up with two sizes of Pasteur pipettes, one about 15 cm (6 inches) and the other about 22 cm (8¾ inches). The 6 inch ones are the ones I use most frequently. The 22 cm (8¾ inches) ones, which are typically used for perfume applications, probably aren't needed, but they have proved useful for sucking up material from deep inside a bottle of the 1.130 or 1.200 solutions when they’re getting empty. If my test tubes were much deeper, the 22 cm (8¾ inches) pipettes also would be needed if I ever needed to draw up material from the bottom of a tube. RETURN

(29) I particularly like the **Rubbermaid 32oz Heavy-Duty Spray Bottle** because it has a strong spray, I find that I need to spray down at a somewhat horizontal angle into the mesh in the party cup, but, given the way the bottle's internal mechanism works, the sprayer stops working as the bottle empties, and, even if it’s still half full, it won't spray. Due to this, I find it's best to have a number of spray bottles, otherwise I need to refill them frequently. About six appears to be the ideal number. RETURN

(30) Changing micron mesh to a new party cup when it has egg bearing material on top of it is a little tricky, since I have to get the rubber band off, transfer the mesh to a new cup, and then get the rubber-band back on, all the while keeping the mesh depressed so that I don’t spill the material that is sitting on top of it. I don’t want the depression in the mesh to become flat. This is really a two person job, and the ideal one-man solution would probably involve some sort of spring loaded device that snapped down around the mesh and held it on the container, but here’s my method for one person doing it while also utilising a rubber band to keep the mesh in place. To ease the task, I created a simple third hand by cutting off the top half of a 0.7 litre (24 Am fl oz) (upside down **conical frustum**-shaped) **yogurt container**. I had to make sure that the cut edges were smooth with no **burrs** so that they won’t later snag the mesh. The resulting half yogurt container's dimensions are about 6.3 cm (2½ inches) tall, with a 9.5 cm (3¾ inches) diameter circular mouth, and an 8.25 cm (3¼ inches) diameter base. The party cups are about 12.7 cm (5 inches) tall, with a 9.5 cm (3¾ inches) diameter circular mouth, and a 5.7 cm (2¼ inches) diameter base. This arrangement leaves about a 1.25 cm (½ inch) circumference gap between the two when the cup is placed down into the container. So I place the cup, with the mesh rubber-banded onto it, down into the container, tucking the mesh fabric down into the gap space that's between them. When I want to transfer the mesh, I hold the top of the mesh in place, cupping one hand down onto it, grasping the top rim of the cup gently but firmly through the mesh. Then, with the other hand, I pull the rubber band away from the side of the mesh on the cup and slip the band out and down onto the outer container, transferring the band to it. Then I lift the cup and set it next to another fresh and empty cup. Next I lift up the mesh and transfer it to the new cup. I then put this down into the half yogurt container, again tucking in the mesh. Then, while holding the top mesh in place as before, I slip the rubber band off the half yogurt container, and up and onto the new inner cup, such that the rubber band is now holding the fabric in place once again. If I do have to use my gloved fingers to adjust and force the mesh back down a bit into the new cup, increasing the depression, I then spray my fingers over the mesh so any eggs that might be clinging to them won’t be lost, but instead will fall into the mesh. RETURN (30a) RETURN (30b)

(31) If I’m doing a lot of scoops of material this will take a lot of water, so, to have enough dechlorinated water, I fill up two 15 litre (16 quart) containers. I use dechlorinated water for this work, although I’m not completely sure that this is necessary. Dechlorinated water is easily prepared by filling a plastic container, 15 litre (4 US gallons) size with cold tap water, and then letting it stand with the lid off for 24 hours. (I always use cold water because there is less risk of contamination. Hot water can much more easily leach contaminates from pipes.) 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 eggs 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. There are jug water filters (**Brita Jug Filters**) and filters that attach to the home tap/faucet (**Home Kitchen Simple Easy Faucet Tap Water Clean Filter Double Purifier Head Pp** / **Culligan FM-15A Advanced Faucet Filter Kit** [**Culligan Compact Faucet Mount Filter Installation and Operation Instructions**]) and some of these claim to remove over 97% of the chlorine, but I haven’t tried any of these to see whether they might work.

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 eggs. 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 this type of product**), 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 - The Tropical Tank / Chloramine Facts - Citizens Concerned About Chloramine (CCAC) / Frequently Asked Questions - Chloramines - The City of Tulsa / Removing chloramines from water - 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 38 litres [10 US gallons] of water) more or less instantly renders this safe for aquatic animals (**How to remove chloramine from tap water?** and **Review- Water Conditioners and Dechlorinators**). **RETURN**

(32) It takes about a litre (quart) of water per scoop of egg-containing solution to work it thoroughly through a 30 micron mesh. This can take a while, and so I find it useful to have several 30 micron meshes with party cups going at once. Since I am first going from 1.270 separated stool, it's likely that, when using a 30 micron mesh, this mesh will become clogged with sediment. If a mesh does becomes clogged I spoon transfer the contents to another 30 micron mesh, and then heavily spray as much of the remaining material as I can through the mesh. Then I remove and clean the mesh.

The meshes need to be hand cleaned with warm soapy water and rinsed thoroughly after, and between, each time they are used. If I'm using them again right away I also then spray rinse them with dechlorinated water, although I doubt this is necessary. If I'm drying them, I hang them with a clip freely suspended in the air, like **photos drying in a dark room** so that the wet meshes do not rest on anything from which they might accumulate contaminants. I would only want to use clips similar or identical to the magnetic clips listed in the Materials section rather than strong metal clips, such as **Binder Clips**, since the area of contact between the jaws of the clip and the mesh needs to be as large as possible to more evenly distribute the pressure on the mesh. A clip that grips in a way that's more like two fingers pressed together is better than one that acts like a knife edge pressed against a hard surface. A hand held blow dryer will dry a micro-mesh very quickly, although **I have to make sure I don't get it too hot**.

When cleaning Pasteur pipettes with washing-up liquid/dish soap and water, I alternately suck and squirt some of the soapy water several times. Then I do the same with clean water. When rinsing them out I find it sometimes useful to point them up into the stream from the tap and repeatedly fill them and then squirt out the liquid rapidly upwards. The 22 cm (8¾ inch) ones can be very difficult to rinse the soap out of. **RETURN**

(33) I have to be careful to not leave a drop on a slide unattended too long, since the heat from my light will evaporate it over time. **RETURN**

(34) I'm aware that each time I handle the eggs I risk losing some, so the fewer times I handle them the better. In any case, to minimize the loses, I rinse my Pasteur pipettes after I've just finished sucking up and expelling a sample so as to not leave any eggs that might be clinging to the pipette's inside or outside. I do this by drawing up fresh dechlorinated water from a shot glass and expelling it into the mesh. **RETURN**

(35) I never squirt any liquid from a Pasteur pipette back into the Chug bottle because there's too much risk of contaminating the solution. Instead, I dispose of it, even if I know it's completely clean. In the case of any larger debris appearing, this can be removed by putting the Chug bottle liquid through a 20 micron mesh. **RETURN**

(36) Later on, when I might be transferring a solution with eggs in it *from* any container, I spray up into the container onto its internal bottom and internal sides a number of times, repeatedly letting the liquid empty. Most of the eggs will typically be on the bottom of the container since they sink in water. When I'm transferring eggs from a microscope slide back onto a mesh I pick up the slide, hold it over the mesh and spray it a number of times, letting the liquid drip off the slide and onto the mesh. **RETURN (36a)** **RETURN (36b)**

(37) I like to get the Chug bottles out and warm them up a little, although I very much doubt this makes any difference. **RETURN**

(38) I've found that storing the 1.130 and 1.200 solutions in the refrigerator for longer than a few weeks isn't worth the trouble. Even in a sealed container, they develop "fibres" in them, which I think are condensed sugar. These can be filtered out with a 20 micron mesh, but then the density can also change slightly, and might need to be adjusted. I find it just as easy to simply make 2 new chug bottles' worth, 2 litres (2 quarts) of it each time. **RETURN**

(39) An unbalanced centrifuge could easily become unstable, and therefore dangerous. **RETURN**

(40) I don't know the eggs' tolerance level for g-forces, but I'm assuming it's reasonably robust. I've read of a specialised method for separating ova using Eppendorf tubes at 13,000 RPM, however I don't have that citation. Other technical sources I've consulted typically talk in terms of units of 2,000 RPM and 500 g-forces. (Bowman DD. Georgis' parasitology for veterinarians, 7th ed. Philadelphia: WB Saunders. 1999. p 287.) Potentially I could address the question of whether or not 1,600 g-forces harm the ova in the same way as I described in note 16. **RETURN**

(41) This filtration step is very likely not necessary. Liquid Rid-x easily pours through the 20 micron mesh with no apparent residue, except for foam created by shaking the bottle. (The instructions on the Rid-x bottle say to shake the bottle, so I do this each time I use some.) RETURN

(42) I'm only using either distilled or dechlorinated water throughout this whole protocol. RETURN

(43) A closed system would become contaminated with breakdown products and toxins. So I need to periodically filter the sample to remove them, and then introduce fresh materials. Also the pH could shift, which could slow or stop growth. Four weeks might be overkill, but I'm being thorough here. Extending the Rid-x treatment beyond 4 weeks does not clean the sample any better. RETURN

(44) I always, and only, **add acid to water**, **never water to acid**. When acid and water mix they create an exothermic reaction, which means the reaction releases heat. If I were to add water to acid this could conceivably boil and splatter the mixture, which could be potentially dangerous, although, since I'm using a mild acid, this possibly isn't extremely dangerous, but I'm following correct lab practice at all times in all of this. And if I ever did use a stronger acid then it indeed would be very dangerous to have that acid splatter. I don't want to get into bad habits that might prove disastrous in the future.

Even though my Ph meter seems flaky, (perhaps all of them are) and will jump around strangely, it eventually settles down such that the results make sense and I believe them. It indicates that the car battery acid is stable and doesn't appear to change pH even over a long period of time. According to the meter, when it's been calibrated, the acid I have in its original container is still a pH of 3 after a year and a half.

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. The acid bath with the eggs in it also remains a pH of 3.0 for the three weeks the eggs are soaking.

- pH = 0 => Battery Acid which is very strong Hydroflouric Acid
- pH = 1 => Hydrochloric Acid secreted by stomach lining
- pH = 2 => Lemon Juice, Gastric Acid, Vinegar
- pH = 3 => Grapefruit Juice, Orange Juice, Soda
- pH = 4 => Tomato Juice, Acid Rain
- pH = 5 => Soft Drinking Water, Black Coffee
- pH = 6 => Urine, Saliva
- pH = 7 => "Pure" Water
- pH = 8 => Sea Water
- pH = 9 => Baking Soda
- pH = 10 => Great Salt Lake, Milk of Magnesia
- pH = 11 => Ammonia Solution
- pH = 12 => Soapy Water
- pH = 13 => Bleach, Oven Cleaner
- pH = 14 => Liquid Drain Cleaner such as "Draino"

When I've looked at the pH that providers and laboratories seem to be using it seems that the ideal pH should be between 0 and 2, inclusive, e.g.,

"... a composition for storage and development of eggs from helminthic parasites, where the composition further comprises a liquid carrier having a pH value of below 7 at a temperature of from 10 C to ambient temperature. The liquid carrier can be sulphuric acid, H2SO4 with a pH in the range of from 0 to 2, ..." (Link)

"… it is then incubated in an one molar sulphuric acid solution for three weeks"…it is then incubated in an aerated one molar sulphuric acid solution for three weeks, then soaked in Chlorhexidine for at least a day, then washed with various antibiotics and then suspended in a buffered saline solution called M9." (Link)

"*Trichuris* infective.- *Trichuris* spp. infective egg, was incubated for a period of four weeks at 26 ºC (78.8 F) "in a solution of 0.1 N" (1 pH) "sulfuric acid, obtaining the development to a stage 2 larva (L2)." (Link)

Note: Talking about Normal acid strength can be confusing, since people do not always distinguish between dissociated sulphuric acid in water, versus non-dissociated pure sulphuric acid not mixed in water. There is a difference in strength of a factor of 2 between these.

"0.12N is indeed 0.12 normal. In short, there are 0.12 moles of hydrogen ions per liter. Since this is sulfuric acid, it is 0.06 moles of" (non-dissociated) "sulfuric acid per liter." (Link)

So these three sources have given pH numbers for soaking eggs as ranging from either 0 to 2, or 0 to 1.

When working with acids it is often necessary to convert from molarity to pH. For this immediate discussion I'm only concerned about sulphuric acid, since how the acid dissociates in water makes a difference. 1 mole of non-dissociated pure H2SO4 that's then put in water, such that it results in 1 litre of total solution, will dissociate into 2 moles of H- ionic atoms and 1 mole of SO4+ ions. Since I get 2 hydrogen ionic atoms and 1 SO4 ion from each H2SO4 molecule this will result in a 2 molar acid from one mole of H2SO4, since the number of H- ion atoms are what determine the strength of the acid. The pH is defined as pH = -log (the molar strength), which equals the (base 10) positive log of (1/molar strength). So for a molar strength of 1, this means the pH will be log (1/1) = 0.

Dissociated Sulphuric Acid in water (Log Base 10 Calculator)

Molar Strength	pH
1	0.0
½	+0.3
¼	+0.6
1/8	+0.9
1/10	+1.0
1/100	+2.0
1/1,000	+3.0

I confirmed these results using this on-line pH Calculator. For each calculation I had to enter 4 pieces of information. For example,
1) The acid was "sulfuric," since another acid might produce a different number of H+ ions per mole from the original non-dissociated molecule.
Under "Volume Method"
2) The concentration of acid, 1.0 moles
3) The volume of acid 1.0 liters
4) The total volume of the final solution 1.0 liters.
This gives a pH of 0.

I also confirmed these results at: Approximate pH for different concentrations of various substances

From the above table it's clear that the pH scale is based on powers of 10. That is, a 3.0 pH acid is 100 times weaker than a 1.0 pH acid, and it is 1,000 times weaker than a 0 pH acid. So, if I'm using sulphuric acid and ideally want the pH to be from 0 to 2, then my molar strength should be from 1 to 1/100 of a mole of H+ ions per litre. Which means the amount of the original H2SO4 non-dissociated molecules should be from ½ of a mole to 1/50th of a mole of them. If I pick a mid-point goal of a pH of 1, since this equals the strength of stomach lining acid, then I would want a 1/10 molar final solution, or 1/20th of a mole of pure H2SO4 that was then dissociated into water, such that it added up to one litre of total final solution.

I considered making my battery acid stronger by boiling it, but this seems dangerous. (How to Make Sulfuric Acid at Home.) Also, the reduction necessary to go from a pH of 3 to 1 would be a factor of 100. So I'd have to buy 100 bottles of battery acid, at a cost of about £640 ($1,000/€895) and then reduce it to 1 bottle. The alternatives of lemon juice or vinegar, both with a reported pH of 2 (see the above table), didn't seem attractive because these might introduce additional biological material contaminates. Also, lemon juice doesn't seem to be a good enough disinfectant. (Myth Buster: Can You Sanitize Kitchen Tools With Lemon Juice and Salt?) White vinegar is reported to be a reasonable disinfectant: "white vinegar is very effective at killing E. coli, salmonella and shigella bacteria," (Do 'Green' Cleaners Work?) But when I tested the pH of the white vinegar I had, it came out as about 6.0, which was worthless. Flinn Scientific sells 1 pH = 0.1 molar sulphuric acid, as does the Seoh Corporation. However, such scientific supply companies typically won't sell such products to individuals, but only to schools and similar institutions. Having said all this, I have never become ill from my dosings when I'm using 3.0 pH battery acid to kill any bacteria and protozoa that might remain in my sample. RETURN

(45) I'm careful to only add a little baking soda at a time, since the reaction generates a lot of foam. I also try to make sure the plastic spoon is non-BPA, types 1, 2, 4, or 5. RETURN

(46) If it becomes necessary to add more water because of evaporation over time, I add distilled water to the Glad container. Using distilled water keeps the mineral concentration from gradually building up. If I thought it was necessary I could filter and change the water with dechlorinated water every year or so. However, whipworm eggs are capable of developing while in salt water, so they must be very tolerant of such mineral build-up.

(I'm assuming that in the following article the term "trichurids" includes *Trichuris trichiura*.)

“The eggs of the ascarids and trichurids attained the mobile larvae stage in 91-94% of the cases by the 25th day. It was thus proved that clean sea water does not prevent development of geohelminth eggs within the optimum range of temperature.”
(Akademiia nauk SSSR. Contributions to helminthology: published to commemorate the 75th birthday of K.I. Skrjabin. 1966. Verusalem: Israel Program for Scientific Translations. p. 802.) RETURN

(47) The life expectancy of the eggs in storage.

“... Likewise from the experiments of Davaine (1863), Morris (1911) and Epstein (1892) in which the eggs of Ascaris and Trichuris remained infective as long as five years in preserved faeces it has been argued that eggs of these species probably remain viable and infective in nature a corresponding length of time. This opinion has been supported further by the results of experiments by Baillet (1866) and Cram (1924) who found Ascaris and Trichuris eggs to be very resistant to freezing temperatures.”

(Harold W. Brown, **Studies on the Rate of Development and Viability of the Eggs of Ascaris lumbricoides and Trichuris trichiura under Field Conditions**. The Journal of Parasitology. 1927 Sep;14(1):1-15)

"These eggs may remain viable outside of the host for many months if they lie in moist areas, but development of the larvae within them may be delayed by dryness or cold, and when a favourable weather change occurs, the bulk of the accumulated organisms continues development, thus making possible a massive infection."

(Noble ER, Noble GA. Parasitology: the biology of animal parasites. Philadelphia: Lea & Febiger. 1964. pp 373-374) RETURN

(48) The quotations below are all from, **Integrated Guide to Sanitary Parasitology**. (World Health Organization, Regional Office for the Eastern Mediterranean, Regional Centre for Environmental Health Activities, Amman – Jordan. 2004. pp 25, 29-31, 36.)

"A comprehensive resume of the time-temperature requirements for the destruction of Ascaris, Trichuris, Taenia and hookworm eggs is given by Feachem et al. (1983). In general, temperatures above 60˚C (140ºF) are rapidly lethal to eggs." p. 25.

"Exposure of eggs and partly developed embryos to freezing temperatures (-9˚C to -12˚C) resulted in a high percentage of fatality in Trichuris but had no apparent effect on Ascaris. It was also found that the further Trichuris eggs are developed, the less resistant they were to freezing" p. 27 (Nolf, 1932)

"Trichuris eggs were much more resistant to the effects of the light. The difference in susceptibility was not definitely understood, but it was suggested that the dark pigmentation of the outer covering of the Trichuris eggs probably offers them considerable protection from the shorter light rays. It was also demonstrated that a very short exposure was sufficient to prevent a large percentage of Ascaris eggs from reaching embryonation, and slightly longer exposure was completely lethal to them" p. 29 (Nolf, 1932)

"Trichuris eggs were killed at slightly lower temperatures (52˚C to 54˚C) for a shorter time than were Ascaris eggs (Nolf, 1932). The additional minutes exposure plus one degree rise in temperature reduced the percentage of surviving Trichuris eggs from 67% to 14% and the Ascaris eggs from 93% to 26%. Further, if the eggs of Ascaris and Trichuris are subjected to temperatures above 52˚C to 54˚C for even a brief period of time, most of them will lose their ability to embryonate." p. 29

“Trichuris eggs required a more highly saturated atmosphere before they could develop than did Ascaris eggs, and the former was less resistant to desiccation. It was evident that under fractional relative humidities, the eggs of Trichuris succumbed more readily than did those of Ascaris. The explanation for the difference may lie in two basic differences in the eggs: (1) the comparative sizes: Ascaris eggs are larger and have a considerably greater surface of the fibrous membrane through which the diffusion of gases occurs; (2) the difference in time required to complete embryonation under optimum conditions: Trichuris eggs require more time to complete their development than do Ascaris." p. 30 (Nolf, 1932).

"Many early researchers noted that Ascaris lumbricoides degenerated rapidly on various soil types when exposed to direct sunlight (Ogata, 1925; Brown, 1928; Caldwell & Caldwell, 1928; and Otto, 31 1929). Studies on the effects of humidity on Ascaris eggs under field conditions having noted that the human ascarid eggs were rapidly killed in faecal cultures on sand in the direct sun (Brown, 1928). It was concluded that desiccation and heat were both important in killing the eggs, and the results indicated that soil type is an important factor in the rate of development and viability of the Ascaris and Trichuris eggs. The cultures on sand in the sun did not produce any embryonated eggs, while those in the shade did. Those in loam, clay and humus soils became embryonated but Ascaris on humus soil was slower in development due to the minimised oxygen." p. 30

“Studies on the effects of humidity on Ascaris eggs under field conditions having noted that the human ascarid eggs were rapidly killed in faecal cultures on sand in the direct sun (Brown, 1928). It was concluded that

desiccation and heat were both important in killing the eggs, and the results indicated that soil type is an important factor in the rate of development and viability of the Ascaris and Trichuris eggs. The cultures on sand in the sun did not produce any embryonated eggs, while those in the shade did. Those in loam, clay and humus soils became embryonated but Ascaris on humus soil was slower in development due to the minimised oxygen. The temperatures on sand frequently rose above 50°C which was found to be lethal to Ascaris eggs while other soils used never reached those temperatures p. 31." (Ogata, 1925).

"It is quite likely that Ascaris eggs have become adapted to developing in nature in a medium, which is not fully oxygen-saturated, with the result that higher oxygen tensions are not necessary for normal development. It was originally thought that the rate of oxygen consumption was constant in embryonating Ascaris eggs (Brown, 1928); but later work by Fairbairn, (1957) showed that the rate decreases rapidly to about half its initial value during the first 36h, then increases steadily to a maximum after 10 days when the embryo is vermiform. From days 10-25 the rate decreases rapidly again, then declines more slowly to a very low level at 140 days. The initial decline was due to an oxygen debt inherited from the essentially anaerobic metabolism of the adult female worm and the second decline could be an adaptation to prolonged survival in the environment, ensuring that food reserves are not expended."

"The oxygen tension in faecal cultures is usually low (Fairbairn, 1957) and the initial decline could also be an adaptation to this situation. The oxygen requirements of Trichuris eggs are not essentially different from those of Ascaris eggs. Carbon dioxide given off, if allowed to remain in close contact with the eggs, will retard their development. No nitrogen is given off during development of Trichuris embryos" p. 36 (Nolf, 1932).

Feachem RG, Bradley DJ, Garelick H and Mara DD. Sanitation and Disease: Health Aspects of Excreta and Wastewater Management. John Wiley and Sons, Chichester, UK. 1983

Nolf, L. O. (1932). Experimental studies on certain factors influencing the development and viability of the ova of the human Trichuris as compared with those of the human Ascaris. (American Journal of Hygiene, 16(1): 288-322.)

Further Reading and Notes:

Spindler, L. A. (1929 b). **The relation of moisture to the distribution of human Trichuris and Ascaris**. American Journal of Hygiene. 10:476-496.

Silverston NA. **Whipworms in a Cambridgeshire Village**. Br Med J. 1962 Dec 29;2(5321): 1726–1728. [**PDF**]

Bundy DA, Cooper ES. **Trichuris and trichuriasis in humans**. Adv Parasitol. 1989;28:107-73.
(Chapter 3 in: Baker JR, Muller R. **Advances in Parasitology**, Volume 28 Elsevier Science. 1989.)

Rogers WP, Summerville RI. **The Infective Stage of Nematode Parasites and Its Significance in Parasitism** Adv Parasitol. 1963;1:109-77 (Chapter 3 in: Dawes B. **Advances in Parsitology** vol 1.)

Jenkins J. **The Humanure Handbook: A Guide to Composting Human Manure**. 1999.

Feachem RG, et al. **At for Water Supply and Sanitation 3, Health Aspects of Excreta and Sullage Management**. World Bank. 1980 Dec.

Ciziulevicius, S. On the Viability of *Ascaris* lumbricoides and *Trichuris trichiura* Eggs in Compost Heaps. Acta Parasitologica Lithuanica. 1961;3:51-60. (English Summary)

Caldwell FC & EL. **Preliminary Report on Observations on the Development of Ova of Pig and Human Ascaris under Natural Conditions, and Studies of Factors Influencing Development.** Journal of Parasitology. 1928 Jun; 14(4):254-260

Desiccation will kill eggs. See p. 168 in:
Muller R. **Worms and Human Disease**, 2nd ed. CABI Publishing. 2002.

Brown, H. **Studies on the Rate of Development and Viability of the Eggs of Ascaris lumbricoides and Trichuris trichiura under Field Conditions**. The Journal of Parasitology. 1927;14(1):1-15.

Developing eggs will die within a week, if subjected to desiccation.
1) Cheng. TC. General Parasitology. 3rd ed. Academic Press. 1986, p 488.
2) Garcia LS, Bruckner DA. Diagnostic medical parasitology. 2nd ed. American Society of Microbiology. 1993. p 198

"... eggs of the genus Trichuris, which seem particularly able to survive under conditions of extreme cold."
Nakladova 1956, Hill 1957, quoted on p 141 of Dawes B. **Advances in Parasitology APL**. 1963. **RETURN**

(49) Whipworm eggs develop according to this schedule:
4-6 months at 15ºC (59ºF)
3 to 4 weeks at 26ºC (79ºF)
17 days at 30ºC (86ºF)
11 days at 35ºC (95ºF)
Muller R. **Worms and Human Disease**, 2nd ed. CABI Publishing. 2002. p 165.

I have it in my notes that they will not develop if the temperature is over 37ºC (98.6ºF), but I don't have the citation for this.

"A review of the literature furnished the following information relative to optimum temperatures for development of several species of parasitic eggs."
Trichuris trichiura 30 C (86 F)
Feachem et al. (1983). **Integrated Guide to Sanitary Parasitology**. (World Health Organization, Regional Office for the Eastern Mediterranean, Regional Centre for Environmental Health Activities, Amman – Jordan, 2004. p 27. Table 2.3. **RETURN**

(50) Opinions vary widely regarding the life expectancy of whipworms in a host.

* "The adult worms normally live for about 1-2 years where there is repeated reinfection, but can survive for much longer in some circumstances."
Muller R. **Worms and Human Disease**, 2nd ed. CABI Publishing. 2002. p 165

* When reinfection does not occur, most worms die within 3 years.
Myers, E. N., Negron, R. and Pearlstein, H. Intestinal parasitoses in Puerto Rican preschool children at Philadelphia, Pennsylvania in 1958. Am J Med Sci. 1959 Jan; 237(1):59–66. (cited in Marcial-Rojas, RA. 1971. Pathology of Protozoal and Helminthic Diseases. Baltimore: Williams & Wilkins. p 664.)

* Whipworms live 4-5 years.
Henry JB. Clinical Diagnosis and Management By Laboratory Methods. Philadelphia: WB Saunders, 1978: 1779-88.

* Lifespan is 4 to 6 years, but can live up to 8 years.
Brown, Harold W., Neva FA. 1983. Basic clinical parasitology. 5th ed. Norwalk, Conn.: Appleton-Centurry-Crofts. 339 p., p 112. **RETURN**

(51) I don't have the citation for this. **RETURN**

Materials List

I've found that the sort of materials listed below are currently adequate for my cleaning of whipworm eggs, 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 whipworm egg cleaning. 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 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 would not 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 almost always use 40X and 100X magnifications, and it would be very unusual for me to need any stronger than this.

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/€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 ($80/€60) (see graphic).

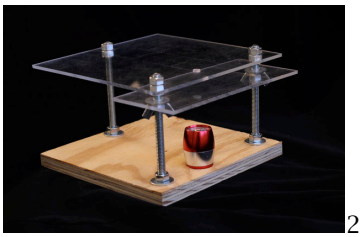
Microscope case

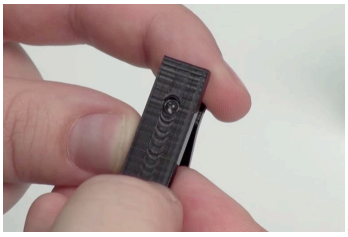
I wasn't sure a microscope case would be necessary, but eventually decided to get one. Various sites suggested that I could save money and yet make a decent case from a tool box with a very inexpensive inner lining made from a one person-sized (approximately 90-96 cm x 190-193 cm [36-38" x 75-76"]) egg 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 that might work include USB microscopes such as the **Plugable USB 2.0 Handheld Digital Microscope** (see graphic 1, and further details **here**); **making a digital microscope using a smart phone** for as little as $10 (see graphic 2); or using a **3D Printed Microscope** with a mobile device, for pennies (see graphic 3).


1


2


3

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., Microscope slides, plain glass, 72/pack (see graphic).

Slide storage box, 25 slides

e.g., Slide storage box, 25 slides (see graphic).

Centrifuge

When getting a centrifuge it was important to anticipate the tubes I would need, since I had to match the tubes to the centrifuge I bought. It turned out that I actually had to modify my tubes by cutting them off, which was a problem, since they turned out to have harness 9, like corundum, sapphire, or ruby; so I had to use a diamond tile saw. I purchased a used centrifuge locally, but, if I hadn't been able to, I could have bought one on eBay. The centrifuge I have is a horizontal one, which is supposed to result in a cleaner separation line, but I'm not sure how much difference this makes. Its relative centrifugal force (RCF) is about 1,600 times gravity, and its speed is about 4,000 RPM. So, if I were to use only Earth's natural gravity, it would take 10 minutes times 1,600 to perform this separation. So 10 minutes X 1,600 = 16,000 minutes, which works out to about 11 days.

I found these guides useful: How to Buy Centrifuges by eBay / Centrifuge Sector Guide / Basics of Centrifugation / Why Horizontal Separation?

Scientists at Harvard have developed a manually-operated, light and portable, very inexpensive centrifuge. (Egg beater as centrifuge: isolating human blood plasma from whole blood in resource-poor settings)

Also: Salad spinner useful to separate blood without electricity in developing countries

Centrifuge Tubes

e.g., **Centrifuge Tubes, plastic** (see graphic).

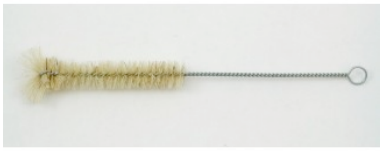
Test Tube Rack

I had to be careful to match the size of my test tube rack to my test tubes. I really like the type shown here.

e.g., **Globe Scientific Inc., 16/17 mm, 60 tube capacity** / **SEOH TEST TUBE RACK PLASTIC For 60 tubes 16mm** (see graphic).

Test tube brush, small

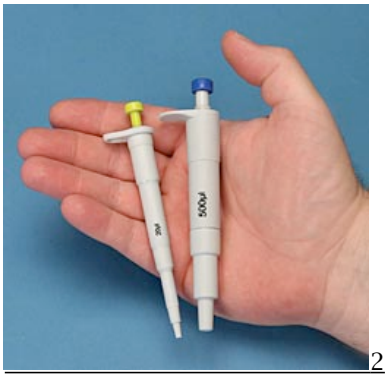
e..g., **Test tube brush, small**



Miinipipette, 100 microlitre

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


1


2

Minipipette tips

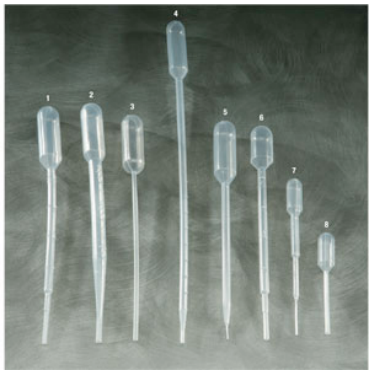
When I bought my mini pipette tips I had to check carefully to make sure that they were compatible with my particular mini pipette, e.g., **MS® Pipette Tips** / **Diamond Tips - Gilson** / **Pipette Tips Company Links** / **Pipettes Suppliers** / **Biohit Optifit Tips** (see graphic 1) / **Pk/100 1-200 Microliter Universal Fit Micropipette Tips, Natural Color** (see graphic 2).


1


2

Pasteur pipettes

e.g., **Disposable 1.0 ml Transfer Pipettes** / **Pipette, disposable, 5ml, 10 pk.** / **Pipette, disposable, 8-3/4 in. long**



Graduated cylinder

e.g., Graduated cylinder, poly, 250 ml and 500 ml: item# CE-CYPP250 (see graphic).

Hydrometer

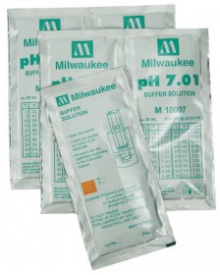
e.g., Wards Natural Science Specific Gravity Hydrometer 1.00 - 2.00 model / 150879 Specific Gravity Hydrometer, 1.00–1.20, 0.002 divisions (see graphic).

pH Meter, Digital, 0-14 pH range

e.g., Milwaukee pH600 pocket digital pH meter | 0-14 pH range (see graphic).

pH 7.0 Calibration Solution, 5 packets

e.g., **pH 7.0 Calibration Solution, 5 packets** (see graphic).



If I happen to have run out of calibration fluid I will calibrate my pH meter by using distilled water, which should be about 7 pH, and newly bought pH 3 battery acid.

Small capacity postal scale

e.g., **Helix Salter FC3250 500x10g Foldable Postal Letter Scale** / **Smart Weigh TZ5000 Sleek Cuisine Stainless Steel Digital Kitchen Scale 5000g X 1g** (see graphic).

Small funnel. I don't currently need to use this size of funnel, but this is the size I would need if, for some reason, I needed to fill test tubes. This type of funnel is typically used for filling perfume bottles, and its neck is small enough to fit into the tube's mouth.

e.g., **Plastic Funnel: 65mm Polypropylene Long Stem Pk 1** (see graphic).

Larger Funnel, for filling the Chug bottles

e.g., 1 x 9" LARGE FOOD GRADE FUNNEL (see graphic).

Set of three nested funnels. The largest of these is probably the right size for filling sprayer bottles.

e.g., Chef Aid 3-Piece Funnel Set, White (see graphic).

Sieve

e.g., Premier Housewares Sieve - 20 cm - Stainless Steel (see graphic). This model looks a lot like the one I bought, before I cut the handle and prongs off with my hacksaw.

Meshes

1) Nylon 6/6 Woven Mesh Sheet, Opaque Off-White, 12" Width, 12" Length, 30 microns Mesh Size, 18% Open Area (Pack of 5) (see graphic).

2) Nylon 6/6 Woven Mesh Sheet, Opaque Off-White, 12" Width, 12" Length, 20 microns Mesh Size, 14% Open Area (Pack of 5)

Clips for holding the meshes

e.g., **Kitchen Craft Set of 4 Magnetic Memo Clips** (see graphic). Although the magnets on these clips are rather weak, they're just strong enough for my purposes. / **Kitchen Craft Magnetic Bag Clips, Multi-Colour** / **OXO Good Grips All Purpose Magnetic Clips, Multi-Colour**.

Trash container (lid type)

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

Stock pot 11 litres (12 quarts)

e..g., **Buckingham Stock Pot with Stainless Steel Lid 26 cm, 11 L** (see graphic).

Plastic containers - 0.5 litre (16 fl oz) - for holding bleach or ammonia, and for use when neutralising the car batter acid.

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).

Soft, thin plastic bowl that has the top 75% cut off, leaving the bottom, and thereby making my hand scoop.

e.g., **Colourworks Melamine Bowls, 15 cm (Set of Four)** (see graphic).

Glad containers

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

Yogurt container/cup, e.g:

Party cups

e.g., Ruby Apple Red American Party Cups - 16oz (455ml) - Disposable Party Cups - Packs of 50 or 100 (50) (see graphic).

Shot glasses, 2 – 4, either plastic or glass

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

Chug Style Bottle 32 ounce/1 quart Rubbermaid "Chug bottles". I need at least two of these.

e.g., Rubbermaid 1 Qt. Sipp'N Sport Chug Style Bottle / Wayfair.com 1 Quart Chuggables Bottle (see graphic).

Spray bottle (for dechlorinated water). I use 6 of these

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.

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

I store the filled Glad containers in an unplugged mini fridge (e.g., the Caldura 17 litre Compact Mini Fridge [see graphic]) on the assumption that, if my home were to burn down, my precious stock of eggs 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. As 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 eggs must use very little air.

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. For example, when I rinse the meshes I run these under the flowing water from the tap. Then I either hang them up, or, if I'm going to use them right away, spray rinse them with the dechlorinated water.

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

Dishrack

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

Bluettes

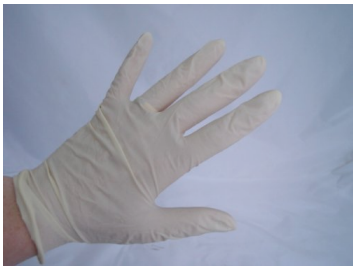
Lehigh Spontex 17005 Bluettes Knit Rubber Glove (see graphic).

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**

Scissors with a fine point

e.g., Nurses Scissors Sharp/Sharp points (331-00) / MagiDeal Stainless Steel Pointed Tip Eyelash Trimmer Eyebrow Scissors (see graphic).

Hacksaw

e.g., Stanley Dynagrip Heavy Duty Hacksaw 1 20 110 (see graphic).

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 making a bed to rest my hydrometer on.

Rubber bands

e.g., Universal Rubber Bands, Size 16, 1lb Pack by Universal (see graphic). From this picture, I'm guessing these are about right.

Zip-lock Bags Ziploc 17.7cm x 19.5cm bags (see graphic).

Disposable plastic spoons

e.g., 100 x Disposable White Plastic High Quality Spoons For Party / Wedding Cutlery (see graphic).

Rid-x. RID-X Septic Tank System Treatment, 3 Month Supply Liquid, 24 Ounce by Rid-X (see graphic).

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

Car battery acid (sulphuric/sulfuric)

e.g., Battery Acid 1 Litre Electrolyte for Dry Batteries car motorbike SULPHURIC (see graphic).

Distilled water

e.g., Distilled Water - 5.5 Litres / 5 Litre Distilled Water / Distilled Water - 20 litres (see graphic).

Terry cloth towels, 2 or 3, small

Paper towels, 1 roll

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

Sugar (sucrose), a number of bags

e.g., Tate & Lyle Granulated Sugar 1KG (see graphic).

Baking soda (sodium bicarbonate/sodium hydrogen carbonate/NaHCO3)

e.g., Arm & Hammer Baking Soda 454 g (see graphic).

Helminth incubation discussion group

The main online venue for discussion about the incubation of all types of therapeutic helminth is the Facebook Helminth Incubation group.

Suggestions/observations

If anyone using this paper 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 edition, August 2015

Last updated, February 2016

Recent changes

The following changes were made at the last update. Many of them are discussed in footnotes 13, 14 and 18, and at the end of footnote 32.

* I'm now centrifuging with a multistep procedure using both 1.130 and 1.200 specific gravity sugar water solutions stored in 2 separate chug bottles.

* When I perform the 1.130 centrifuging I'm pulling the eggs up from the bottom of the tube with a non-cut-off Pasteur pipette.

* When making the Sheather's solution, instead of 2.270 kg (5lbs) of table sugar and 1,775 ml (60.02 U.S. fluid ounces) of distilled water, I'm now using 2.721 kg (6 lbs) of table sugar and 2,130 ml (72.02 U.S. fluid ounces) of distilled water.

* After I've mixed the Sheather's solution in with the stool, I'm now double checking and doing a density check on the stool-laced solution.

* To reduce the possibility of an accident, I'm now storing my Glad container within an open topped square plastic container.

* I'm often using a hand held blow dryer to dry my micro meshes, while being careful to not get them too hot.

* A Waterpik is very useful for cleaning the fecal material out of the bottom of centrifuge tubes.

* Eggs that have clumped together in a container can be separated by a 30-20-30 procedure.
