Standard Operation Procedure – Helminths

1. Scope and Field of Application

The prevalence of helminth infections in people living with basic water and sanitation in third-world countries like most of those in Africa is generally high. Due to the extreme hardiness of the eggs of the roundworm, *Ascaris lumbricoides*, they are used in the sanitation field as a "marker for the safe re-use of human waste". Other commonly found helminths are *Trichuris trichiura*, *Taenia* sp. and in areas with very sandy soils, hookworm sp. It is generally accepted that if any of the various waste treatments used are successful in inactivating *Ascaris* eggs, then all harmful bacteria and viruses should also be killed.

2. Principles

Helminth eggs are thought to adhere to soil particles, possibly as a result of charge interactions with or adsorption of eggs to the particles. Many waste samples, even if they are not from Urine-Diversion Toilets, are often contaminated with silica particles, hence the use of ammonium bicarbonate as a wash solution. Laboratory testing for helminths is based on four main principals: washing, filtration, centrifugation and flotation of the eggs to remove them from the various waste mediums.

- Ammonium bicarbonate is used as both a wash solution and also to dissociate the eggs from the soil particles.
- Filtration, using 100µm and/or 20µm sieves is used to separate larger and smaller particles from the eggs both after washing and after flotation.
- Centrifugation to 1. Sediment the deposit and remove the water before flotation, 2. Aid the separation process during flotation and 3. Sediment the final sieved and washed eggs retrieved during flotation.

- Flotation, using a solution of zinc sulphate at a specific gravity of 1.3 is used to float eggs with a relative density of <1.3 out of the matter retained with them on the 20µm sieve.

3. Storage of Samples

After taking samples from the various waste materials, these should be stored as is, at approximately 4 - 10ºC. Processing is always best as soon after sampling as possible, but providing that there is sufficient moisture and the samples are fairly large, the eggs should be unharmed and development will be arrested at these low temperatures.

4. Laboratory Safety

- Always wear gloves and laboratory coat or plastic apron while processing the samples
- After testing, wash and rinse sieves and beakers and leave to drain on draining rack
- Dispose of used gloves after completion of processing the samples
- Wash hands using antiseptic soap.

5. Apparatus

- Compound microscope with 10x and 40x objectives
- Bench-top centrifuge with a swing-out rotor that can spin a minimum or 8 x 15ml plastic conical test tubes (Falcon tubes)
- Sink with hose attached to tap for washing using high water pressure
- Top balance (scale for weights up to 200gm and accurate to 2 decimal places)
- Magnetic stirrer and bar magnets
- Vortex mixer
- Hydrometer that can measure SG between 1.2 and 1.3 or 1.3 and 1.4

- 100µm mesh stainless steel flat sieve, diameter 200mm
- 20µm mesh stainless steel flat sieve, 200mm
- 20µm mesh stainless steel flat sieve, 100mm
- Plastic test tube racks to hold the 15ml Falcon tubes
- Plastic 200ml beakers
- Plastic “hockey-stick ” shaped stirring rods
- Plastic 3ml pipettes
- Non-sterile gloves
- Applicator sticks and wooden tongue depressors
- Microscope slides (76x26x1.2mm)
- Cover glasses 22 x 40mm

6. Reagents

Ammonium Bicarbonate (AmBic)

Dissolve 119gm of ammonium bicarbonate in 1lt de-ionized water (use a magnetic stirrer and bar magnet) – store in a glass jar

Zinc Sulphate (ZnSO4)

Dissolve 500gm zinc sulphate in approximately 800ml de-ionized water (use magnetic stirrer and bar magnet) and adjust SG using more of the chemical or water to raise or lower the SG to 1.3

7. Procedure

- Weigh 10 or 20gm into a 200ml plastic beaker, on a top-pan balance
- Add 50-80ml AmBic + a magnetic stirring bar, mix on magnetic stirrer for 20 min
- Pour mixture over 100µm sieve which fits on top of a 20µm sieve (wet sieves with tap H2O first)
- Rinse beaker with tap H2O & pour over sieves
- Wash magnet & remove, wash 100µm sieve well (using “hockey -stick” or gloved hand) over 20µm filter, checking bottom sieve for fluid build-up
- Separate sieves and then rinse the 20µm sieve well & wash the material to one side of the sieve
- Rinse all material off 20µm filter into original rinsed-out beaker
- Pour beaker contents into 4 x 15ml conical test tubes
- Centrifuge at 3000rpm (1308g) in centrifuge with swing-out rotor for 5 min
- Pour off supernatant: deposits left in 4 test tubes
- Place test tubes in rack with applicator stick in each (as a stirring rod) & pipette in ZnSO4, 3ml at a time, vortexing in between addition of the chemical, until tubes are filled to 14ml mark
- Centrifuge at 2000rpm (581g) for 5 min
- Pour supernatant flotation fluid over smaller diameter 20µm sieve. Wash out test tubes & keep one aside for re-use
- Wash material on sieve well with tap water & rinse it down to one side of the sieve for collection. Using a 3ml plastic pipette, transfer the material back into the test tube kept aside
- Centrifuge at 3000rpm for 5min to obtain the final deposit

- Pour off supernatant water and pipette up the deposit, place it on a microscope slide, place a 22x40mm coverslip on top, examine & count every *Ascaris* egg, classifying them as viable, potentially viable or dead. *Trichuris* and *Taenia* can also be counted and assessed in a similar manner as for *Ascaris*.

References:

Pebsworth, P.A., Archer, C.E., Appleton, C.C. and Huffman, M.A., 2012. Parasite transmission risk from geophagic and foraging behavior in Chacma baboons. *American journal of primatology*, *74*(10), pp.940-947.