

Appendix 2: Methods

The modified combined formol ether concentration technique

- Add 1 spoonful (approximately 2 g) of fresh stool
- Add 7ml of 10% formol
- Shake for 1 minute
- Check the 15ml centrifuge tube is securely attached to the FPC stainer and pull out the vent-straw in the strainer unit out approximately 1 inch.
- Attach the FPC strainer tightly to the flat bottomed tube contained the faecal specimen and invert with the conical end pointing downwards
- Shake the specimen through the strainer into the 15ml centrifuge tube, tapping the centrifuge tube lightly if necessary
- Unscrew the FPC strainer with the flat bottomed tube still attached
- Top up the resulting solution to 7ml (original volume) with 10% formol
- Add 3ml diethyl ether, a lipid extraction agent
- Cap the 15ml tube and shake for 1 minute
- Centrifuge, using the hand centrifuge for 3 minutes
- After centrifugation the specimen should appear clearly separated into four layers; sediment, formalin, faecal debris and fat, diethyl ether.
- Rim the debris layer using an applicator stick
- Pour off the debris and supernatant fluid.
- Use 10% formol re suspend sediment, making it up to 1ml
- Mix by aspiration with a pipette
- Add 2-3 drops to a slide
- Apply coverslip and view under a microscope

Kato-Katz Technique

PREPARATION

- Attach three pieces of nylon mesh (40x80mm) by one edge to a piece of wooden board, such that when held up by one hand they will act as a sieve for faeces.
- Cut an appropriate number of pieces, 35 mm long, from a roll of hydrophilic cellophane and place them in a jar containing glycerol malachite and leave for at least 24 hours.
- Prepare a plastic Kato template with have a hole of 6mm on a 1.5mm thick template, delivering 41.7g of faeces.

- **PROCEDURE**

- Mix faecal sample thoroughly.
- Label a glass microscope slide with the date, sample identification number and repeat number.
- Use a plastic spatula to place a small amount of faeces on the nylon sieve.
- Use the spatula to press the faecal material through the mesh so that sieved material accumulates on the underside of the mesh.
- Use another plastic spatula to scrape across the lower surface of the mesh to collect the sieved faeces.
- Place a plastic template on the centre of a microscope slide.
- Add faeces from the spatula to the hole on the template so that the hole is completely filled.
- Pass the side of the spatula over the top of the template to remove excess

faeces.

- Remove the template and spatula leaving a disk of faecal material on the slide.
- Place a pre-soaked cellophane strip over the faecal material. The strip must be very wet if the faeces are dry and less if the faeces are soft.
- Invert the microscope slide and press the faecal sample firmly and evenly against the cellophane strip on a smooth hard surface. The faecal material should spread evenly between the slide and the cellophane strip such that it is possible to read a newspaper print through the smear.
- Carefully remove the slide and place downwards on a piece of absorbent paper, allowing excess fluid to be removed whilst the glycerol clears the faeces.
- Examine the slide initially within 30-60 minutes in order to look for hookworm eggs which clear rapidly and will no longer be present after this time.
- Other parasite eggs such as schistosoma sp., ascaris lumbricoides and trichuris trichiura eggs will remain visible and recognisable for many months in this preparation and the slide should be examined again after 1 hour to look for these eggs after full clearing.
- The slide should be examined in a systematic manner in order that the number of eggs of each species can be recorded.
- Multiply the number of eggs of a particular species in each slide by 24 in order to obtain the number of eggs per gram of faeces.