Kato-Katz technique – cellophane faecal thick smear

Materials and reagents
1. Applicator sticks, wooden.
2. Screen, stainless steel, nylon or plastic, 60-105 mesh (Fig. 1).
3. Template, stainless steel, plastic, or cardboard (Fig. 1). Templates of different sizes have been produced in different countries. A hole of 9 mm on a 1 mm thick template will deliver 50 mg of faeces; a hole of 6 mm on a 1.5 mm thick template, 41.7 mg; and a hole of 6.5 mm on a 0.5 mm thick template, 20 mg. The templates should be standardized in the country and the same size of templates should always be used to ensure repeatability and comparability of prevalence and intensity data.
4. Spatula, plastic (Fig. 1).
5. Microscope slides (75 x 25 mm).
6. Hydrophilic cellophane, 40-50 μm thick, strips 25 x 30 or 25 x 35 mm in size (Fig. 2).
7. Flat-bottom jar with lid (Fig. 2).
8. Forceps.
9. Toilet paper or absorbent tissue.
10. Newspaper.
11. Glycerol-malachite green or glycerol-methylene blue solution (1 ml of 3% aqueous malachite green or 3% methylene blue is added to 100 ml of glycerol and 100 ml of distilled water and mixed well). This solution is poured onto the cellophane strips in a jar and left for at least 24 h prior to use.

Procedure
1. Place a small mound of faecal material on newspaper or scrap paper and press the small screen on top so that some of the faeces are sieved through the screen and accumulate on top (Fig. 3).
2. Scrape the flat-sided spatula across the upper surface of the screen to collect the sieved faeces (Fig. 4).
3. Place template with hole on the centre of a microscope slide and add faeces from the spatula so that the hole is completely filled (Fig. 5). Using the side of the spatula pass over the template to remove excess faeces from the edge of the hole (the spatula and screen may be discarded or, if carefully washed, may be reused).
4. Remove the template carefully so that the cylinder of faeces is left on the slide.
5. Cover the faecal material with the pre-soaked cellophane strip (Fig. 6). The strip must be very wet if the faeces are dry and less so if the faeces are soft (if excess glycerol solution is present on upper surface of cellophane wipe with toilet paper). In dry climates excess glycerol will retard but not prevent drying.
6. Invert the microscope slide and firmly press the faecal sample against the hydrophilic cellophane strip on another microscope slide or on a smooth hard surface such as a piece of tile or a flat stone. The faecal material will be spread evenly between the microscope slide and the cellophane strip (Fig. 7). It should be possible to read newspaper print through the smear after clarification (Fig. 8).
7. Carefully remove slide by gently sliding it sideways to avoid separating the cellophane strip or lifting it off. Place the slide on the bench with the cellophane upwards. Water evaporates while glycerol clears the faeces.
8. For all except hookworm eggs, keep slide for one or more hours at ambient temperature to clear the faecal material prior to examination under the microscope. To speed up clearing and examination, the slide can be placed in a 40 °C incubator or kept in direct sunlight for several minutes.
9. Ascaris and Trichuris eggs will remain visible and recognizable for many months in these preparations. Hookworm eggs clear rapidly and will no longer be visible after 30-60 minutes. Schistosome eggs may be recognizable for up to several months but it is preferable in a schistosomiasis endemic area to examine the slide preparations within 24 hours.
10. The smear should be examined in a systematic manner (see Plate 1, Fig. 4) and the number of eggs of each species reported. Later multiply by the appropriate number to give the number of eggs per gram of faeces (by 20 if using a 50 mg template; by 50 for a 20 mg template; and by 24 for a 41.7 mg template). With high egg counts, to maintain a rigorous approach while reducing reading time, the Stoll quantitative dilution technique with 0.1 mol/litre NaOH may be recommended (see Basic laboratory methods in medical parasitology, WHO, 1991).