# An Ultra-rapid Apparatus for Diagnosing Intestinal Parasitoses: a Larvoocyst Detector*

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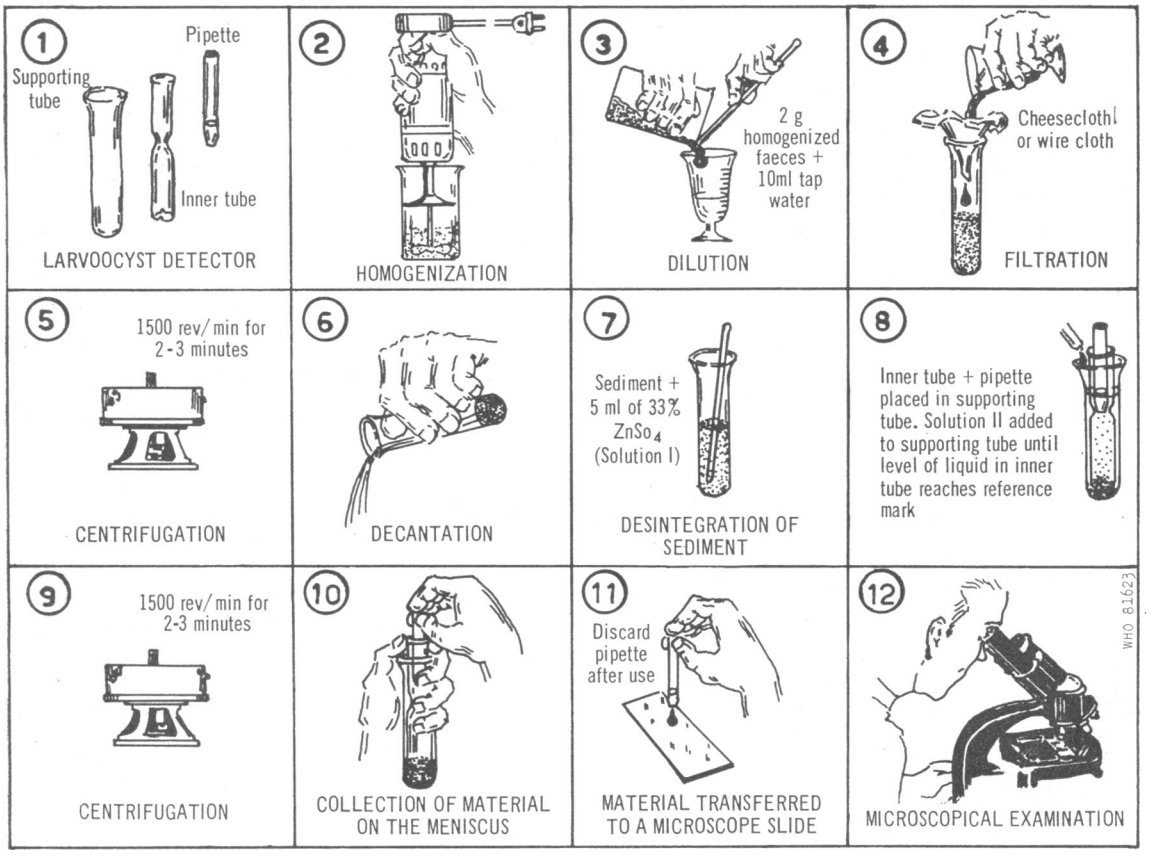
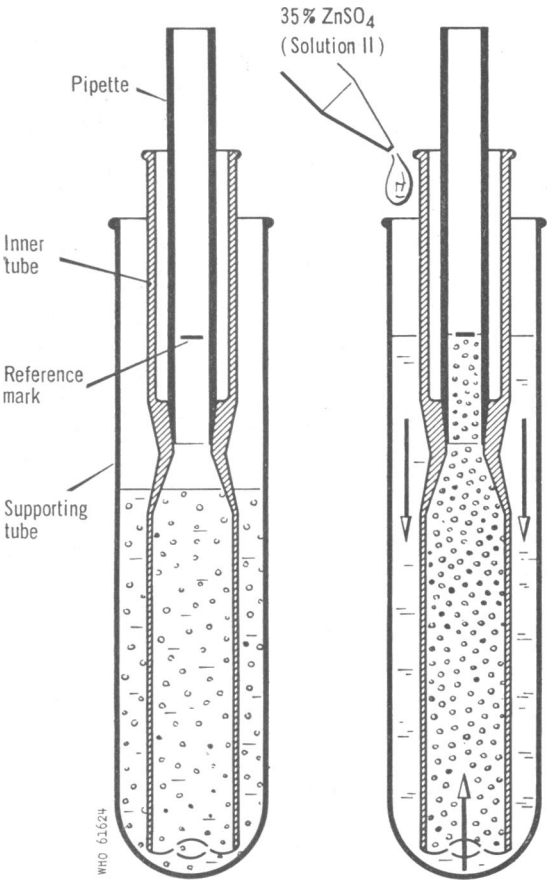
The larvoocyst detector, which is constructed from glass or plastic, consists of 2 concentric tubes: a supporting tube resembling a test-tube, and an inner tube open at each end—the circumference of the bottom edge being notched and the tube drawn into a constriction one-third of the way down. The constriction of the upper third of the inner tube has the inside surface ground with emery in order to connect with the ground tip of a small pipette (Fig. 1 (1); Fig. 2). The internal diameter of the pipette is about 2 mm. There are two forms of pipette; one form has a subterminal enlargement to collect the whole meniscus from a sample, the other

FIG. 1
SEQUENCE OF STEPS IN SAMPLING FAECES WITH THE LARVOOCYST DETECTOR




* This paper has also been published, in part, in *Hospital (Rio de J.)*, 1967, 72, 1509 (in Portuguese).

FIG. 2
ENLARGEMENT OF FIG. 1 (8) TO SHOW LARVOOCYST DETECTOR ASSEMBLED AND SOLUTION II BEING ADDED TO THE SUPPORTING TUBE




form has no enlargement but an outside mark indicates the level to which it should be filled.

With this larvoocyst detector it is possible to carry out very efficiently the method of Faust et al.[a, b] of centrifugal-flotation for concentrating parasite objects in samples of faeces but a denser suspension of faeces can be used together with 2 different concentrations (33% and 35%) of zinc sulfate solution. The method is thus a polyvalent concentration of protozoan cysts and helminth eggs (including *Schistosoma mansoni* in recently passed stools).

[a] Faust et al. (1938) *Amer. J. trop. Med.*, **18**, 169-183.
[b] Faust et al. (1939) *J. Parasit.*, **25**, 241-262.

The use of modern statistical methods in the analysis of research results makes an immediate change in routine techniques for sampling faeces an essential policy. Older techniques use, at most, a few grams of faeces although about 150 g are discharged in a period of 24 hours by an adult on an average diet. Furthermore, the eggs of some helminths such as *S. mansoni* are scattered on the surface of faeces while those of other helminths (e.g., *Ascaris*, *Necator*) are discharged in the upper parts of the alimentary canal (duodenum) and consequently disseminated more uniformly in the mass. The eggs of oxyurids are discharged in the anal region and eggs of *Taenia* spp. are found only when gravid proglottids break away and rupture or when the eggs are discharged through openings in the gravid proglottids—leading in both cases to non-uniform distribution of eggs. It is clear, therefore, that the whole faecal mass must be perfectly homogenized so that all samples will be representative and will contain nearly equal numbers of eggs and cysts of intestinal parasites that are present in the host.

The device illustrated in Fig. 1 (2) homogenizes the whole mass of faeces rapidly. The electrically powered stirrer is of stainless steel and the container is plastic; the whole apparatus is easily cleaned with a detergent solution.

*Procedure* (Fig. 1)

Before the sampling procedure is commenced, the clean, dry supporting tube, inner tube and pipette are each carefully weighed. Two grams, or an equivalent volume, of homogenized faeces are placed in a suitable container and diluted with 10 ml of tap water. The suspension is then filtered through either cheesecloth folded into 4 thicknesses or wire cloth with 60–120 meshes per linear inch (24–48 meshes per linear centimetre) into the supporting tube of the detector that must then be filled up to within 1 cm of the top with tap water. The supporting tube and contents are weighed and then centrifuged for 2–3 minutes at 1500 rev/min. The supernatant is decanted and approximately 5 ml of 33% zinc sulfate solution (solution I) are added to the sediment. The sediment is broken up with a glass rod and uniformly mixed in the solution I. The inner tube, together with a pipette, is placed inside the supporting tube and 35% zinc sulfate solution (solution II) is added to the supporting tube until the level of liquid in the pipette reaches the reference mark (Fig. 2). The apparatus plus the

faecal suspension is again weighed and centrifuged for 2–3 minutes at 1500 rev/min. The pipette is removed from the inner tube—the top being closed with a finger-tip—and the contents are run on to a microscope slide and covered with a cover-glass. The preparation may then be examined under the microscope.

It is important that the pipette should be discarded (and preferably broken to avoid accidental re-use) after each sampling because it is extremely difficult to remove all parasite eggs and cysts from a narrow-bore glass tube.

*Discussion*

The method described by Faust et al.[a, b] for the detection of helminth eggs and larvae and cysts of protozoa in faeces is one of the best that has so far been described, but the method uses only a small (about 1 g) sample of unhomogenized faeces, the sediment is washed 2 or 3 times with tap water and only a small fraction of the material floating on the meniscus is removed with a bacteriological wire-loop. The method described here also employs centrifugation and flotation with zinc sulfate solutions but a 24-hour volume of faeces is homogenized and a representative sample is treated. No washing of the sediment is required (thus economizing in time) and the whole fraction of material floating on the meniscus is collected. This method is therefore more accurate and quicker (taking approximately half the time) than the older technique.

It is of interest to note that Dr L. F. Ferreira [c] compared the results obtained by several sampling methods, including a prototype model of the larvoocyst detector. He concluded that for protozoan cysts, the detector and the MIFC method gave the most satisfactory results and that the detector gave very good results with helminth eggs, except those of *S. mansoni*. The reader's attention is drawn particularly to Tables IV and VII in Dr Ferreira's paper.

[c] Ferreira, L. F. (1966) *Hospital (Rio de J.)*, **70**, 347-368.

# Titration of Antibody to Soluble Antigens of the Cholera Vibrios by Passive Haemagglutination

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Passive haemagglutination has been widely used as a convenient *in vitro* serological method for the titration of antibody to soluble antigen. Soluble antigens, including toxic substances of *V. cholerae*, have become of increasing interest in relation to immunity to cholera. While antibody to toxins may be titrated as neutralizing antibody to the cholerageni toxin [b] and to the permeability factor demonstrable by intradermal inoculation,[c] the associated bioassay techniques are time-consuming and cumbersome, particularly in the case of the choleragenic toxin demonstrable in the rabbit ileal loop.

The present report is concerned with the possible applicability of the passive haemagglutination reaction to the titration of antibody to toxin and other soluble antigens of the cholera vibrio.

*Material and methods*

The rabbit-passaged toxigenic 569B strain of Inaba serotype [d] was used for the preparation of soluble antigens. The most complex preparation was whole-cell lysate (WCL), prepared by ultrasonic lysis of agar-grown vibrios. The lysate was cleared by

[a] The work described was carried out during this author's tenure of a WHO Senior Research Training Fellowship at the University of Chicago.

[b] Kasai, G. J. & Burrows, W. (1966) *J. infect. Dis.*, **116**, 606–614.

[c] Craig, J. P. (1965) In: *Proceedings of the Cholera Research Symposium, Honolulu*, Washington, D.C., US Government Printing Office, pp. 153–158.

[d] Dutta, N. K. & Habbu, M. K. (1955) *Brit. J. Pharmacol.*, **10**, 153–159.