

Technical methods

Further observations on the formol-ether concentration technique for faecal parasites

A. V. H. ALLEN AND D. S. RIDLEY *From the Hospital for Tropical Diseases, St Pancras Way, London*

The simplified version of Ritchie's formol-ether technique for the concentration of faecal cysts, ova, and larvae (Ridley and Hawgood, 1956), which is no less effective than the original, has been quite widely adopted as a useful routine diagnostic procedure. With further experience, however, three objections have become apparent, which apply also to some other concentration methods: (1) The degree of concentration of *Taenia* and *Ascaris* (especially infertile *Ascaris*) ova is unsatisfactory in some cases; and the concentration of *Schistosoma* ova is less than optimum. (2) It is difficult to make the concentrated deposit adhere to a microscope slide for fixation and staining. (3) The concentration of faecal specimens, fixed in bulk and stored for a period of days or weeks, is unsatisfactory.

The object of the present paper is to describe further simple modifications which overcome the first two of these objections.

Method

REAGENTS

- 1 10% formalin (1 vol of 40% formaldehyde diluted with 9 vol distilled water)
- 2 Ethyl ether.

APPARATUS

Centrifuge; centrifuge tubes, conical, approximately 15 ml capacity; swab sticks, long enough to reach to bottom of centrifuge tube; copper-wire gauze, mesh 40 to the inch (15 per cm) (any other mesh is unsuitable) evaporating basin.

PROCEDURE

- 1 Take specimen of faeces about the size of a pea on the end of a swabstick and emulsify it in 7 ml of formalin in centrifuge tube.
- 2 Sieve by pouring whole contents of tube through wire gauze into an evaporating basin. Wash out the tube.
- 3 Return the fluid in the evaporating basin to the centrifuge tube. Add 3 ml of ether. Shake *vigorously* for a full 30 seconds.
- 4 Centrifuge. Set the regulator of the centrifuge to a

mark corresponding to 3,000 rpm; switch off after exactly 60 seconds. If the centrifuge is only capable of a speed of 2,000 rpm spin for one and a half minutes. 5 A layer of debris will have accumulated at the interphase between the two liquids. Loosen it by passing a swabstick gently round the circumference of the tube. Pour the whole contents of the tube down the sink, allowing only the last one or two drops to return to the bottom, where there will be a small deposit. 6 Shake up the deposit. Pour the whole, or most of it, on to a slide and examine under a cover slip. If the inside of the tube is dirty, wipe with cottonwool, or alternatively transfer the deposit to the slide with a Pasteur pipette.

For convenience the 40 mesh wire gauze may be soldered to a short section of a copper cylinder (about 3 in in diameter) to make a sieve¹ that will sit in the evaporating basin. Another improvement, though it is only justified if faecal concentration is a frequent procedure, is to use a Whirlimixer² instead of manual shaking (step 3). Fifteen seconds' mechanical shaking in a large-bore tube is better than 30 seconds' manually.

Common Faults

A dirty deposit (too much debris, or deposit too large to examine) may be due to taking too large a specimen, but is more likely to be due to insufficiently vigorous shaking if this has been done manually.

An excess of blastocysts in the deposit (a troublesome complication in certain parts of the tropics) can be remedied by a preliminary washing in distilled water, allowing time for lysis to occur.

To Make a Permanently Stained Preparation.

When it is desired to stain the concentrated deposit $\frac{1}{2}$ % mercuric chloride solution in water should be substituted for 10% formalin in the concentration procedure.

To make the concentrate adhere to the slide, first add 1 drop of 1% bovine albumin to the deposit and then smear it on a slide coated with egg albumen.

While the smear is still moist fix it in Schaudinn's fluid (saturated mercuric chloride 2 parts, absolute alcohol 1 part) for 10 minutes. Transfer to 70% alcohol for at least 10 minutes. Wash gently in distilled water.

Stain with iron haematoxylin or phosphotungstic acid haematoxylin.

Results

The concentration procedure described here differs from Ridley and Hawgood's technique in two details: the use of formalin in water instead of formol saline, and centrifugation at 3,000 rpm instead of 2,000. The modified procedure has now been in regular routine use at this hospital for five years and has been found to give good results with all types of protozoal cysts and ova and with the larvae of *Strongyloides*. Some representative results are given in the Table. The main advantage of the new modification is in the concentration of *Ascaris*, *Taenia*, and *Schistosoma* ova, but the

¹Suitable sieves are supplied by Endecotts (Test Sieves) Ltd, 9 Lombard Road, London SW19.

²Supplied by Scientific Industries International Inc. (UK) Ltd.

	Number of Cysts, Ova, Larvae Found by		
	Direct Examination	Concentration (Old Method)	Concentration (New Method)
<i>E. histolytica</i>	13	418	540
<i>E. coli</i>	3	43	60
<i>G. lamblia</i>	35	177	929
<i>Ascaris</i>	65	54	1,187
<i>Trichuris</i>	8	225	262
<i>Hookworm</i>	27	817	1,259
<i>Strongyloides</i>	15	138	236
<i>Schistosoma</i>	5	11	76
<i>Taenia</i>	—	32	433

Table The concentration of various cysts, ova, and larvae by two methods¹

¹The figures for the cysts represent the mean of three counts of 25 fields each. The figures for ova are the counts per coverslip.

results are appreciably better also with most other ova and cysts, and worse with none. The concentration achieved varies from about 15 to 50 times, depending on the type of parasite, and to some extent on the individual specimen.

Comment

The superiority of formol-ether concentration over the well known zinc sulphate flotation method was demonstrated by Ritchie, Pan, and Hunter (1952). In the latter method the heavier ova (*Schistosoma*, *Fasciola*) fail to rise to the surface; with formol-ether, a sedimentation technique, all parasites go to the bottom.

The increased yield of positive findings with all types of faecal parasites, the relatively clean deposit, and the enhanced visibility of the structural detail of cysts obtained by this method justify its use as a routine diagnostic procedure.

Unformed stools need to be examined by direct microscopy also for trophozoites, but formed stools without mucus or blood need only be examined by concentration (Ridley and Hawgood, 1956).

We have made many attempts further to improve the efficiency of the method by the addition of wetting or mucolytic agents but none has been a consistent success.

References

- Ridley, D. S., and Hawgood, B. C. (1956). The value of formol-ether concentration of faecal cysts and ova. *J. clin. Path.*, 9, 74-76.
- Ritchie, L. S., Pan, C., and Hunter, G. W. (1952). A comparison of the zinc sulfate and the MGL (formalin-ether) techniques. *J. Parasit.*, 38, Suppl. to No. 4, 16.

A screening technique for the detection of nasal carriers of antibiotic-resistant *Staphylococcus aureus*

E. R. STOKES, H. R. INGHAM, AND J. B. SELKON
From the Regional Public Health Laboratory,
General Hospital, Newcastle upon Tyne

Most staphylococci giving rise to epidemic infection in hospital today are resistant to a number of antibiotics (Williams, Blowers, Garrod, and Shooter, 1966) and these patterns of resistance can serve as a useful discriminant in the selection of staphylococci for routine phage typing. In this laboratory all coagulase-positive staphylococci (*Staph. aureus*) resistant to penicillin, tetracycline, and streptomycin are routinely phage typed. The nasal carriage of *Staph. aureus* has been shown to bear an important relationship to the occurrence of wound infection, in some circumstances by autoinfection (Weinstein, 1959; Williams, Jevons, Shooter, Hunter, Girling, Griffiths, and Taylor, 1959; Williams, Noble, Jevons, Lidwell, Shooter, White, Thom, and Taylor, 1962; Rountree and Beard, 1968) and in other circumstances by dispersal through the ward environment to result in cross infection of other patients (Stokes, Hall, Richards, and Riley, 1965; Williams *et al.*, 1962). The detection of nasal carriers of *Staph. aureus* resistant to multiple antibiotics either as part of a programme of routine surveillance or during an actual outbreak of infection due to these organisms results in a considerable increase in the work load on the laboratory and this suggested the need for a simple screening technique. Since none of the many existing selective media for the isolation of *Staph. aureus* (Cruikshank, 1965) was sufficiently selective for multiply drug-resistant strains, the medium reported here was developed.

Medium and Methods

Nutrient agar plates were prepared containing 5 µg/ml of streptomycin and 5 µg/ml tetracycline plus 0.01% phenol red and 1% mannitol to indicate the growth of *Staph. aureus* (Chapman, Berens, Nilson, and Curcio, 1938). The medium was stable for one week when stored at 4°C. Nasal swabs were inoculated first onto 5% horse blood agar, two per plate, by a member of the laboratory staff and then on to mannitol-antibiotic agar, eight swabs per plate, by one of us. A strain of *Staph. aureus*, resistant to penicillin, tetracycline, and streptomycin, was inoculated onto each mannitol-antibiotic plate as a control. Both series of plates were incubated at 37°C for 24 hours when they were examined for the presence of colonies resembling *Staph. aureus*; the mannitol-antibiotic plates were incubated for a further 24 hours and re-examined. The blood agar plates were read by the laboratory staff and the mannitol-antibiotic plates by the sister in control of infection who then referred any plates on which colonies thought to be *Staph. aureus* were growing to the laboratory for further investigation. Colonies resembling *Staph. aureus* were sub-cultured onto nutrient agar containing 6% lysed horse