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Comparison of four techniques for the routine diagnosis of *Trichomonas vaginalis* infection

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SYNOPSIS Specimens from 495 patients attending Johannesburg hospitals and family planning clinics were examined for *Trichomonas vaginalis* by microscopy of Giemsa (GS), Papanicolaou (Pap), and acridine-orange (AO) stained smears, and by culture in Feinberg-Whittington medium. Culture, Pap and GS stained smears from vaginal swabs yielded fewer positives than AO stained smears. Although Pap-stained cytological smears gave the highest number of positives, in 30% of these cases the presence of *T.vaginalis* could not be confirmed by examination of vaginal swabs. Of the positive AO-stained smears, 93% were also positive by at least one other technique.

Since the introduction of metronidazole for the treatment of trichomoniasis (Durel *et al*, 1960), a major problem in the control of this disease has been the accuracy of diagnosis (Hughes *et al*, 1966). A large number of culture and staining techniques have been designed to increase the reliability of laboratory diagnoses. Rayner (1968) compared the (FW) medium of Feinberg and Whittington (1957) with the cysteine-peptone-liver-maltose (CPLM) medium described by Johnson and Trussel (1943). Rayner found that CPLM was the most reliable, and further observations (Rayner and Lowe, unpublished results quoted by Lowe (1972a)) described it as being as sensitive as a semi-solid agar (SSA) introduced by Lowe in 1965. Whittington (1957) had, however, previously described the FW medium as being more reliable than CPLM.

Hess (1969) found that a commercial *Trichomonas* medium (Oxoid No. 2) gave more positive isolations than did CPLM, but Lowe (1972a) found it less reliable than SSA. Cox and Nicol (1973) found FW medium to be better than Oxoid No. 2.

Contradictory findings have also been published in comparisons of culture and microscopic techniques. Lowe (1972b) regarded cultural methods as being more sensitive than microscopy of either fresh or stained material, a view shared by Hess (1969) and McCann (1974). A number of authors (Woodcock, 1972; Morton, 1972), however, have found simple microscopy to be as reliable as culture. Lowe (1972b) regarded direct microscopy as being useful only in cases where rapid diagnosis was required.

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For direct microscopy only fresh specimens are of value (McCann, 1974), and with the large numbers of patients attending gynaecological clinics, an 'on the spot' examination of a vaginal swab is virtually impossible. Stained smears have the advantage that there can be considerable delay between preparation and staining and examination of a smear without loss of reliability in diagnosis, providing the smear has been adequately fixed. Specimens can thus be sent to a central laboratory for processing. Among the stains commonly used for *Trichomonas* diagnosis are Papanicolaou (Hughes *et al*, 1966) and Giemsa (Freeman, 1958) or other Romanowsky stains (Lowe, 1972b).

Acridine orange (AO) is a compound which differentially stains DNA and RNA (Von Bertalanffy and Bickis, 1956); the former fluoresces yellow-green and the latter bright red under ultraviolet illumination. Dart and Turner (1959), in an examination of the application of AO to cytological staining, mentioned that *T.vaginalis* stained characteristically.

We have used AO in the routine laboratory diagnosis of trichomonal infections, and this report presents the results of a comparison between this technique and other stains (Giemsa and Papanicolaou) and culture on FW medium.

Material and Methods

SPECIMENS

Vaginal swabs were obtained from patients attending

the gynaecological outpatients department of the Johannesburg General Hospital, from patients in the gynaecological wards of hospitals in Johannesburg, and from family planning clinics. The swabs were kept in sterile physiological saline at room temperature until collection and were brought to the laboratory within three hours of being taken. Smears of the vaginal exudate were made on clean glass slides, after which the swab was put into a culture bottle, as described below.

Giemsa Stain

As soon as the smear was dry it was fixed by immersion for one minute in absolute ethanol and allowed to dry. The following day it was stained with Giemsa, diluted 1 part to 19 parts 1/15M phosphate buffer, pH 7·2 for 10 minutes. (Preliminary experiments had shown this to be the optimum dilution and staining time for *T.vaginalis*.) After the stain had been washed off and the slide had dried, it was scanned for trichomonads at 160× magnification. At least 30 fields were examined before a negative finding was recorded.

Papanicolaou Stain

Cervical material for routine cytological examination was taken from about 60% of the patients from whom swabs had been taken. In these cases a copy of the cytologist's report was obtained and the presence or absence of trichomonal infection was noted. In those cases where cytological investigations were not required, smears were prepared from vaginal swabs and fixed while still wet with a commercial fixative (Fencott, Cape Town, South Africa). These smears were subsequently stained by a standard Papanicolaou method (Culling, 1963) and examined for *T.vaginalis* organisms.

Acridine Orange

Smears prepared for AO staining were not fixed and were stained the next day using a standard technique (Culling, 1963). The smears were examined immediately on a Zeiss fluorescence microscope, using a BG12 exciter filter, and 44 and 53 barrier filters. Details of this technique are given by Fripp *et al* (1975).

Culture

After the smears had been prepared, the swab was broken off and put into a culture bottle containing about 5 ml of Feinberg-Whittington medium and incubated at 36°C. Specimens were withdrawn from the medium at 48 hr, 72 hr, and 96 hr post-inoculation and examined for motile trichomonads.

Results

Of the 495 patients examined, 231 (47%) were found to be harbouring *T.vaginalis* by at least one of the four techniques used. The largest number of positive findings (171) was recorded with Papanicolaou-stained smears (table I), though this was not significantly different from the number (164) recorded with Acridine-orange stained smears ($\chi^2 = 0{\cdot}16$, $P > 0{\cdot}6$). There were quite marked differences between the infection rates revealed by these two tests and with culture (109/495) and Giemsa-stained smears (95/495), both of which were negative in more than half the cases where trichomonads could be demonstrated by at least one other technique.

Comparison between those Papanicolaou stained smears examined parasitologically (Para-pap) and the cervical smears examined cytologically (Cyto-pap) also revealed marked differences. Of the former, 24% of 182 specimens were positive compared with 40% of 313 Cyto-paps.

In 8/44 (18%) of those Para-paps showing *T.vaginalis* the organisms were not demonstrated by any other test, compared with 38/126 (30%) of the Cyto-paps. Unconfirmed cases diagnosed by Giemsa-staining made up 4%, culture 3%, and acridine-orange staining 7% of their respective totals (table II).

Discussion

The highest percentage of positives in this comparison were found by examination of Papanicolaou-stained cervical smears. This is in agreement with the findings of Thin *et al* (1975) and Hughes *et al* (1966), who compared cytological, clinical, and culture techniques. The comparison between Para-pap and Cyto-pap smears confirmed the suggestion by Hughes *et al* (1966) that the advantages lie in the collection of the specimen rather than the stain used.

In the present study, infection rates in Para-pap smears (24%) were similar to those demonstrated by culture (22%) and Giemsa-staining (19%). Although the highest rates were recorded by Cyto-pap examination, only 70% of these could be confirmed by other techniques, whereas 93% of positive AO-stained smears were positive by other methods. Perl (1972) found that *T.vaginalis* could not be cultured from 37% of 666 patients diagnosed as having an infection by examination of Papanicolaou-stained cervical smears. On the basis of her results she suggested that positive findings in such examinations should be treated with reserve. Re-examination of cervical smears was possible in 24 cases in the present study, and *T.vaginalis*

Stain	No. of Specimens examined	Positive		
		No.	%	Percentage of Total Positives by Any Test
Giemsa	495	95	22	47
Culture	495	109	19	47
Para-pap[1]	182	44	24	52
	495	171	34	74
Cyto-pap[1]	313	127	40	88
Acridine-orange	495	164	33	71

Table I *Diagnosis of* T.vaginalis *from vaginal swab and cytological smears*

[1]See text for explanation

Stain	No. Positive	Confirmed No. Positive	Confirmed Positives as % of Total Positives
Giemsa	95	91	96
Culture	109	106	97
Para-pap[1]	44	36	82
Cyto-pap[1]	127	89	70
Acridine-orange	164	153	93

Table II *Cases of* T.vaginalis *infection confirmed by at least one other method*

[1]See text for explanation

infection could be confirmed in our laboratory in 20 (83%) of these.

The infection rate recorded by AO-staining (33%), although lower than that recorded by cytological investigations, was higher than either culture or other vaginal swab examinations.

AO is a fluorescent nucleic acid stain, which stains *T.vaginalis* a characteristic brick-red colour with an oval yellow-green nucleus (Fripp *et al*, 1975). Although the flagellae and other cell organelles do not stain, the fluorescence of the cytoplasm and nucleus is distinctive enough to facilitate recognition of organisms from other cellular material in the smear even at low magnifications. Unfixed smears can be kept at room temperature for 10 to 24 hours without a significant reduction in the reliability of dagnosis. If a longer delay is anticipated, the fixative-coated slides described by Amies and Garabedian (1965) maintain the morphological and staining characteristics of *T.vaginalis* for at least five days.

The major disadvantage of the technique is that the slides do not keep after staining, and a permanent record is not possible. The rapidity, ease, and reliability of AO-staining, however, justify its use in the routine laboratory diagnosis of trichomonal infections.

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