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Evaluation of the Haematocrit Centrifuge and Other Techniques for the Field Diagnosis of Human Trypanosomiasis and Filariasis

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Introduction

Human trypanosomiasis in Africa though under control still remains at an irreducible level in many areas. These endemic foci are due mainly to the presence of animal reservoirs and to "apparently healthy carriers". If the true incidence of the disease in an area is to be known it is necessary that (1) surveys include the examination of the blood of the entire population (sick or otherwise), and (2) the use of sensitive and efficient techniques.

Ideally, a good field diagnostic technique has to be relatively simple to operate, sensitive, rapid, and preferably a parasitological diagnosis. It should be portable enough so that the survey team can go from village to village in a land rover or some other mode of transport without too much trouble or advance notification.

The haematocrit centrifuge technique for the detection of small numbers of trypanosomes in the blood (Woo 1969) is more rapid and reliable than those presently used in the laboratory for the diagnosis of African trypanosomiasis at the E.A.T.R.O. hospital (Woo 1970). The purpose of the present study is to evaluate the feasibility and reliability of this and other techniques under field conditions where large numbers of people have to be examined in a relatively short period.

The Lugala area in south Busoga, Uganda, was chosen for this study as it has been an endemic *Trypanosoma rhodesiense* area. In the month of December 1970, five cases had been diagnosed at the E.A.T.R.O. hospital from this area.

Materials and Methods

Haematocrit Centrifuge Technique (H.C.T.): One heparinized capillary tube was filled with blood (about 0.06 ml) from each person from a finger prick; one end of the tube was then sealed with plasticine. The sealed capillary tubes (in batches of 10 or 15) were then centrifuged in an International Micro-capillary Centrifuge (Model MB) for four minutes at 12,000 rpm. The power for the running of the centrifuge and the light for the microscope was supplied by a portable Honda generator (Model E 15,000). During continuous use of the centrifuge, heat was built up on the rotating plate of the centrifuge (this might affect

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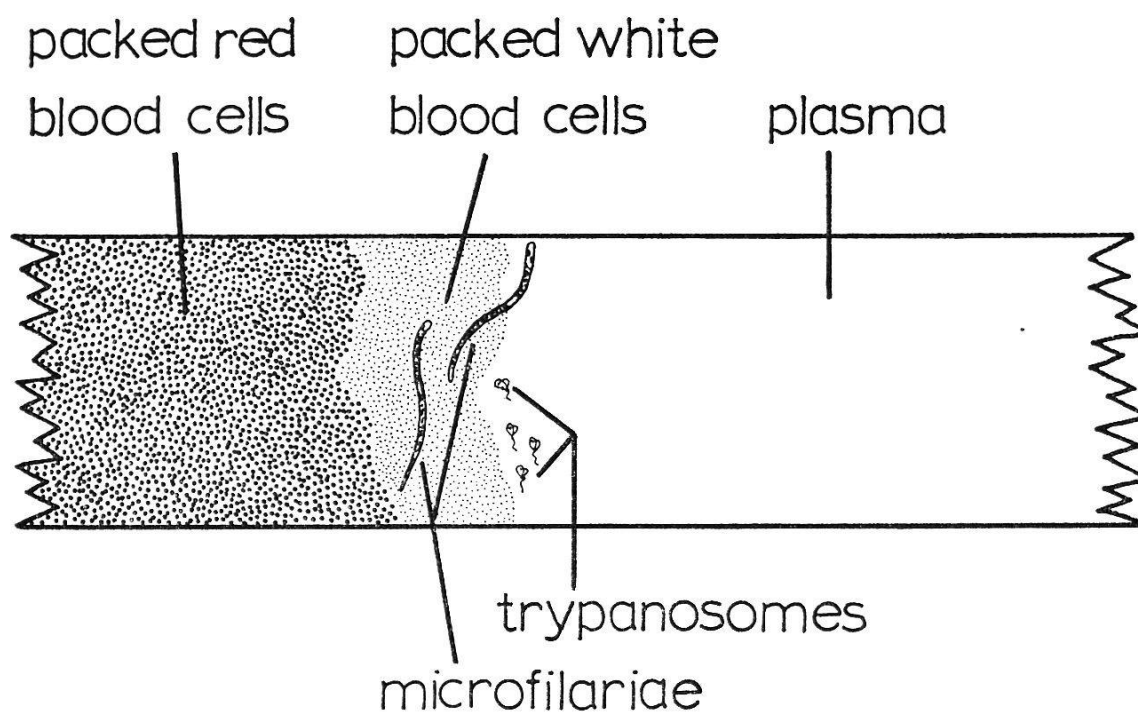


Fig. 1.

the viability of the trypanosome in the tubes); to prevent this heat build up, the lid was not completely closed during centrifugation or it could be replaced by a lid that will allow air to circulate freely e.g. one that is made of strong wire mesh. After centrifugation the capillary tube was then placed in a capillary tube holder along with a drop of immersion oil and examined under a microscope using $12.5\times$ eye piece and $10\times$ objective. The capillary tube should be rotated from time to time during the examination so as to ensure that all sides of the tube have been examined.

In a positive diagnosis, trypanosomes are found "wriggling" at the junction of the "buffy" layer (packed white blood cells) and the plasma; in filariasis, the microfilariae can also be found in the packed white cells (Fig. 1).

Thick Blood Smear: A thick smear was made from each person whose blood was examined by the H.C.T. The smears were air dried, later stained in Giemsa's stain and examined in the laboratory under high power ($8\times$ eye piece and $40\times$ objective) by experienced technicians for trypanosomes and microfilariae.

Estimation of IgM Content in Blood: Blood from each person was collected on Whatman No. 4 filter paper to saturate a circle about 2 cm in diameter, air dried, put in plastic bags and stored at -20°C until used. The immunoglobulin M (IgM) content of the blood was determined in the laboratory at E.A.T.R.O. according to the method of CUNNINGHAM et al. (1967). The blood of those people with elevated IgM was further tested for anti-trypanosomal antibody.

Indirect Fluorescent Antibody Test (I.F.A.T.): The I.F.A.T. was used on the blood of those people with elevated IgM; those who showed a strong serological reaction ($3+$, $4+$ fluorescence) were recalled two weeks later.

Mouse Inoculations: Two mls. of blood were taken from each person who showed a strong serological reaction by the I.F.A.T. and positive by the H.C.T.; the blood was inoculated intraperitoneally into two mice. Tail blood of these mice was examined by wet preparation every other day for at least 60 days. All negative mice were destroyed at the end of 60 days.

Results

The blood of 413 individuals of different ages were examined by the various techniques outlined (Table 1).

Haematocrit Centrifuge Technique: Of the 413 individuals whose blood was examined by this technique, one 11-year girl (Field No. 305) had trypanosomes in her blood. She did not show obvious signs of the infection at the time her blood was examined, but developed a slight fever (99°F) the following morning at the E.A.T.R.O. hospital (Hospital Admission No. SS980). Through her mother, it was found that she had a seven month history of fever, headache, and limb and joint pains. No trypanosomes were found in gland juice nor in cerebrospinal fluid by the centrifuge technique. This was later confirmed by animal inoculation. The cerebrospinal fluid cell count was 34 and protein content 25 % mg. Trypanosomes were frozen (CUNNINGHAM et al. 1963) directly in the patient's blood (E.A.T.R.O. 1184) and a second isolate (E.A.T.R.O. 1186) was frozen from an inoculated rat 13 days later; the prepatent period in the rat was seven days.

Microfilariae were found in the blood of 17 people. The infection was diagnosed as *Dipetalonema perstans*. The microfilariae of *D. perstans* can be distinguished from those of *Wuchereria bancrofti* and *Brugia pati* by the absence of a sheath which can be seen, if it is present, during the microscopic examination of the centrifuged tubes.

Thick Smear Examinations: Although the whole thick smear (about 3 cm radius) was examined after staining, none of the 17 microfilariae infections was detected. Two trypanosomes were found on the thick smear from the patient previously diagnosed by the centrifuge technique; no other trypanosome infection was found.

Table 1. Results obtained by the different techniques used in the survey

Technique used	Number of individuals examined	Results
1. Haematocrit Centrifuge Technique	413	1 positive for trypanosome 17 positive for microfilariae
2. Thick Smear	413	1 positive for trypanosome * none positive for microfilariae
3. IgM Estimation	413	281 with elevated IgM
4. Indirect Fluorescent Antibody Test	281	9 with 3+ reaction 3 with 4+ reaction
5. Animal Inoculation	11	1 positive for trypanosome *

* Already diagnosed by H.C.T. in the field.

Table 2. Results of the serological tests as confirmed or negated by animal inoculation

Igm estimation		I.F.A.T.		Mouse inoculation	
Diameter of precipitation ring (in mm)	Number of individuals with elevated IgM to number tested	Number of individuals with strong serological reaction to number tested	<i>Degree of fluorescence</i>	Blood of individuals with strong serological reaction	Number of positive individuals
		+++	++++		
4.0-4.9	166/413	7*/281	1/281	7	0
5.0-5.9	85/413	2/281	2/281	4	1 **
6.0-6.9	27/413	0	0	0	0
7.0-7.9	1/413	0	0	0	0
8.0-8.9	2/413	0	0	0	0
Total	281/413	9/281	3/281	11	1 **

* One individual could not be located.

** Already diagnosed by H.C.T. in the field.

Estimation of IgM in Blood: Two hundred and eighty one out of 413 had elevated IgM (precipitation ring greater than 4.0 mm) in their blood. This included the diagnosed case (precipitation ring 5.0 mm). The blood of these 281 people was further tested for anti-trypanosomal antibodies by the I.F.A.T.

Indirect Fluorescent Antibody Test: Twelve people gave a strong serological reaction (degree of fluorescence 3+ and 4+); of these four had been previous trypanosomiasis patients who had been treated at the E.A.T.R.O. hospital during the previous 18 months. The patient (SS 980) had a 3+ fluorescence. The results of the two serological tests are summarized in Table 2.

Mouse Inoculations: Of the 12 people whose serum showed a strong reaction by the I.F.A.T., one is the already confirmed case. Ten of the remaining 11 people could be traced and their blood inoculated intraperitoneally into mice. None of the mice inoculated with blood from the 10 individuals was positive at the end of 60 days.

Post-survey Information: None of the 412 individuals (not found to be infected with trypanosomes by H.C.T.) examined during the survey was admitted to the E.A.T.R.O. hospital for trypanosomiasis 4 weeks after their blood was checked; i.e. probably none of them were infected at that time. This, along with the results of the other tests suggest that the H.C.T. is relatively reliable for the field diagnosis of trypanosomiasis.

Discussion

In the absence of an efficient and reliable field technique for the parasitological diagnosis of trypanosomiasis many of the surveys undertaken use either serological and or the more conventional techniques (e.g. animal inoculations or thick smear). This would mean that the results of the survey would not be known for sometime after the collection of the blood samples. As the current serological tests are only sensitive enough for screening people, the number of

individuals giving a positive serological reaction greatly exceeds the number in which trypanosomes were isolated by other techniques. Hence, follow-up studies (e.g. by animal inoculations) have to be done to pick out the infected people. This has not always been possible either because of the lack of time and/or because some of these people cannot be located. On the other hand, animal inoculations although sensitive (for *Trypanosoma rhodesiense* but not for all strains of *T. gambiense*) is not feasible for large or widely scattered populations, while the microscopic examinations of blood (either wet preparation or thick smear) or lymph node fluid can be used for large numbers of people, it is grossly insensitive and time consuming (SCHOENAERS et al. 1953; PAUTRIZEL et al. 1960; MATTERN 1964; WOO 1970).

The H.C.T. as shown by this and an earlier study (Woo 1970) is a more rapid and sensitive technique compared to those that are currently being used. It is simple and can be mastered easily by technicians and its operation does not involve elaborate preparations or post-sampling duties e.g. the examinations of materials in the laboratory or the cleaning of glassware. This would mean that the number of workers involved in the survey need not be large and that the survey team can be out in the field every day. A team of 2 or 3 workers can easily process and examine the blood of 250–300 people in a normal working day (about 50 people in an hour). Since this permits an on-the-spot parasitological diagnosis, infected persons can be removed from the area the same day the infection is diagnosed and transported back to the hospital for treatment. Also this would ensure that they no longer serve as sources of infections for more flies.

In an earlier study (Woo 1970) it has been shown that this technique can be used to detect *T. brucei*, *T. congolense* and *T. vivax* infections in experimentally infected cattle 6 to 10 days before the infections can be demonstrated by either thick smear or wet preparations. Hence this technique would be useful for surveys of trypanosomes especially those that are non-infective to laboratory animals by blood inoculations (e.g. *T. vivax* and certain strains of *T. gambiense* and *T. congolense*).

Preliminary studies have shown that this technique can also be used for the early diagnosis of *T. cruzi* infections in laboratory infected monkeys (MARSDEN 1970) hence it is conceivable that it can also be used as a survey technique for south American trypanosomiasis.

Besides trypanosomiasis, the H.C.T. can also be used for the detection of microfilariae in the blood when the number is too small to be detected by thick smear examinations. Further critical study e.g. comparing this technique with the millipore filtration technique (BELL 1967; CHULAERK & DESOWITZ 1970) is needed to assess the efficiency and reliability of this as a field technique for the diagnosis of human filariasis (both periodic and non-periodic infections).

Another promising technique for the diagnosis of trypanosomiasis is the DEAE-cellulose column technique as described by LANHAM (1968). It is quite sensitive for the detection of small numbers of trypanosomes in the blood (LANHAM 1969; GODFREY & LANHAM 1970). As a field technique the H.C.T. seems better because it is simpler to operate, more rapid, and does not necessitate as much advance preparation. Further comparative field trials of the two techniques are needed, however.

The sensitivity of the H.C.T. can be increased if more than one centrifuge tube (about 0.06 ml of blood) is examined. Since it takes approximately half a minute to examine a tube, examinations of more tubes from each individual or animal would not be too time consuming. Under laboratory conditions we (WOO & ROGERS 1971) have shown that when 3 tubes are examined, we can consistently demonstrate trypanosomes in at least one or two tubes when there are about 10 trypanosomes per cubic millilitre of blood.

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