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A comparative evaluation of the parasitological techniques currently available for the diagnosis of African trypanosomiasis in cattle

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Summary

The parasitological techniques currently in use for the diagnosis of African trypanosomiasis were compared in a series of experiments for their capacity to detect *Trypanosoma congolense, T. vivax* and *T. brucei* in the blood of cattle. The darkground/phase contrast buffy coat method proved to be more sensitive than the haematocrit centrifugation technique, thick, thin and wet blood films in detecting *T. congolense* and *T. vivax*. On the other hand with *T. brucei*, mouse inoculation was the most sensitive method, followed by the haematocrit centrifugation technique. In a further series of experiments involving cattle infected with either *T. congolense* or *T. vivax*, the darkground/phase contrast buffy coat method was consistently more sensitive in detecting parasites than haematocrit centrifugation, capillary concentration using glycerol and miniature anion-exchange/centrifugation techniques. As well as showing superior sensitivity, the darkground/phase contrast buffy coat method allowed species identification, estimation of parasitaemia and simultaneous assessment of anaemia (packed red cell volume).

Key words: parasitological diagnosis; cattle; *Trypanosoma congolense; T. vivax; T. brucei.*

Introduction

The diagnosis of a current infection with African trypanosomes in livestock can only be made at present by demonstrating the parasite in the blood or tissue

fluids of infected animals. The sero-diagnostic techniques which are available depend on the demonstration of circulating antibody and as a result cannot by themselves confirm an active infection. However, the reliability of many of the parasitological techniques currently in use in detecting trypanosomes in naturally-occuring field cases, where parasitaemias are often low and sporadic, is often questioned.

The present paper evaluates the sensitivity of the parasitological diagnostic techniques which are commonly used for the detection of African trypanosomes in blood samples and pays particular attention to their capacity to detect *Trypanosoma congolense* and *T. vivax*, the main pathogens of cattle. The techniques investigated included 1. thick, thin and wet blood films, 2. inoculation of blood into susceptible mice and 3. trypanosome concentration methods, including haematocrit centrifugation (Woo, 1970), capillary concentration using glycerol (Walker, 1972), the darkground/phase contrast buffy coat method (Murray et al., 1977) and the miniature anion-exchange/centrifugation technique (Lumsden et al., 1977). Consideration was also given when using each test to the ease and confidence with which trypanosome species identification could be made.

Materials and Methods

Diagnostic techniques

Thick, thin and wet blood films. For each of these tests, 5 μl of blood was used. Following rapid air drying, the thick film preparation was placed in distilled water for 5 min to dehaemoglobinise, while thin films were fixed for 3 min in methanol. Thick and thin films were then stained in 10% Giemsa for 30 min. Subsequently, 200 fields were viewed under oil immersion using a NPL Oel 100/1.30 objective with periplan NF 10 × eyepieces. The wet film was examined by phase contrast microscopy as described later.

Inoculation of blood into mice. This method was tested only with blood infected with *T. brucei.* Seventy μl of blood in 0.2 ml phosphate saline glucose (PSG), pH 8.0, were inoculated intraperitoneally into each of 2 mice. Wet preparations of mouse tail blood were examined 3 times per week for 30 days before the animals were declared not infected.

Concentration techniques: a) Haematocrit centrifugation technique (HCT) (Woo, 1970). Following the standard 5 min centrifugation, the micro-haematocrit capillary tube[1] containing the blood sample was placed on a glass slide prepared as follows: 2 rectangular pieces of glass 25 × 10 × 1.2 mm thick from a microscope slide were fixed 1.5 mm apart on a microscope slide. The capillary tube was placed in the groove formed by the rectangular glass pieces under a coverslip and the space between the slide and coverslip was flooded with water. By slowly rotating the tube, the buffy coat-plasma junction was examined using the following microscopic set up: a Leitz SM Lux microscope and a combination of a Phaco L 20/0.32 long working distance objective, darkground or phase contrast illumination from a Zernicke 402 condenser, and periplan NF 10 × eyepieces. The use of the long working distance (6.7 mm) allowed considerable depth of focus through the capillary, unlike a standard objective where the average working distance is approximately 0.5 mm.

b) *Capillary concentration technique (CCT)* (Walker, 1972). Because of the tendency of *T. congolense* to be retained amongst red blood cells, this technique was designed to create a large dif-

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[1] The micro-haematocrit capillary tubes used measured 75 mm in length and had an internal diameter = 1.2 mm.

ferential density between the red blood cells and the parasites. By mixing infected blood with a strongly hypertonic non-toxic medium prepared as follows: 9% glycerol, 9% magnesium sulphate, 0.1% TRIS buffer, pH 8.0 to 8.2, and phenol red 1:100,000, it is possible to alter the specific gravity of erythrocytes. On centrifugation, the denser red cells separate from the trypanosomes which display normal motility.

Samples were prepared by mixing 50 μl of diluent and 50 μl blood in a microtitre titration plate. After being allowed to stand for a minimum of 15 min plain capillary tubes were three-quarter filled from the wells, sealed and spun for 2 min in a micro-haematocrit centrifuge. Capillaries were placed on a clean microscope slide (up to 12 at a time) and the buffy coat zone covered with a few drops of diluent beneath a coverslip. Capillaries were examined in turn with the same microscope set up used from the HCT.

c) *Miniature anion-exchange/centrifugation technique (m-AEC)* (Lumsden et al., 1977, 1979). Anion-exchange columns were prepared in the barrels of 2 ml disposable syringes using DEAE-cellulose (DE52), as described by Lanham and Godfrey (1970). The column was primed by filling to the brim with PSG and allowing to drain twice. PSG (ionic strength 0.145) was prepared by mixing 4 volumes of PBS with 6 volumes of distilled water, and adding glucose to a final concentration of 1% (w/v); the ionic strength of 0.145 was considered appropriate for bovine blood (Lanham and Godfrey, 1970). The blood sample (100 μl) was then discharged from a capillary tube and drained into the DEAE-cellulose bed. Four drops of PSG were added to the top of the column and the reservoir attached and immediately filled with PSG (i.e. a Pasteur pipette containing approximately 2.5 ml PSG connected to the column through a perforated syringe piston). The eluate was collected in a prepared centrifuged tube (a Decon-cleaned, drawn out, sealed Pasteur pipette, capacity 2.5 ml, protected by a plastic pipette tip).When drainage was complete, this pipette was centrifuged at 525 g for 5 min. The drawn out tip was examined microscopically for trypanosomes under a coverslip with PSG using the optical system employed for the HCT and the CCT.

d) *Darkground/phase contrast buffy coat technique (DG)* (Murray et al., 1977). The buffy coat zone, prepared in a micro-haematocrit capillary tube filled with 70 μl of blood and centrifuged for 5 min, was examined for trypanosomes as follows: the capillary tube was cut with a diamond-pointed pen 1 mm below the buffy coat to incorporate the uppermost layer of erythrocytes, and 1 cm above to include some plasma. Using a capillary tube holder, the contents of the tube were gently expressed on to a clean slide, mixed and covered with a 22 × 22 mm coverslip. The preparation was examined using a Leitz SM microscope with a combination of Phaco 2 NPL 25/0.50 objective, a Zernicke 204 condenser and periplan NF 10× eyepieces: alternatively, a Heine condenser with a PV 25/0.50 objective was employed (Leitz Wetzlar, Germany). Both these condensers provide phase contrast and darkground illumination. Two hundred microscopic fields were examined. The use of 10× eyepices in combination with a 25× objective gave optimal viewing, by allowing large visual fields and sufficient magnification for ready indentification of trypanosomes.

Enumeration of trypanosomes

Haemocytometer. Trypanosomes were counted by a modification of the method of Belding (1965); 5 μl blood samples were mixed with 45 μl of the following solution: 10 μl 40% formaldehyde, 40 μl glacial acetic acid, 30 μl carbol fuchsin and 430 μl distilled water. Trypanosomes were then enumerated at an appropriate dilution. Using this technique, parasites are detectable to the level of 2.5×10^4 trypanomes/ml, although we found that with parasitaemias of less than 10^6 trypanosomes/ml consistent results were not obtained.

DG system. Because of the low parasitaemia often encountered in trypanosome-infected cattle and because of the poor sensitivity of the haemocytometer, a scoring system based on the DG technique was developed for estimating low parasitaemias. The system and its relationship to trypanosome numbers are shown in Table 1. While only giving an estimated parasitaemia, this method was found to give reproducible results when evaluated by appropriate serial dilutions of samples containing parasites quantified by the haemocytometer.

Table 1. Darkground/phase contrast buffy coat parasitaemia scoring system

Score	Trypanosome/field*	Estimated parasitaemia trypanosomes/ml
6+	Swarming >100	$>5 \times 10^6$
5+	>10	$>5 \times 10^5$
4+	1–10	$10^4–5 \times 10^5$
3+	1 per 2 fields to 1 per 10 fields	$5 \times 10^3–5 \times 10^4$
2+	1–10 per preparation	$10^3–10^4$
1+	1 per preparation	$10^2–10^3$

* magnification = $\times 250$

Preparation of blood samples used in comparative sensitivity studies

Jugular blood samples[2] were collected from cattle infected with *T. congolense*, *T. vivax* and *T. brucei*. Where possible, cattle with parasitaemias of more than 10^6 trypanosomes/ml, as estimated by the haemocytometer, were used. The concentration was then adjusted to 5×10^4 parasites/ml by adding the appropriate volume of normal uninfected bovine blood. The dilution of 5×10^4 trypanosomes/ml was chosen on the basis of preliminary findings that all the parasitological techniques being evaluated were positive at this level or above. The samples were then diluted sequentially as shown in the results.

All experiments were repeated at least 5 times. However, because of the low parasitaemia ($<10^6$ trypanosomes/ml) encountered especially in *T. congolense* and in *T. brucei* infected cattle, the results obtained were compared with samples collected from infected mice; similar findings were obtained with samples from cattle and mice for each trypanosome species.

In each experiment, all tests were carried out concurrently on the same sample.

Results

A comparison of the commonly used parasitological diagnostic techniques

In the first part of this study, the sensitivity of the standard trypanosome detection methods (STDM) as defined by Wilson (1969), namely, wet, thin and thick blood films and mouse inoculation, were compared with the HCT and DG concentration techniques.

Detection of T. congolense. The DG technique proved to be more sensitive in detecting *T. congolense* than any of the other 4 techniques evaluated. The result of one experiment is shown in Table 2. The DG test identified parasites to an estimated level of 2.5×10^2 trypanosomes per ml. The HCT test was the next most sensitive at 6.25×10^3 parasites per ml and was marginally superior to the thick smear at 8.33×10^3 trypanosomes per ml. These results were confirmed in both cattle and mice.

Detection of T. vivax. The results for *T. vivax* were similar to those obtained for *T. congolense* (Table 2). No difference was found between *T. vivax* infected

[2] The anticoagulant used was the disodium salt of ethylenediaminetetra-acetic acid (EDTA) at a concentration of 2 mg/ml of blood.

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Table 2. Sensitivity of different parasitological techniques in detecting *T. congolense*, *T. vivax* and *T. brucei*

Trypanosomes per ml	DG*			HCT			Wet film			Thick film			Thin film			Mouse sub-inoculation
	Tc	Tv	Tb	Tc	Tv	Tb	Tc	Tv	Tb	Tc	Tv	Tb	Tc	Tv	Tb	Tb
5.0×10^4	4+	3+	4+	+	+	+	+	+	+	+	+	+	+	+	+	ND
2.5×10^4	3+	2+	3+	+	+	+	+	+	+	+	+	+	+	−	+	ND
1.25×10^4	2+	2+	2+	+	+	+	+	−	+	+	+	+	−	−	−	ND
8.3×10^3	2+	2+	2+	+	+	+	−	+	+	−	+	+	−	−	+	ND
6.25×10^3	2+	2+	1+	−	+	+	−	−	−	−	−	+	−	−	−	+
5.0×10^3	2+	1+	1+	−	+	+	−	−	−	−	−	+	−	−	−	+
2.5×10^3	2+	1+	−	−	+	+	−	−	−	−	−	−	−	−	−	+
1.25×10^3	1+	1+	−	−	−	+	−	−	−	−	−	−	−	−	−	+
8.3×10^2	1+	1+	−	−	−	+	−	−	−	−	−	−	−	−	−	+
6.25×10^2	1+	1+	−	−	−	+	−	−	−	−	−	−	−	−	−	+
5.0×10^2	1+	1+	−	−	−	+	−	−	−	−	−	−	−	−	−	+
2.5×10^2	1+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
1.25×10^2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
8.3×10	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

* Scoring system only applied to DG

ND = not done
DG = Darkground/phase contrast buffy coat technique
HCT = Haematocrit centrifugation technique (Woo)
Tc = *T. congolense*
Tv = *T. vivax*
Tb = *T. brucei*

cattle and mice and in both cases the DG technique proved the most sensitive, detecting trypanosomes at a level of 5.0×10^2 parasites per ml. As with *T. congolense*, the HCT was the next most sensitive at 1.25×10^3 trypanosomes per ml.

Detection of T. brucei. In contrast to the results achieved with *T. congolense* and *T. vivax*, the HCT proved consistently more sensitive in detecting *T. brucei* than all other techniques, except mouse inoculation (Table 2). HCT was sensitive to a level of 5×10^2 parasites per ml, while thick film and DG were found to be similar at 5×10^3 trypanosomes per ml. For the detection of *T. brucei*, inoculation of mice with infected blood proved the most sensitive technique. However, it must be emphasised that the results obtained following inoculation varied considerably between samples, probably as a result of the variation in the number of infective forms present in the inoculum.

A comparison of concentration techniques

The DG and HCT proved the most sensitive tests in the first section of this study. We, therefore, compared them with 2 other recently developed concentration techniques, namely, m-AEC and CCT in a further series of experiments. In our hands, HCT proved to be consistently more sensitive than the DG in detecting *T. brucei*. At the same time, Lumsden et al. (1979, 1981) provided conclusive evidence that the m-AEC technique was more sensitive than the DG and HCT in detecting *Trypanozoon* subspecies. Therefore, in this part of our study we confined our investigations to *T. congolense* and *T. vivax*, the major pathogenic trypanosomes of cattle. Only infected bovine blood was investigated.

The DG technique proved to be the most sensitive of the 4 tests used, both for *T. congolense* and *T. vivax* (Table 3).The DG detected trypanosomes to an estimated level of just over 10^2 parasites per ml. The CCT proved the next most sensitive, while there was little difference between HCT and m-AEC tests. We experienced problems with the m-AEC technique, as cellulose debris deposited at the extreme end of the centrifuged pipette tended to partly obscure the viewing area, possibly masking the presence of parasites.

Species identification

Using the DG technique, it has proved possible to identify trypanosome species on the basis of size and movement (Murray et al., 1977). Species identification using the other techniques was possible in some cases but generally more difficult to assess. The wet film was as good as the DG in this respect, but suffered from vastly inferior sensitivity, as did the stained thin film which is normally employed for morphological studies. Stained thick films often produced distortion of the trypanosomes, thus hampering identification. In the hands of an experienced microscopist, species identification was possible with the HCT and CCT. However, as these techniques do not give as high optical resolution as the DG, identification was more difficult.

Table 3. Sensitivity of different concentration techniques in detecting *T. congolense* and *T. vivax* in bovine blood

Trypanosomes per ml	DG*		HCT		CTT		m-AEC	
	Tc	Tv	Tc	Tv	Tc	Tv	Tc	Tv
5.0×10^3	2+	2+	+	+	+	+	+	+
2.5×10	2+	2+	+	+	+	+	+	+
1.25×10^3	2+	2+	−	+	+	+	−	−
6.0×10^2	1+	1+	−	−	+	+	−	−
3.0×10^2	1+	1+	−	−	−	−	−	−
1.5×10^2	1+	1+	−	−	−	−	−	−
7.5×10	−	−	−	−	−	−	−	−

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*     Scoring system only applied to DG
  DG  =  Darkground/phase contrast buffy coat technique
  HCT =  Haematocrit centrifugation technique (Woo)
  CTT =  Capillary concentration technique
m-AEC =  Miniature anion-exchange/centrifugation technique
   Tc =  T. congolense
   Tv =  T. vivax.
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Discussion

The DG technique proved consistently to be more sensitive than STDM (Wilson, 1969) and the HCT (Woo, 1970) in detecting *T. congolense* and *T. vivax* organisms. On the other hand, the HCT was superior for the diagnosis of *T. brucei*, apart from the inoculation of parasitaemic blood into mice. These findings are in agreement with the preliminary observations of Murray et al. (1977, 1979), who found the DG better than the HCT, thick, and wet blood films when sampling naturally-infected N'Dama cattle in The Gambia. Similarly, Dillmann and Townsend (1979), while conducting a survey of wild animals in Zambia using a technique similar to the DG, reported the superiority of this test over wet smears, dry stained films and the HCT. These authors emphasised particularly the technique's advantage over the HCT in the detection of *T. congolense* organisms because of their sluggish movement.

While inoculation of infected blood into mice proved to be the most sensitive technique for the detection of *T. brucei*, results will vary according to the phase of parasitaemia and the ratio of infective to non-infective forms present in the donor's circulation. The value of inoculation of suspected *T. congolense* or *T. vivax* infected material is more limited as not all field isolates become established in rodents. Thus, when comparing diagnostic techniques in trypanosome-infected pigs and dogs, Mehlitz (1979) showed that inoculation of the rodent *Mastomys natalensis* was only as sensitive as thick film examination for *T. congolense*, but significantly more sensitive than either thick film or HCT for *T. bru-*

cei. Moreover, Leeflang et al. (1978) found mouse inoculation to be significantly less efficient for the diagnosis of *T. vivax* in comparison with other diagnostic tests, including HCT, thick and thin blood smears.

In the second part of the study when DG, HCT, CCT and m-AEC concentration techniques were compared, the DG test again proved to be the most sensitive in detecting *T. congolense* and *T. vivax*. Walker (1972) estimated that detection of *T. congolense* was possible at a concentration of 35 parasites per ml with the CCT; however, in our hands, the technique did not approach this level of sensitivity.

The m-AEC has been extensively utilised both in the laboratory (Lumsden et al., 1977, 1978, 1979) and in the field (Lumsden et al., 1981) for the detection of *Trypanozoon* parasites. It proved better than the DG in detecting *T. brucei* in mice (Lumsden et al., 1979), although not as sensitive as inoculation of mice (Joshua et al., 1979). In the field, m-AEC was more sensitive than HCT and thick film in detecting *T. gambiense* in human cases. During our investigations using the m-AEC, we encountered problems concerning the presence of deposits in centrifuged pipettes undergoing visual examination for trypanosomes. As all glassware was scrupulously cleaned prior to use, the debris was probably cellulose emanating from the DE52 columns. It is possible that this debris reduced the sensitivity of the m-AEC in detecting trypanosomes. The sensitivity of this test was probably affected also by the propensity of *T. congolense* for adhering to bovine erythrocytes (Banks, 1979).

As part of the diagnostic procedure, it is also important to identify the trypanosome species, where possible. The STDMs proved to be insensitive in detecting trypanosomes for identification, or distorted trypanosome morphology to the extent that species identification could not be made. The DG test overcame these constraints by allowing identification of trypanosome species even at very low concentrations. Each species was recognised by its size and the way in which it moved, as described by Murray et al. (1977). Species identification also proved possible using the HCT and CCT. However, with mixed infections identification is more easily accomplished by the DG (data not shown).

Current methods for quantifying trypanosomes such as haemocytometer or automated procedures (Mills and Valli, 1978) are not accurate or sensitive at less than 10^6 parasites per ml. This is a major constraint to both field and experimental studies involving cattle, particularly the trypanotolerant breeds, in which parasitaemias of this level are rare. Thus, a further advantage of the DG technique was that it provided in conjunction with the scoring system described in Table 1 reliable approximations of parasitaemia levels. With the HCT, on the other hand, there was little correlation between the concentration of trypanosomes seen in capillary tubes and the corresponding dilution.

An advantage offered by the DG and the HCT is that a packed red cell volume (PCV) can be read prior to carrying out the parasitological examination. Estimation of the degree of anaemia is probably the most reliable indication of

the progress of the disease in trypanosome-infected cattle (Hornby, 1952). At the same time, it has been shown that PCV is an accurate reflection of total red cell volume (Dargie et al., 1979) and, therefore, its recording forms an important aspect of any study involving diagnosis of bovine trypanosomiasis.

The disadvantage of the concentration techniques described in this paper is that they must be read as soon after blood sample collection as possible, at least within 4 to 6 h, otherwise the number of detectable trypanosomes in the sample declines.

In conclusion, the DG technique was found to be the best all around parasitological test available for the diagnosis of African trypanosomiasis in cattle, as judged by sensitivity, trypanosome species identification, quantification of parasitaemia, and simplicity. Nevertheless, on the basis of the present study, we would agree with Wilson et al. (1975) that no single technique meets all requirements for the parasitological diagnosis of trypanosomiasis in cattle and that where possible, a combination of methods, including laboratory animal inoculation, should be used. The current study shows that for any new diagnostic technique to be worthy of consideration, it will require to operate efficiently at levels of less than 10^2 trypanosomes per ml.

Banks K. L.: In vitro binding of *Trypanosoma congolense* to erythrocytes. J. Protozool. *26*, 103–108 (1979).

Belding D. L.: Textbook of parasitology, 3rd ed. Appleton-Century-Crafts, New York 1965.

Dargie J. D., Murray P. K., Murray Max, McIntyre W. I. M.: Bovine trypanosomiasis. The red cell kinetics of N'Dama and Zebu cattle infected with *Trypanosoma congolense*. Parasitology *78*, 271–286 (1979).

Dillmann J. S. S., Townsend A. J.: A trypanosomiasis survey of wild animals in the Luangwa Valley, Zambia. Acta trop. (Basel) *36*, 349–356 (1979).

Hornby H. E.: Animal trypanosomiasis in East Africa. Her Majesty's Stationery Office, London 1952.

Joshua R. A., Herbert W. J., White R. G.: Diagnosis of African trypanosomiasis. Trans. roy. Soc. trop. Med. Hyg. *73*, 602–603 (1979).

Lanham S. M., Godfrey D. G.: Isolation of salivarian trypanosomes from man and other mammals using DEAE-cellulose. Exp. Parasit. *28*, 521–534 (1970).

Leeflang P., Buys J., Blotkamp C.: Studies on *Trypanosoma vivax:* comparison of parasitological diagnostic methods. Int. J. Parasit. *8*, 15–18 (1978).

Lumsden W. H. R., Kimber C. D., Strange M.: *Trypanosoma brucei:* detection of low parasitaemias in mice by miniature anion-exchange/centrifugation technique. Trans. roy. Soc. trop. Med. Hyg. *71*, 421–424 (1977).

Lumsden W. H. R., Kimber C. D., Doig S.: *Trypanosoma brucei:* use of miniature anion-exchange/centrifugation technique to recover trypanosomes from tissue suspensions. Trans. roy. Soc. trop. Med. Hyg. *72*, 214 (1978).

Lumsden W. H. R., Kimber C. D., Evans D. A., Doig S. J.: *Trypanosoma brucei:* miniature anion-exchange/centrifugation technique for the detection of low parasitaemias: adaptation for field use. Trans roy. Soc. trop. Med. Hyg. *73*, 312–317 (1979).

Lumsden W. H. R., Kimber C. D., Dukes P., Haller L., Stanghellini A., Duvallet G.: Field diagnosis of sleeping sickness in the Ivory Coast. 1. Comparison of the miniature anion-exchange/centrifugation technique with other protozoological methods. Trans. roy. Soc. trop. Med. Hyg. *75*, 242–250 (1981).

Mehlitz D.: Trypanosome infections in domestic animals in Liberia. Tropenmed. Parasit. *30*, 212–219 (1979).

Mills J. N., Valli V. E. O.: A cytofluorimetric method of counting trypanosomes. Tropenmed. Parasit. *29*, 95–100 (1978).

Murray Christine M., Murray Max, Murray P. K., Morrison W. I., Pyne C., McIntyre W. I. M.: Diagnosis of African trypanosomiasis in cattle. Improved parasitological and serological techniques. International Scientific Council for Trypanosomiasis Research and Control. 15th Meeting, Banjul, The Gambia, 1977. OAU/STRC. Publication No. 110, p. 247–254 (1979).

Murray Max, Murray P. K., McIntyre W. I. M.: An improved parasitological technique for the diagnosis of African trypanosomiasis. Trans. roy. Soc. trop. Med. Hyg. *71*, 325–326 (1977).

Walker P. J.: Capillary concentration technique applicable to infections of *T. congolense* in cattle. Trans. roy. Soc. trop. Med. Hyg. *66*, 348 (1972).

Wilson A. J.: Value of the indirect fluorescent antibody test as a serological aid to diagnosis of *Glossina*-transmitted bovine trypanosomiasis. Trop. anim. Hlth Prod. *1*, 89–95 (1969).

Wilson A. J., Paris J., Dar F. K.: Maintenance of an herd of breeding cattle in an area of high trypanosome challenge. Trop. anim. Hlth Prod. *7*, 63–71 (1975).

Woo P. T. K.: The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. Acta trop. (Basel) *27*, 384–386 (1970).