

Adaptation of a 5CG-Desiccator for *in vitro* cultivation of *Trypanosoma brucei brucei*

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Abstract

Objective: The paper report an attempt to adapt a "5CG-desiccator" as a simple and less expensive *in vitro* cultivation system for *T. brucei brucei*.

Methods: The ability of 5CG-desiccator to sustain the growth and survival of *T. brucei brucei* was investigated using different media in various gaseous environment and concentrations, different glucose supplementation, different temperature and pH using haemocytometer counts of parasite density. The morphology and infectivity of the cultured trypanosomes were also studied.

Results: Optimization study revealed that the parasite grow better in 20 % horse or goat serum when supplemented with 0.1-1 % (w/v) glucose at 27 °C and when the pH of the culture medium ranges between 7.2 to 8.0. Cultured parasites from this system were infective to laboratory mice, morphologically identical to blood-stream forms, and could be subcultured in 96-wells microtiter plate.

Conclusion: We conclude that this approach will prove invaluable in field work and in the micro assessment of EC₅₀ /IC₅₀ values of plant-derived extracts.

Key words: "5CG-Desiccator", *in vitro* cultivation, *Trypanosoma brucei brucei*,



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Introduction

African trypanosomiasis, also known as sleeping sickness in human and Nagana in animal, is a parasitic disease caused by various salivarian species of *trypanosome*, a protozoa whose complex life cycle revolves around a mammalian host and an intermediary vector, the tsetse fly. The bloodstream forms of trypanosome could change the antigenic character of their glycoprotein surface coat, a process that allow them to evade immune response [1]. This phenomenon which is often referred to as antigenic variation, has rendered the development of trypanosomal vaccine almost impossible leaving chemotherapy as the only viable alternative.

There is an urgent need for the development of new trypanocidal drugs as current treatments agents are scarce, toxic and several species have exhibited resistance to them [2, 3, 4]. In addition, there are no drugs in routine use to prevent transmission during blood transfusion [5]. *In vitro* culture techniques are require for screening new compounds and plant-derived drugs for their antitrypanosomal effect and will permit close study of the immunology as well as the biochemistry of the parasite even though a lot have already been established in this regard. This will be particularly useful to developing countries that heavily rely on costly orthodox medications.

At present most of the *in vitro* screening of plant extracts conducted locally employed shorter time (from 5 minutes to

1 hour) for studies [6, 7]. This duration is not adequate for drug-parasite interaction and so results from such studies may lead to wrong or misleading conclusions. One way out of this is to develop techniques that allow for the cultivation of trypanosome in vitro for longer period of time.

Internationally, several scientists have developed *in vitro* culture techniques for various stages of the parasite in the presence of feeder cells [8, 9] and in a variety of axenic media [10]. These techniques, however, use CO₂ incubator, which is expensive and out of reach of researchers in developing countries. Not only that, such advanced equipment requires steady source of electricity and hence not handy for field work. There is need for a simpler and cheaper *in vitro* culture system that will overcome the barriers set by cost, the need for advance equipment and much reliance on electricity among developing nations. Earlier preliminary study in this laboratory shows improvement in the survival rate of trypanosome parasites when slight amount of laboratory-prepared CO₂ was introduced into a culture bottle containing the parasite suspension when compared to control bottles exposed totally to air. Since most standard *in vitro* cultivation techniques for trypanosomes utilizes CO₂ incubator [11, 12], it is felt that pursuing this observation further could provide a promising outcome. In the present work, we have tried to improve the culture methods for *Trypanosoma brucei brucei* in order to address some of the above concerns

It is in view of these that we attempt to adapt a "5CG-desiccator" as a simple and less expensive *in vitro* cultivation system for *T. brucei brucei*.

Methods

Animals and parasite collection

Rats were obtained from the animal laboratory in Kaduna State University, Nigeria. The *T. brucei brucei* was obtained from Nigerian Institute for Trypanosomiasis Research, Kaduna and were maintained in the laboratory by serial passage.

Sourcing of various sera

Fresh blood samples of horse, goat, sheep and cow were obtained from main abattoir in Tudun Wada, Kaduna-Nigeria while human blood was collected from a healthy volunteer.

Chemical /Reagents

Diethylaminoethyl cellulose (DE52) and Tris base were from Sigma Chem. Co. (USA). Amino acids, Sodium Chloride, Sodium Dihydrogen Phosphate (NaH₂PO₄.2H₂O), Disodium

Hydrogen Phosphate (Na₂HPO₄.12H₂O), Glucose, Antibiotics (ampicilline, streptomycin), Ethylenediamine tetraacetic acid (EDTA), Chloroform, Glycerol, Giemsa powder, Calcium carbonate (Ca₂CO₃) were all of analytical grade.

Preparation of Sera

Fresh blood samples from horse, sheep, goat, cow and human were collected in sterile centrifuge tubes. The tubes were allowed to stand for 5 minutes after which they were centrifuge at 2000 rpm for 10 minutes. The red cells settle down the centrifuge tube and the serum supernatant collected using a pasture pipette into separate storage sample containers and stored at -20°C until required.

Maintainance of *Trypanosoma brucei brucei* in laboratory rats

The parasite *T. brucei brucei* was maintained by regular checking of the parasitaemia in infected rats using the Herbert and Lumsden method of counting parasite in the five squares of a 16 squares haemocytometer and once the parasitaemia is found to be massive in any of the infected rats, 2 to 3 drops of the infected blood from the animal tail was collected in a glass container and mixed with little phosphate buffer saline glucose and healthy animals were inoculated with 0.2 ml of the mixed solution. The procedure is repeated when it becomes necessary.

Isolation of blood stream trypanosome from infected rats

Infected rats were sacrificed after chloroform anesthesia and blood collected in syringe containing 0.2 ml 1% (w/v) EDTA solution in 0.1M phosphate buffer saline. Pure parasites were isolated from an infected blood using ion exchange chromatographic method of Lanham and Godfrey [13]. The resin, DE52 cellulose in phosphate buffer saline glucose was transferred into a 20 ml syringe whose outlet was blocked with little cotton wool. The 20 ml syringe was fixed to a retort stand and a small clean beaker was placed at the bottom of the syringe. Before the application of the blood, the column (a 20 ml syringe whose plunger has been removed) is equilibrated with phosphate buffer saline glucose (pH 8.0). Thereafter, 2 ml of infected blood from the animal with rising parasitaemia (between 30 to 60 parasites per microscopic field) was then added to the column and eluted with the same PBSG. By this procedure, the red cells stick to the resin while the parasites come down in the eluent as a thick white suspension. The column is continually washed until eluent is transparent again.

Daily monitoring of Parasitaemia in infected rats

The parasitaemia in infected animals was monitored using the rapid matching method of Herbert and Lumsden [14]. Briefly, tail of infected animal is punctured with needle and a drop (about 5 μ l) of blood collected on a clean slide, covered with a cover slip and viewed under microscope at X400 magnification.

The number of parasites counted in a field of the microscope is then compared with standard table provided by Herbert and Lumsden from where the corresponding absolute number of trypanosomes per ml of blood is extrapolated [14].

Counting of parasite in suspension and culture media

Parasite densities in the suspension as well as in the culture media were determined as described previously [15]. This involves counting the number of live trypanosomes (as judged by their motility) from 5 squares of the red blood cells counting region of a New Improved Neubauer counting chamber. The parasite density is then calculated using the formula below:

$$\text{Density (cells/ml)} = \text{Number counted} \times 50 \times 1000 \times \text{Dilution Factor employed.}$$

Laboratory preparation of gaseous carbon dioxide

About five grams of calcium carbonate was transferred into a glass bottle after which 2M hydrochloric acid was pumped from a wash bottle (A) into the reaction glass unit (B). The gaseous carbon dioxide released was passed through rubber tubing into a deflated polythene gas reservoir (C) until it was fully inflated as shown in **Fig 1**. The chemical equation for the reaction is:



Preliminary screening of various sera for in vitro survival of trypanosomes

Ten clean culture tubes (3 ml capacity) were labelled; cow, horse, goat, sheep and human in duplicate. Two ml of each heat inactivated serum was pipetted into the appropriate labelled cultured tubes. Five micro liter of antibiotic solution (to give an effective antibiotic concentration of 100 μ g/ml) and fifty micro liter of the pure parasite suspension (6 x 10⁷ parasites/ml) were added into all the cultured tubes with gentle shaking before they were incubated in a candle jar. The experiments were monitored at 6 hour intervals.

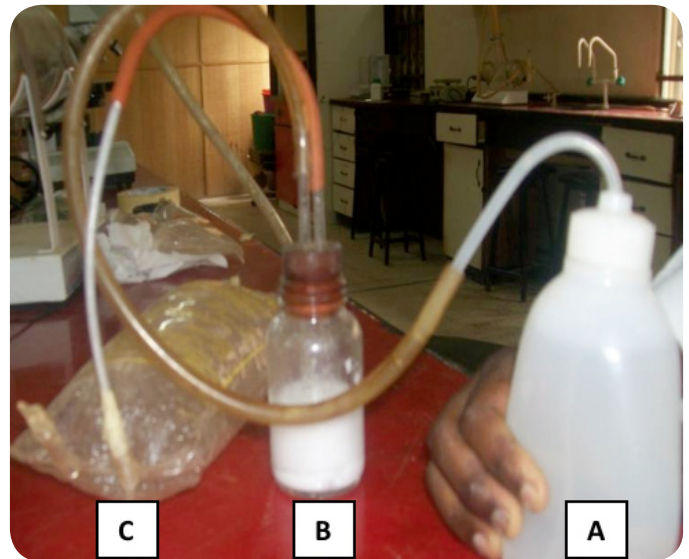


Figure 1. The components required for laboratory preparation of carbon dioxide **A** (Wash bottle containing 2M HCl); **B** (Reaction glass bottle, where the acid reacts with the base); **C** (carbon dioxide storage chamber).

Study on the effect of various gaseous environments on the survival of trypanosomes

Exactly 50 μ l of parasite suspension (8 x 10⁴ parasite per bottle), supplemented with 0.5% glucose, and 10 μ l of antibiotic mixture (streptomycin and penicillin, each at 10 μ g/ml) were dispensed into 9 glass bottles (of 8 ml capacity) containing 200 μ l of heat inactivated horse serum. Of the nine bottles, three (A) were plugged with cotton wool and kept under atmospheric air. The next set of three bottles were left open in a 2000 ml desiccators, little amount of carbon dioxide, prepared in the laboratory, was introduced to the desiccators and immediately covered and sealed with petroleum jelly to prevent gaseous exchange with the exterior of the desiccators. The last set of bottles was kept open in a candle-jar (a similar glass desiccator into which a burning candle is allowed to extinguish by itself). The candle-jar system, also referred to as high oxygen environment, has been estimated to contain 16-18% oxygen [16, 17]. These setups were kept at room temperature (27-28 $^{\circ}$ C) and their densities determined for 18 hours at interval of 6 hours.

Study on the effect of various carbon dioxide concentrations in the 5CG-desiccator

Four desiccators of varying capacity were employed in this study. Three bottles containing culture mixture as described earlier were placed in each of the desiccators. Different

amounts of laboratory prepared CO₂ were added to three of the desiccators in three different concentrations (8, 20 and 50%) of the gas (within the desiccators). The fourth desiccator was left as control (0% CO₂). Mean parasite densities were determined after 24 hours and compared.

Study on the effect of glucose on cultivation of *Trypanosoma bucei bucei*

Exactly 200 µl of horse serum were placed in eight wells of 96-well microtiter plate then 10 µl of antibiotic mixture and 50 µl of parasite suspension were added. The parasite density in each well is about 1.65 x 10⁶ parasites/ml. The culture media in the wells adjusted into four sets of glucose concentrations; 10, 1.0, 0.1 and 0.01 % (w/v) and were incubated in 5% CO₂ gased-desiccator (from where we coined the caption "5CG-Desiccator") at 27°C. Each concentration was in duplicates. Parasite densities were determined from all the wells at 6 hours intervals for twelve hours.

Study on the effect of temperature on parasites *in vitro* survival

In this experiment four desiccators of identical capacity were used and all conditions (capacity of desiccators, 5% CO₂, and initial parasite densities) were kept constant but the temperatures of cultivation were deliberately varied (20, 28, 30, and 37 °C). The ability of parasites to grow at these temperatures were observed by monitoring a decline in parasite densities after 24 hours.

Study on the effect of pH on *in vitro* survival of *Trypanosoma bucei bucei*

To each well of microtiter plate, 100 µl of heat-inactivated horse serum, 100 µl each of phosphate buffers (pH 6.0, 6.4, 6.8, 7.0, 7.2, 7.4) and Tris buffers (pH 7.8 and 8.0), 20 µl of parasite suspension and 10 µl of antibiotic solution (containing 100 µg/ml of penicillin and streptomycin) were added and mixed with the aid of pasteur pipettes. Each experiment was carried out in triplicate, incubated in 5CG-desiccator and parasite densities determined after 12 hr incubation period.

Study on the survival of *Trypanosoma bucei bucei* in various media/solutions

Eagle Minimum Essential medium (containing Amino Acids, Vitamins and Inorganic salts) as described by Eagle [18]; Amino Acid solutions (Eagle medium without vitamins) and Vitamin solution (Eagle media without Amino acids) are three laboratory prepared media that were screened for their ability to sustained *T. b. bucei invitro*. The experiments were conducted in triplicate against controlled solution (Phosphate

Buffer Saline). For each experiment, 50 µl of parasite suspension (8 x 10⁴ parasites/ml) was dispensed into 200 µl of the medium in wells of micro titre plates and the parasite density determined before and after incubation period of 24 hours.

Morphological observation of cultured parasite

Smears of the bloodstream parasite isolate and those of parasites cultivated under different CO₂ concentrations were prepared on glass slide, allowed to dry in air and then fixed by dipping in methanol and stained with giemsa stain. Stained parasites were observed under the compound microscope using oil immersion.

Infectivity study of cultured *Trypanosomes* in mice

Infectivity studies were conducted to know if the cultured parasite still retain the ability to infect a host or not. Eight mice were divided into four groups of two mice per group. Each group was inoculated intraperitoneally with parasite cultured in different carbon dioxide and parasitaemia concentrations (0%, 8%, 20%, 50%) followed daily until the death of the animals.

Result and discussion

This study has successfully adapted a glass desiccator gassed with laboratory prepared 5% CO₂ as an *In vitro* culture system for trypanosomes (5CG-Desiccator).

This study came as a result of our preliminary observation that when slight amount of laboratory-prepared CO₂ was introduced into a culture bottle containing parasite suspension, parasite survived better than control bottles exposed totally to air. Since most standard *in vitro* cultivation techniques for trypanosomes used CO₂ incubator [11, 12], it is felt that pursuing this observation further could provide a promising outcome.

When the parasite *T. bucei bucei* was grown under different gaseous atmosphere, results (**Fig. 1**) revealed that the parasite densities in reaction bottle of both the Candle-jar and the room-atmosphere dropped greatly in comparison with those exposed to laboratory-prepared CO₂ after 12 hours of incubation. This difference in survival is attributable to the differences in the amount of carbon dioxide in each gaseous environment; the composition of the atmosphere itself is about 21 % oxygen, 78 % nitrogen, and less than 1 % carbon dioxide while the candle-jar system, is known to have high oxygen environment of between 16-18% oxygen [16, 17]. The 5CG-desiccator system, however, employs 95 %

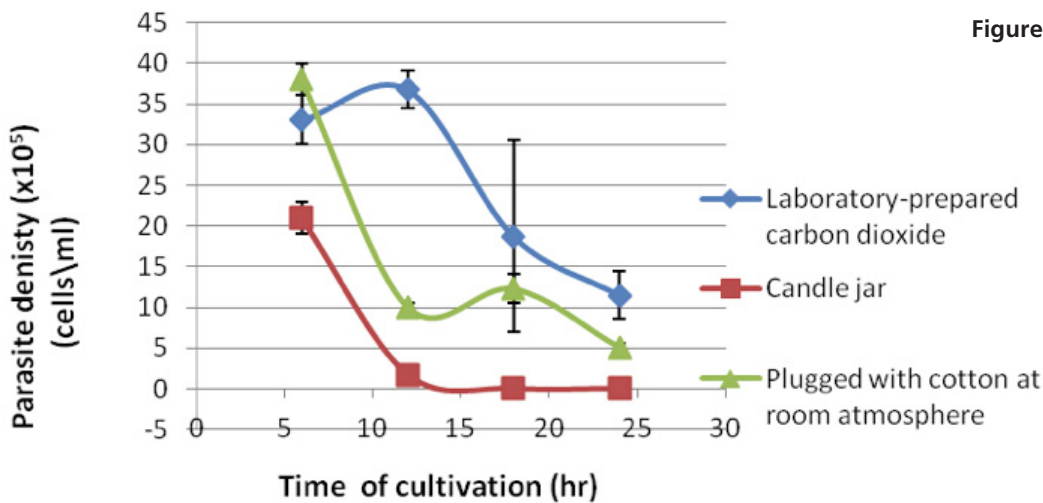


Figure 1. The components required for laboratory preparation of carbon dioxide **A** (Wash bottle containing 2M HCl); **B** (Reaction glass bottle, where the acid reacts with the base); **C** (carbon dioxide storage chamber).

air and about 5 % carbon dioxide. Further comparison was made of the capacity of the gas reservoir employed relative to the capacity of glass desiccator by setting up glass desiccators at different concentrations of carbon dioxide: the results of the effects of different concentrations (%) of the laboratory-prepared CO₂ in culture bottles (**Fig. 2**) shows that CO₂ is very essential in the *in vitro* maintenance of the parasite *T. brucei brucei*. Even though the parasites grown for 24 hours under different CO₂ concentrations proved infective (**Table 1**), higher concentrations of CO₂, however, were found to suppress the growth and survival of the parasite (**Fig. 2**), in addition to, affecting their morphologies when compared with bloodstream forms thus an excess of CO₂ result in inhibiting growth and survival.

Another major aspect of *in vitro* cultivation of trypanosomes is supplementing the culture media with glucose since trypanosomes rely on exogenous glucose as energy source [19, 20]. **Figure 3** shows that glucose concentration between 0.01 and 1.0 % (w/v) gave better results than that obtained with 10%. This range is thought to be only good for bloodstream forms and not the procyclic forms as the later are very sensitive to glucose at low levels (0.2-0.5%) and so must be grown in glucose-free medium [21].

Temperature dependence study (**Fig. 4**) gave optimum growth at 27°C. Several culture temperatures have been used by different research workers, from as low as 24 °C up to 37 °C [8, 10, 12, 22, 23]. Study of pH-dependence (**Fig. 5**) indicated possibility of the parasite surviving well in a medium with pH

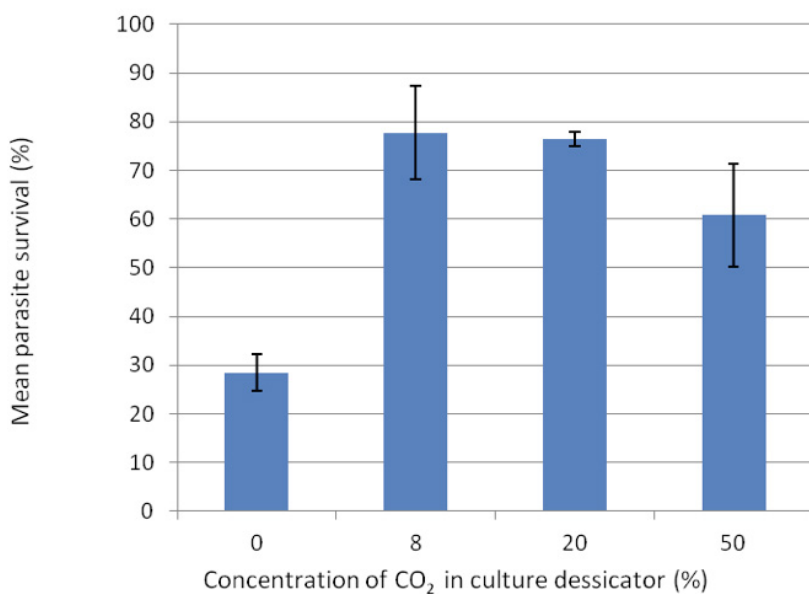
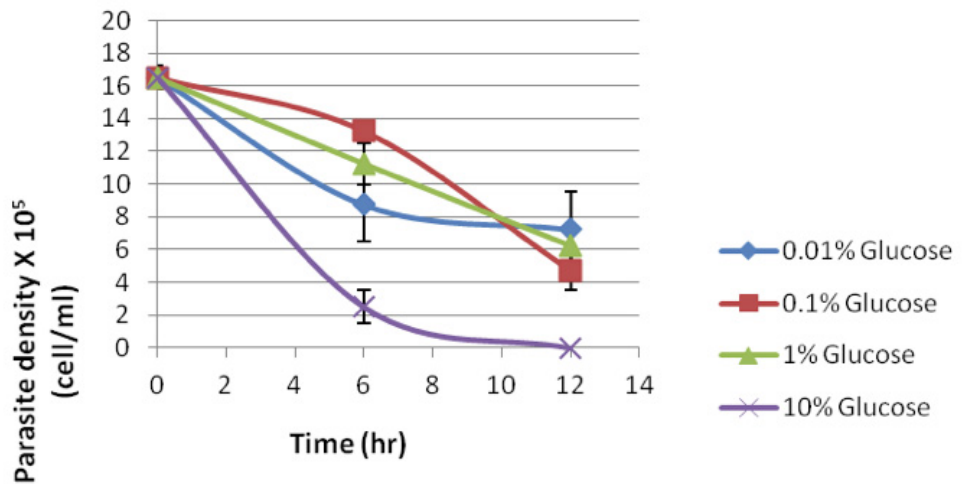


Figure 2. Effect of different concentration of carbon dioxide on *in vitro* survival of *T. brucei brucei*.

Figure 3. Effect of glucose supplementation on *in vitro* survival of parasite.



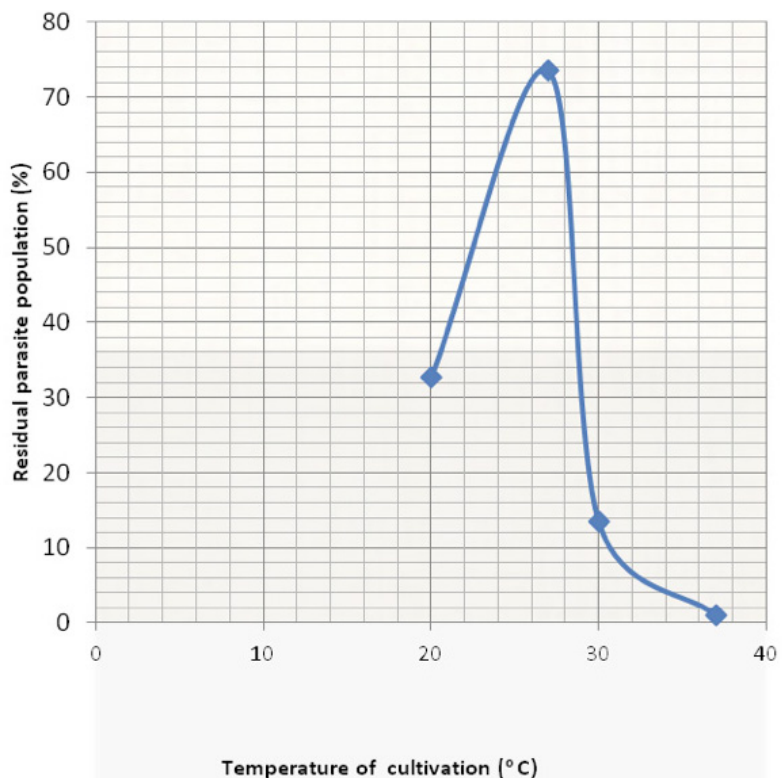
range of 7.0 – 8.0. Higher parasite densities were observed between 7.8 – 8.0, where the medium was buffered with Tris. This may also indicate the superiority of Tris over Phosphate when used to buffer *in vitro* cultivation processes.

Various media/solutions were also screened for their ability to support parasite *in vitro*. The study revealed that the parasites cultivated in laboratory-prepared Eagle's minimum essential amino acid medium survived best (Fig. 6). Supplementing this medium with heat-inactivated horse serum even gave better result (Fig. 7) which does not seem to vary much when either 10%, 20% or 50% serum was used. Initiation of culture with

different parasite densities, prepared by dilution suggests that it is safe to initiate culture with parasite density ranging from 4.5 x 10⁶ to 1.7 x 10⁷ cell/ml in order not to have number of parasites too few or too many on the haemocytometer counting field which will require further dilution, a practice that should be avoided.

Finally, preliminary subculturing experiment (Table 2) shows the possibility of further subculturing already cultured population in microtiter plate using the presented setup. More studies, however, are required to optimize this system. Trying other media with this technique are hereby recommended.

Figure 4. Effect of temperature on *in vitro* survival of *T. brucei brucei* cultivated in 5CG-dessicator.



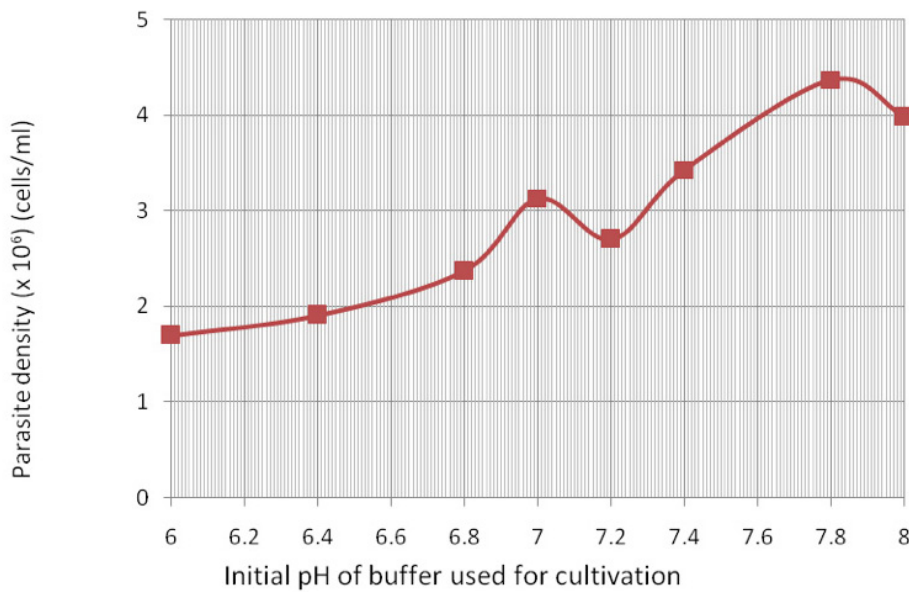


Figure 5. Effect of pH on *in vitro* survival of *T. brucei brucei*.

It is significant to note that the pH study was actually carried out in a 96 wells microtiter, which means that this culture approach could be handy when the need for *in vitro* screening of agents suspected to have antitrypanosomal potentials are used. We are already using this "5CG-desiccator" together with EC₅₀ method of Huber and Koella [24] to determine the IC₅₀ values of various plant extracts with known *in vivo* trypanocidal activities, a step which will no doubt provide a standard comparative assessment for trypanocidal efficacy of local plants. Additionally, the system does not involve the use of complex equipment such as CO₂ incubator and so not quite cumbersome, therefore, could easily be transported and used in areas whose ambient temperatures do not exceed 30°C. This setup, hence, may be very useful to scientists who are involved in field researches.

Table 1. Infectivity potential of *Trypanosoma brucei brucei* cultured under different percent of CO₂ in 5CG-desiccators.

| | | Parasitaemia in mice | | | | | | | |
|----------------------------------|---|----------------------|------|------|------|------|------|------|------|
| | | 0 | | 8 | | 20 | | 50 | |
| Animal | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Duration post inoculation (days) | 1 | - | - | - | - | - | - | - | - |
| | 2 | - | - | - | - | - | - | - | - |
| | 3 | - | - | - | - | - | - | - | - |
| | 4 | + | + | + | + | + | + | + | + |
| | 5 | + | + | + | + | + | + | + | + |
| | 6 | + | + | Dead | + | Dead | Dead | + | + |
| | 7 | Dead | + | | Dead | | | Dead | Dead |
| | 8 | | Dead | | | | | | |

* + presence of parasite in the blood - absence of parasite in the blood.

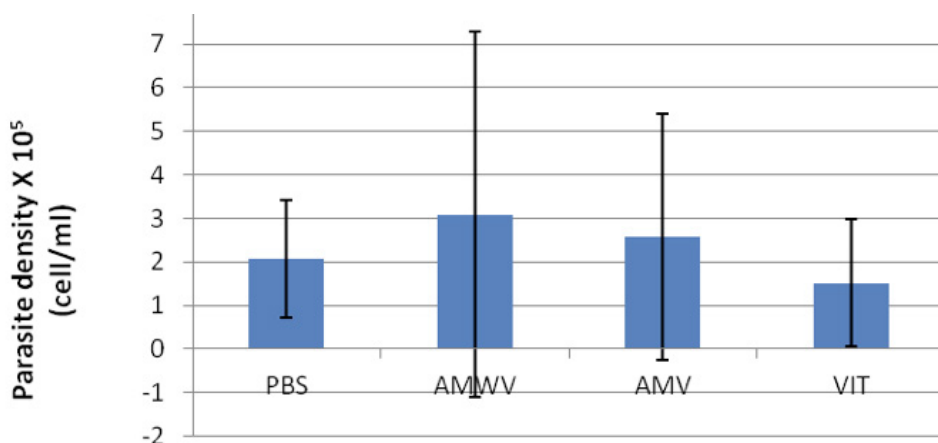


Figure 6. The ability of various media to sustain *T. brucei brucei* *in vitro*. (PBS, phosphate saline; AMWV, Eagle's minimum essential aminoacid without vitamins; AMV, Eagle's minimum essential aminoacid with vitamins; VIT, Eagle's vitamins solution only).

Figure 7. The effect of different proportions of serum in the culture medium on *in vitro* growth of *T. brucei brucei*.

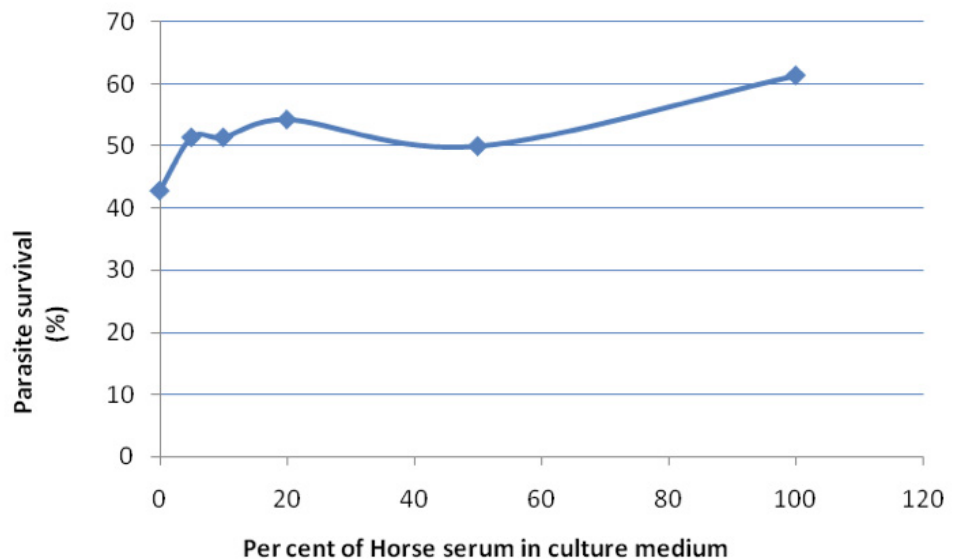


Table 2. Subculturing result of 24 hrs – cultured *Trypanosoma brucei brucei* in 96-well microtiter plate.

| Parasite treatment | Age of parasite in medium (hr) | Mean parasite survival (%) |
|-------------------------------|--------------------------------|----------------------------|
| Maintained in old medium | 48 | 0 |
| Subcultured into fresh medium | 24 | 44.44 |

References

- Vickerman, K. (1978) Antigenic variation in trypanosomes. *Nature* 273: 613-617.
- Onyeyilli, P.A. and Egwu, G.O. (1995) Chemotherapy of African Trypanosomiasis: a historical perspective. *Protozool Abst* 19(5): 229-241.
- Smith, D.H, Pepin, J, and Stich, A.H. (1998) Human African trypanosomiasis: an emerging public health crisis. *Br Med Bul* 54(2): 341-55.
- Nok, A.J. (2005) Effective measures for controlling trypanosomiasis. *Expert Opin Pharmacother* 6(15): 2645-2653.
- Gutteridge, W.E. (1985). Existing chemotherapy and its limitations. *Br Med Bul* 41(2): 162-168.
- Atawodi, S.E., Bulus, T., Ibrahim, S., Ameh, D.A., Nok, A.J., et al. (2003) In vitro trypanocidal effect of methanolic extracts of some Nigerian Savannah plants. *Afr J Biotechnol* 2(9): 317-321.
- Brun, R. and Balmer, O. (2006). New developments in human African Trypanosomiasis. *Curr Opin Infect Dis* 19(5): 415-20.
- Hirumi, H., Doyle, J.J. and Hirumi, K. (1977) African trypanosomes: cultivation of animal-infective *Trypanosome brucei in vitro*. *Science* 196: 992-994.
- Wink, M. (1979) *Trypanosoma theileri*: In vitro cultivation in tsetse fly and vertebrate cell culture systems. *Int J Parasitol* 9: 585-589.
- Baltz, T., Baltz, D., Giround, C.H. and Crockett, J. (1985) Cultivation in a semi-defined medium of animal infective forms of *Trypanosoma brucei*, *T. equiperdum*, *T. evansi*, *T. rhodesiense*. *EMBO J* 4(5): 1273-1277.
- Hirumi, H. and Hirumi, K. (1989). Continuous cultivation of *Trypanosoma brucei brucei* blood stream forms in a medium containing a low concentration of serum protein without feeder cell layers. *J Parasitol* 75(6): 985-989.
- Hirumi, H. and Hirumi, K. (1990) In vitro cultivation of *Trypanosoma congolense* blood stream forms in the absence of feeder cell layers. *Parasitology* 102: 225-236.
- Lanham, S.M. and Godfrey, D.G. (1970) Isolation of salivarian trypanosomes from man and other animals using DEAE-cellulose. *Exp Parasitol* 28: 521-534.

14. Herbert, W.J. and Lumsden, W.H. (1976). *Trypanosomal brucei*: a rapid "marching method for estimating the host parasitaemia. *Exp Parasitol* 40: 247-431
15. Turgeon, M.L. (2007). *Clinical laboratory science the basics and routine techniques*. Mosby-Elsevier, pp 263-275.
16. Jensen, J.B. and Trager, W. (1977). *Plasmodium falciparum* in culture: use of outdated erythrocytes and description of the candle-jar method. *J Parasitol* 63: 883-886.
17. Scheibel, W., Ashton, S.H., and Trager, W. (1979). *Plasmodium falciparum*: microaerophilic requirements in human red blood cells. *Exp Parasitol* 47: 410-418.
18. Eagle, H. (1955). The specific amino acid requirement of a human carcinoma cell (Strain HeLa). *J Exp Med* 102: 37-48.
19. Fairlamb, A.H., Operdoes, F.R., and Borst, P. (1977) New approach to screening drugs for activity against *trypanosomes*. *Nature* 265: 270-271.
20. Bossche, H.V. (1978). Chemotherapy of parasitic infections *Nature* 273(22): 626-10.
21. Simpson, A.M., Hughes, D, and Simpson, L. (1985) *Trypanosoma brucei*: differentiation of *in vitro* grown blood stream trypomastigotes into Procyclic forms. *J Parasitol* 32(4): 672-677.
22. Pizzolatti, M.G., Koga, A.H., Grisard, E.C., and Steindel, M. (2002) Trypanocidal activity of extract from Brazilian Atlantic Rain Forest plant species. *Phytomedicine* 9: 422-426.
23. Shuaibu, M.N., Kanbara, T.Y., Icjinoase, A., Ameh, D.A., and Nok, A.J. (2003) *In vitro* trypanocidal activity of dibutyltin dichloride and its fatty acid derivatives. *Parasitol Res* 91: 5-11.
24. Huber, W. and Koella, J.C. (1993) A comparison of three methods of estimating EC50 in studies of drug resistance of malaria parasites. *Acta Trop* 55: 257-261.

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