World Journal of Biology and Medical Sciences
Published by Society for Advancement of Science®
ISSN 2349-0063 (Online/Electronic)
Volume 4, Issue- 2, 15-23, April to June, 2017
Journal Impact Factor: 4.197

Optimization of *in vitro* Culture of *Giardia lambelia* using Different Type Media

Anas Mahadi Elnazeer, *Mubark Mustafa and **Khitma Hassan EL Malik
Department of Microbiology, Faculty of Pure Applied Sciences,
International University of Africa
*National Center Research, Tropical Medicine Institute, Sudan, Khartoum
** Department of Preventive Medicine, Faculty of Veterinary Medicine,
University of Khartoum

ABSTRACT

The culture of a protozoan flagellates always used for research purposes, in this work a different types of media used to optimize the in vitro culture of giardia-lambelia from human fecal samples. A 15 positive fecal samples were collected and purified by gradient methods a trophozoites obtain either directly from fecal samples or by exocystation, and cultured directly in different types of media with small modification, peptone water, nutrient broth, thioglycolate media and RPMI media were used, cyst stage were counted and detected their viability by stain exclusion method and excystation by acid induction method then cultured in above media .all sample were counted by Neubauer chamber before cultured to detect the inoculums tables (1,). The results the cyst viability percentage ranged from 54%to88% while the exocysted trophozoite percentage ranged from (66 to 85%) tables (2, 3) this may affect to parasite culture.

A pre test of the culture both RPMI and Thioglycolate medium looks better than other table (4) for this reason used in culture establishment table (5-6) failure and success showing that , An average increasing number $A = (56 + 2)/2 = 29\%$ ***we suggest that 29%±5% of increasing number it lower end of culture success for each sample . According to this rule we conclude that the percentage of success culture of batch (1&2) =40% that only in 8 sample from 15 (sample 1, 2, 5, 8, 11, 12, 13 and 14).***

Keywords: optimization, *Giardia –Lambelia* and *In Vitro Culture*.

INTRODUCTION

Cultivation of protozoan parasites involves highly complex procedures and the primary aim of the culture *in vitro* is to produce artificial conditions under which the life cycle of the parasite can be entirely completed outside the host under controlled conditions. Moreover, the rate of development, maturation and reproduction should at least be equal to that which takes place *in vivo* **(**Abaza, 1999**)**. Once a parasite can be cultured *in vitro* even for short periods, the horizon of research on the basic biology of the organism is greatly extended. There are three basic types of culture systems: **(***i***)** xenic: in which the parasite is grown in the presence of an undefined flora; **(***ii***)** monoxenic: in which the parasite is grown in the presence of a single additional species; and **(***iii***)** axenic: in which the parasite is grown in the absence of any metabolizing agents **(**Clark and Diamond, 2002**)**. Giardia parasite was first cultivated by Karapetyan **(**1960**)** in a mixed culture with *Candida gulliermondi* and chick fibroblasts. Meyer **(**1970**)** was the first to report axenic cultivation of *Giardia* from small mammals and later also grew the parasite from human material. Bingham and Meyer **(**1979**)** reported that treatment of mature cysts with hydrochloric acid (pH=2) improved excystation of the parasite. The advantages of *in vitro* culture can be summarized as the following, **(**production of antigens used to prepare monoclonal and polyclonal antibodies against the organism for use in immunologic tests, determine the nutritional requirements of the parasite inoculums, understand the ultra structural organization, assess functional antibodies and cell-mediated protective systems against the parasites, screen drugs *in vitro* in order to identify potential therapeutic agents differentiation of susceptible from resistant isolates so the advances in chemotherapy can be made, production of vaccines and vaccine efficacy, since it can only be done using intact parasites that can be obtained in large quantities and without contaminating influences of host components. It can also provide the parasite inoculums used for the experimental study of the biochemistry, physiology and metabolism of the parasites **(**Visvesvara and Garcia,2002**)** the aim of this study is to optimize suitable medium for in *vitro* culture of giardia -spp *vs* failure and success.

MATERIAL AND METHODS

The fecal samples were collected under medical supervision from Heath Center of international university of Africa (I.U.A) and Ibrahim Malik-Bashayer-ALsheakh Hospitals in Khartoum state, during the periods august2016 to January 2017 and purified by modified, continuous gradient method (Roberts-Thompson et *al,* 1976). The purified cyst suspension was diluted 1:10 in normal saline. A 10*µL* aliquot of the dilution was transferred to a Neubauer counting chamber and counted. To ascertain whether cysts would be capable of excysting or not, their viability was assessed by the stain exclusion method (Bingham *et al,* 1979).The Percentage of viability was determined by the number of unstained and stained cysts in the four large squares of the counting chamber and expressing these as percentage viability as the formula % *Viability = unstained cysts/total cysts x 100*Viability was

determined for every sample. *In vitro* exocystation was done by the Acid Induction method 1M Hcl and incubation in 37°C for 1h (AI Tukhi et *al,* 1991) Standard equipment used in a microbiology laboratory were used, All material, chemical and reagent for media preparation were used to prepare suitable media as described by (SIGMA-ALORICH RPMI CHEMIE GmbH Germany) OR (Hi media, India) and modified by a researcher., prepared sterile bovine serum by (Gibco, India) was used to supplement the media as 10%. Other reagents and stains also were used eosin, and trypan blue stain. Cultures initiation by preparation of inoculums, (the possible viable number of trophozoites in etch sample per µl)**.** All *Trophozoites* that were obtained from fecal samples were inoculated aseptically in duplicate at 4set of tissue culture flasks each of them contained 10-12 ml of different media, RPMI medium ,Peptone water , N. broth and thioglycolate medium, with antibiotics (gentamycin and streptomycin) with 10% bovine serum. They were incubated at 37°C anaerbically and examined daily by using an inverted microscope at 40x magnification for three days. At 24- 48 or 72hour intervals, and the parasite were counted. Old culture medium was carefully decanted and 12ml of fresh medium was added daily. For estimate the success culture we chose only media that gives +ve growth and estimate the average of increasing number by counting the parasite in sub culture in 2 different batch as the same procedure above , this work was done at first time in microbiology lab faculty of pure and applied sciences department of microbiology (I.U.A) Khartoum. Sudan.

RESULTS

a total of parasite number that used in this study , their viability and exocystation were obtain in the following tables ,variation of parasite number in the samples may due to severity of infection in tabe(1) cyst viability percentage rang 54%-88% . a high number of give high percentage of exocysted trophozoites (85%) in table (3) . pre test for media screening and culture initiation only 2 type of media obtain result in table (4) culture failure and success in table(5-6) only 8 samples obtain result in success rate 40-53% .

Table 1. The first total of parasite number in different samples per /ml of purified fecal samples.

Number	Stage	Parasite number/ml
1	Trophozoite	30×10^2
2	Trophpzoite	40×10^2
3	Cyst	85×10^2
4	Cyst	67×10^2
5	Cyst	100×10^2
6	Cyst	46×10^2
7	Trophozoite	8×10^2
8	Trophpzoite	70×10^2
9	Cyst	7×10^2
10	Cyst	9×10^2
11	Trophozoite	80×10^2
12	Trophozoite	54×10^2
13	Trophozoite	65×10^2
14	Trophozoite	70×10^2
15	Trophozoite	54×10^2

Table 2. The cyst viability.

Cyst sample	Total of cyst /ml	Total of viable cyst/ml and percentages %	Total of non viable cysts and percentage %
1	85×10^2	53×10^2 - 62%	32×10^2– 38%
2	67×10^2	41×10^2 - 61%	26×10^2 - 39%
3	100×10^2	70×10^2 - 70%	30×10^2 - 30%
4	46×10^2	25×10^2 - 54%	21×10^2 - 46%
5	7×10^2	5×10^2 - 71%	2×10^2 - 28%
6	9×10^2	8×10^2 - 88%	1×10^2 - 11%

Table 3. The excystation of the viable cysts.

Sample number	Total viable cysts /ml	The total of Excysted trophozoites /ml	percentages %
1	53×10^2	35×10^2	66%
2	41×10^2	27×10^2	65%
3	70×10^2	60×10^2	85%
4	25×10^2	20×10^2	74%
5	5×10^2	0	0
6	8×10^2	0	0

Table 4. Pre test for culture initiation to select what is suitable media.

Number	Stage	Parasite number/ml±5 (inoculium)	Medium culture	Parasite growth
1	Trophozoite	30×10^2	RPMI Tioglycolate N. broth peptone water	+ + _ _
2	Trophpzoite	40×10^2	RPMI Tioglycolate N.broth peptone water	++ + _ _
3	Cyst →(exocysted trophozoite)	35×10^2	RPMI Tioglycolate N.broth peptone water	insignifi cant growth __ _
4	Cyst→(exocysted trophozoite)	27×10^2	RPMI Tioglycolate N.broth peptone water	insignifi cant growth fungi&y east
5	Cyst→(exocysted	60×10^2	RPMI	+++

	trophozoite)		Tioglycolate N.broth peptone water	++ __ _
6	Cyst→(exocysted trophozoite)	20×10^2	RPMI Tioglycolate N.broth peptone water	insignificant growth fungi&yeast
7	Trophozoite	8×10^2	RPMI Tioglycolate N.broth peptone water	_ _ __ _
8	Trophpzoite	70×10^2	RPMI Tioglycolate N.broth peptone water	+++ ++ __ _
9	Cyst→(exocysted trophozoite)	000	RPMI Tioglycolate N.broth peptone water	_ _ _ _ __ _
10	Cyst→(exocysted trophozoite)	000	RPMI Tioglycolate N.broth peptone water	_ _ _ _ __ _
11	Trophozoite	80×10^2	RPMI Tioglycolate N.broth peptone water	+++ ++ __ _
12	Trophozoite	54×10^2	RPMI Tioglycolate N.broth peptone water	++ + __ _
13	Trophozoite	65×10^2	RPMI Tioglycolate N.broth peptone water	+++ ++ __ _
14	Trophozoite	70×10^2	RPMI Tioglycolate N.broth peptone water	+++ ++ __ _
15	Trophozoite	54×10^2	RPMI Tioglycolate N.broth peptone water	++ ++ __ _

Table 5. Culture establishment the failure and success batch (1).

Number	Stage	Parasite numb/ *m*l±5 (inoculi um)	Medium culture	Parasite number/ml±5in 3days			Mean &percentages of increasing number% /samples in 3days			
				D1	D2	D3	mea n	D1	D2	D3
1	Trophozoite	30×10^2	RPMI	60×10^2	75×10^2	90×10^2	75×10^2	50 %	20 %	16%
			Tioglyco late	56×10^2	80×10^2	88×10^2	74×10^2	46 %	30 %	9%
2	Trophpzoite	40×10^2	RPMI	75×10^2	90×10^2	95×10^2	86×10^2	46 %	16 %	5%
			Tioglyco late	70×10^2	80×10^2	85×10^2	78×10^2	52 %	12 %	5%
3	Cyst→(exocy sted trophozoite)	35×10^2	RPMI	_	- -	--	-	-	-	-
			Tioglyco late	_	--	--	-	-	-	-
4	Cyst→(exocy sted trophozoite)	27×10^2	RPMI	-	- -	_	-	-	-	-
			Tioglyco late	_	_	_	-	-	-	-
5	Cyst→(exocy sted trophozoite)	60×10^2	RPMI	60×10^2	75×10^2	75×10^2	70×10^2	0%	20 %	0%
			Tioglyco late	64×10^2	70×10^2	75×10^2	69×10^2	6%	8%	6%
6	Cyst→(exocy sted trophozoite)	20×10^2	RPMI	-	-	-	-	-	-	-
			Tioglyco late	-	-	-	-	-	-	-
7	Trophozoite	8×10^2	RPMI	-	-	-	-	-	-	-
			Tioglyco late	-	-	-	-	-	-	-
8	Trophpzoite	70×10^2	RPMI	77×10^2	90×10^2	100×10^2	89×10^2	9%	14 %	10%
			Tioglyco late	90×10^2	98×10^2	110×10^2	99×10^2	22 %	8%	10%
9	Cyst→(exocy sted trophozoite)	0	RPMI	-	-	-	-	-	-	-
			Tioglyco late	-	-	-	-	-	-	-
10	Cyst→(exocy sted trophozoite)	0	RPMI	-	-	-	-	-	-	--
			Tioglyco late	-	-	-	-	-	-	-
11	Trophozoite	80×10^2	RPMI	85×10^2	99×10^2	110×10^2	98×10^2	5%	14 %	10%
			Tioglyco late	75×10^2	90×10^2	120×10^2	95×10^2	0%	16 %	25%
12	Trophozoite	54×10^2	RPMI	65×1	88×1	95×1	82×1	16	26	7%

				0^2	0^2	0^2	0^2	%	%	
			Tioglycolate	55×10^2	70×10^2	88×10^2	71×10^2	2%	21%	20%
13	Trophozoite	65×10^2	RPMI	75×10^2	87×10^2	77×10^2	79×10^2	13%	13%	0%
			Tioglycolate	65×10^2	77×10^2	85×10^2	75×10^2	0%	15%	9%
14	Trophozoite	70×10^2	RPMI	75×10^2	60×10^2	60×10^2	65×10^2	6%	-%	-%
			Tioglycolate	72×10^2	75×10^2	80×10^2	75×10^2	3%	4%	6%
15	Trophozoite	54×10^2	RPMI	65×10^2	77×10^2	67×10^2	69×10^2	15%	16%	-%
			Tioglycolate	55×10^2	60×10^2	70×10^2	61×10^2	2%	8%	14%

Table 6. Culture establishment the failure and success batch (2) created from sub culturing of batch (1).

No	Stage	Parasite numb/*m*l ±5 (inoculium)	Medium culture	Parasite number/ml±5 in3days			Mean &percentages of increasing number% /samples in 3days			
				D1	D2	D3	mean	D1	D2	D3
1	Trophozoite	90×10^2	RPMI	120×10^2	190×10^2	190×10^2	166×10^2	25%	-%	-%
		88×10^2	Tioglycolate	106×10^2	180×10^2	188×10^2	158×10^2	16%	41%	4%
2	Trophpzoite	95×10^2	RPMI	175×10^2	190×10^2	195×10^2	186×10^2	45%	7%	2%
		85×10^2	Tioglycolate	170×10^2	280×10^2	185×10^2	211×10^2	50%	39%	-%
3	Cyst→(exocysted trophozoite)	--	RPMI	_	- -	--	-	-	-	-
		--	Tioglycolate	_	--	--	-	-	-	-
4	Cyst→(exocysted trophozoite)	_	RPMI	-	- -	_	-	-	-	-
		_	Tioglycolate	_	_	_	-	-	-	-
5	Cyst→(exocysted trophozoite)	75×10^2	RPMI	160×10^2	175×10^2	175×10^2	170×10^2	53%	8%	-%
		75×10^2	Tioglycolate	140×10^2	170×10^2	175×10^2	161×10^2	46%	17%	3%
6	Cyst→(exocysted trophozoite)	-	RPMI	-	-	-	-	-	-	-
		-	Tioglycolate	-	-	-	-	-	-	-
7	Trophozoite	-	RPMI	-	-	-	-	-	-	-

		-	Tioglycolate	-	-	-	-	-	-	-
8	Trophpzoite	100×10^2 110×10^2	RPMI	177×10^2	190×10^2	180×10^2	182×10^2	43 %	6%	-%
			Tioglycolate	190×10^2	198×10^2	150×10^2	179×10^2	43 %	4%	-%
9	Cyst→(exocysted trophozoite)	- -	RPMI	-	-	-	-	-	-	-
			Tioglycolate	-	-	-	-	-	-	-
10	Cyst→(exocysted trophozoite)	- -	RPMI	-	-	-	-	-	-	--
			Tioglycolate	-	-	-	-	-	-	-
11	Trophozoite	110×10^2 120×10^2	RPMI	185×10^2	200×10^2	190×10^2	191×10^2	40 %	8%	-%
			Tioglycolate	175×10^2	190×10^2	200×10^2	188×10^2	31 %	8%	5%
12	Trophozoite	95×10^2 88×10^2	RPMI	98×10^2	125×10^2	195×10^2	139×10^2	3%	32 %	35%
			Tioglycolate	90×10^2	170×10^2	98×10^2	119×10^2	2%	25 %	-%
13	Trophozoite	77×10^2 85×10^2	RPMI	175×10^2	187×10^2	177×10^2	179×10^2	56 %	5%	-%
			Tioglycolate	95×10^2	117×10^2	125×10^2	112×10^2	10 %	12 %	6%
14	Trophozoite	60×10^2 80×10^2	RPMI	75×10^2	90×10^2	110×10^2	91×10^2	20 %	16 %	18%
			Tioglycolate	90×10^2	120×10^2	120×10^2	110×10^2	11 %	25 %	-%
15	Trophozoite	67×10^2 70×10^2	RPMI	85×10^2	97×10^2	110×10^2	97×10^2	21 %	12 %	11%
			Tioglycolate	77×10^2	90×10^2	99×10^2	88×10^2	9%	13 %	9%

DISCUSSION

The introduction of methods for the axenic cultivation of *G. lamblia*, improvements in culture media and development of techniques for the *in vitro* excystation of the parasite have made it possible to obtain many axenic isolates. Although a good medium was described, an easy method for the routine cultivation of *G. lamblia* of human origin was not available. The technique presented in this study therefore may prove to be an important advance in fundamental Giardia research. we aim to optimize different media that may support the parasites growth ,so 4 types of media were used only two can obtain a result and support parasite growth (RPMI&Thioglycolate media) no recent study in Sudan, but there are little scale study by (*Koko et,al* 2012) in Aromatic plant research institute-National Research Center they used RPMI media to maintain the parasite used in *in vitro* susceptibility test of plant extract with out optimize to nutritional factor or environmental factors (serum,amino acid,Co2,O2,and temp) which were studded by *(Anas et,al 2015)* .in

recent study out of 15 human faecal samples containing *G. lamblia* , we were able to obtain 8 *G. lamblia* axenic cultures, representing an overall success rate of 40 -53%. This is similar to result found by **(***Agostinho Cruz et, al2002***)** A high concentration number and viability of cysts in the faeces was needed for successful excystation and axenization (tabe 1-2-3). During the process of excystation, trophozoites hatched from their cysts, in successful cultures, trophozoites began to divide and multiply within 2–3 days and subsequently formed a monolayer on the surface of the tissue culture flask. A significant number of purified cyst samples were not established as cultures. The three major reasons for this were,no excystation, persistent microbial contamination and failure of the trophozoites to establish the monolayer, in the latter case, the number of trophozoites increased during the first 24 h, but then declined. All these finding similar to that result obtain by (Anas et,al 2015).

CONCLUSION

An average increasing number $A = (56 + 2)/2 = 29\%$ I suggest that 29%±5% of increasing number it lower end of culture success for each sample. According to this rule the percentage success culture of batch (1&2) = 40-53% that only in 8 sample from 15 (sample 1-2 -5-8-11-12-13-14).

ACKNOWLEDGMENTS

To all participate and facilitate this work at I.U.A. and hospitals.

REFERENCES

Abaza, B. E. (1999). Cultures of human parasites, methods and applications, Review Article 1-45.

Agostinho, C., Sousa, M. I., Zaida, A., Emilia, L.J.C., Sousa, F. and Miguel, C. (2003). Isolation, excystation and axenization of *Giardia lamblia* isolates *in vitro* susceptibility to metronidazole and albendazole, *Journal of Antimicrobial Chemotherapy,* 51, 1017–1020.

Al-Tukhi, M.H., Al-Ahdal, M.N. and Peters, W. (1991). A simple method for excystation of *Giardia lamblia* cysts. *Annals of Tropical Medicine And Parasitology.* 85, (4) 427- 431.

Anas, M. El, Khitma, H.E.L. (2015). Establishment of in vitro culture of *Giardia*-sp from fecal samples of suspected patients, *Journal of faculty of sciences*, I.U.A , 4, p 14-34.

Bingham, A.K., Jarroll, EL, Jr. Meyer, E.A. and Radulescu, S. (1979). *Giardia* Sp.: Physical factors of excystation *in vitro,* and excystation vs eosin exclusion as determinants of viability. *Experimental Parasitology.* 47,284 -291.

Bingham, A.K. and Meyer, E.A. (1979). *Giardia excystation* can be induced *in vitro* in acidic solutions. *Nature.* 277, 301-302.

Clark, C. G. and Diamond, L. S. (2002). Methods for cultivation of luminal parasitic protists of clinical importance. *Clin. Microbiol. Rev.* 15(3), 329-341.

Rodney D. Adam (1991). The Biology of Giardia spp American Society for Microbiology, *Microbiological Reviews*, p. 706-732.

Corresponding author: Anas Mahadi Elnazeer, Department of Microbiology, Faculty of Pure Applied Sciences, International University of Africa.
Email: anaselnazeer7@yahoo.com