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A using of modified Cystein-Peptone-Liver infusion-Maltose medium for cultivation of Trichomonas vaginalis

Wilai Saksirisampant *

Charnchai Saksirisampant **

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Objective : *To introduce a modified medium for cultivation of T. vaginalis from clinical specimens by in vitro cultivation.*

Design : *Prospective study.*

Setting : *Department of Parasitology, Faculty of Medicine, Chulalongkorn University. Gynecological Clinic of Lerdsin General Hospital.*

Subject : *One hundred and fifty-two women with vaginal symptoms.*

Methods : *All 152 clinical specimens from women with vaginal symptoms were transported in Hank's balanced salt solution and examined for T. vaginalis under the microscope. Only the positive specimens were cultivated in CPLM, modified CPLM and Trichosel medium. The inoculation size was $1x10^4$ in each. The parasites were detected and counted after 48 hr in a 36.5°C 5% CO_2 incubator.*

Result : *Twenty-one clinical specimens contained T. vaginalis. The modified CPLM medium showed high efficiency for organism reproduction. The mean increasing number of T. vaginalis were 10.47, 54.33 and 11.23 times in CPLM, modified CPLM and Trichosel medium respectively.*

* Department of Parasitology, Faculty of Medicine, Chulalongkorn University

** Department of Obstetrics and Gynecology, Lerdsin General Hospital

Conclusions : *This modified formula of a standard CPLM medium may be used as a readily available, low-cost medium, particularly suitable for laboratories with budgetary restraints and can eliminate the problem of the expiratory date. The efficient reproduction of T. vaginalis in this modified formula may also be suitable for antigen preparation or in vitro drug sensitivity studies.*

Key words : *Trichomonas vaginalis, Cystein-Peptone-Liver infusion-Maltose medium (CPLM).*

Reprint request : Saksirisampant W, Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

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วิไล ศักดิ์ศิริสัมพันธ์, ชาญชัย ศักดิ์ศิริสัมพันธ์. อาหารเลี้ยงเชื้อชนิด Cystein-Peptone-Liver infusion-Maltose สูตรดัดแปลง เพื่อการเพาะเชื้อ Trichomonas vaginalis. จุฬาลงกรณ์เวชสาร 2544 ม.ค; 45(1): 31 - 8

วัตถุประสงค์ : *ทำการดัดแปลงสูตรอาหารเลี้ยงเชื้อชนิด Cystein-Peptne-Liver infusion Maltose (CPLM) แล้วนำมาใช้เพาะเชื้อ T. vaginalis โดยเปรียบเทียบกับการใช้อาหารเลี้ยงเชื้อสูตรมาตรฐานเดิม CPLM และสูตรทางการค้าสำเร็จรูป Trichosel*

รูปแบบการวิจัย : *การศึกษาแบบไปข้างหน้า*

สถาบันการศึกษา : *ภาควิชาปรสิตวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย และกลุ่มงานสูติ - นรีเวช โรงพยาบาลเลิดสิน*

ผู้เข้าร่วมการศึกษา : *หญิงไทย จำนวน 152 ราย ที่มีอาการคัน ตกขาว*

วิธีวิจัย : *ชีววัตถุตกขาวจากหญิงไทย 152 ราย ถูกลำเลียงใน Hank's balance salt solutium เพื่อนำไปตรวจหาเชื้อ T. vaginalis ภายใต้กล้องจุลทรรศน์ ชีววัตถุรายใดที่พบเชื้อจะถูกเพาะเลี้ยงในอาหารเลี้ยงเชื้อทั้ง 3 ชนิด คือ CPLM, modified CPLM และ Trichosel โดยมีจำนวนเริ่มเพาะที่ $1x10^4$ ตัว เท่า ๆ กัน แล้วศึกษาดูจำนวนเชื้อที่ เพิ่มขึ้นหลังจากอบไว้ 48 ชม. ที่อุณหภูมิ 36.5 °C มี CO_2 จำนวน 5 %*

ผลการวิจัย : *มีผู้ป่วย 21 ราย ที่พบว่าติดเชื้อ T. vaginalis หลังจากเพาะเลี้ยงแล้วพบว่าอาหารเลี้ยงเชื้อชนิด CPLM สูตรดัดแปลง มีประสิทธิภาพของการเพิ่มปริมาณเชื้อสูงมาก กล่าวคือ ค่าเฉลี่ยของเชื้อที่เพิ่มขึ้นคิดเป็น 10.47, 54.33 และ 11.23 เท่าใน CPLM สูตรมาตรฐาน, CPLM สูตรดัดแปลง และ Trichosel ตามลำดับ*

วิเคราะห์สรุปผล : *CPLM สูตรดัดแปลง มีความสะดวก ราคาถูก เหมาะสมกับห้องปฏิบัติการที่มีงบประมาณจำกัด ตัดปัญหาเรื่องอายุการใช้งาน และที่สำคัญ คือสูตรดัดแปลงนี้ให้การเพิ่มจำนวนของเชื้อมากกว่า เหมาะอย่างยิ่งที่จะใช้ในงานที่ต้องการเชื้อมาก ๆ เช่น การเตรียมแอนติเจน การศึกษาประสิทธิภาพของยาในหลอดทดลอง เป็นต้น*

Trichomoniasis is a widespread sexually transmitted disease, with prevalence of 5 % in asymptomatic subjects attending family planning clinics. [1] Clinical manifestations usually associated with trichomoniasis, such as a yellow-green discharge, pruritis, vaginal malodor and abdominal pain, are nonspecific and cannot be relied on for an accurate diagnosis. [1-3] Clinically-based laboratory diagnosis is unsatisfactory. The vaginal pH is elevated above 4.5 in as many as 90 % of cases.[4] This finding, however, is nonspecific, because 90 % of women with bacterial vaginosis also have an elevated vaginal pH. [5] A fish odor after application of 10 % potassium hydroxide is present in 50 % of patients. [6] The time-honored approach for the diagnosis of trichomonal infections has been microscopic evaluation by the wet-mount method. This procedure, however, detects only 35 – 80 % of cases, depending on the expertise of the microscopist. [7,8] Thus, culture in order to multiply the organism remains the most accurate method for detection of T. vaginalis organisms in patient samples. [7-9] For this purpose, the axenic liquid medium of Diamond and Cystein-Peptone-Liver infusion-Maltose (CPLM) are considered the the "gold standard".[10-11]

Additionally, several commercial liquid media are available including Trichosel Broth (BBL) and Trichomonas medium (Oxoid). Among the disadvantages of these commercial alternatives are that they are expensive and not readily available, especially in developing countries.

This study attempted to compare the usefulness of a minor modification formula of CPLM medium with those of the standard CPLM and commercial Trichosel Broth for detecting T. vaginalis from clinical samples.

Materials and Methods

Three kinds of axenic liquid medium including CPLM, modified CPLM and Trichosel were used for the in vitro cultivation of T. vaginalis and compared for their efficiency of organism reproduction. The ingredients of each kind were shown in Table1.

Inactivated steriled horse serum or human serum or amino acids (BME, BBE, Gibco Grand Island, NY., U.S.A.) were added after the medium was autoclaved. Antimicrobial agents were added aseptically afterwards. Five ml. of medium was dispensed in 15x130 mm. screw-cap tubes.

Women with vaginal symptoms attending the Gynecological clinic of Lerdsin General Hospital from February 1997 to October 1998 were examined for T. vaginalis infection.

A total of 152 women were studied. Vaginal fluid samples were obtained from the posterior fornix with cotton swabs and transported in 1 ml of steriled Hank's balanced salt solution (HBSS) containing penicillin G, streptomycin sulfate and amphotericin B at the final concentration of 400 unit, 400 μg and 100 μg respectively.

21 samples (13.8 %) in transported medium were positive for T. vaginalis. Under microscopic evaluation the jerky or nondirectional motility characteristic of viability were clearly shown.

All the 21 positive samples in HBSS were counted in a white blood count chamber and then inoculated in the three kinds of medium. The inoculation size was $1x10^4$ in each medium. The parasites were detected and counted after cultivation for 48 hr. at 36.5 °C in a 5 % CO_2 incubator.

Table 1. Compositions of CPLM, modified CPLM and Trichosel medium per 1 liter of purified water.

CPLM		Modified CPLM		Commercial Trichosel	
Bacto peptone	32 g	Bacto peptone	32 g	Trypticase	20 g
Bacto agar	1.6 g	Bacto agar	1.6 g	L-cysteine	1.5 g
Cystein HCl	2.4 g	Cystein HCl	2.4 g	Maltose	1.0 g
Maltose	1.6 g	Maltose	1.6 g	Agar	1.0 g
Liver infusion	4.7 g	Liver infusion	4.7 g	Chloramphenicol	0.1 g
NaCl	4.5 g	NaCl	4.5 g	Methylene blue	3.0 g
KCl	0.21 g	KCl	0.21 g	Unpublished amino-	
$CaCl_2$	0.24 g	$CaCl_2$	0.24 g	acid and vitamin	
$NaHCO_3$	0.10 g	$NaHCO_3$	0.10 g	Human serum	50 ml
Horse serum	100 ml.	Amino acid (BME, BBL)	1.8 g	PH	6.0 ± 0.2
Amphotericin B	1 mg	Human serum	100 ml.		
Penicillin G	400,000 U	Amphotericin B	1 mg		
Streptomycin	0.4 g	Penicillin G	400,000 U		
PH	6.0 ± 0.2	Streptomycin	0.4 g		
		pH	6.0 ± 0.2		

Results

A total of 152 women were studied. Twenty-one (13.8 %) were infected with T. vaginalis. Full concordance of organism reproduction was observed among the three kinds of culture medium. The times to identity of the protozoan were also similar.

Optimal growth and reproduction of T. vaginalis requires anaerobic conditions. For in vitro cultivation, essential nutrients, including carbohydrate, amino acids and minerals are mandatory.[12] Although the optimal medium for culturing of T. vaginalis has been defined the Diamond's or CPLM. We thought that CPLM medium, the standard broth for anaerobic cultures, might be suitable alternative to Diamond's medium. Furthermore, these two media have in common several ingredients.[13] We chose to make the minor modifications in the CPLM formula instead of in the Diamond's.

In the present study, we demonstrated that modified CPLM medium showed a high potential for organism reproduction (Table 2). Isolated strains of T. vaginalis showed good reproduction in all these three media. However the highest number was found in modified CPLM medium. The mean increasing numbers of T. vaginalis were 10.47, 54.33 and 11.23 times in CPLM, modified CPLM and Trichosel respectively. Interestingly, there was one specimen that exhibited low number and non-active organism with numerous white blood cells. This could be negatively diagnosed if it was examined by the un-expertise technician. We did cultivate this specimen by using three of these media. After 48 hr of cultivation, we could detect the active organism in modified CPLM and the commercial Trichosel but not in CPLM. The number of T. vaginalis from this specimen highly increased when the sub-cultivation were made.

However, the increasing mumber in modified CPLM was more than in the commercial Trichosel. Most importantly, the cost of purchasing modified CPLM medium was lower than the cost of material in preparing the same amount of CPLM and Trichosel, because, normal human serum can be obtained from the Blood-Bank Center. These human sera are separated in the process of packed red blood cell preparation. Moreover, horse serum is usually too expensive for a laboratory low budget.

Table 2. The number of T. vaginalis after cultivation at 36.5 °C, 48 h in 5% CO_2 with the inoculation size of $1x10^4$ cells.

No.	Number of organisms..... $x10^4$		
	CPLM	Modified CPLM	Trichosel
1.	21	71	19
2.	15	93	12
3.	8	27	6
4.	8	36	10
5.	5	24	9
6.	20	102	17
7.	7	35	10
8.	14	66	16
9.	3	27	5
10.	3	20	5
11.	5	27	10
12.	8	53	12
13.	24	121	22
14.	10	42	16
15.	10	74	9
16.	9	35	5
17.	5	95	13
18.	7	39	6
19.	9	37	8
20.	22	87	18
21.	7	30	8
Mean	10.47	54.33	11.23

Table 3. The mean increasing numbers of T. vaginalis in medium after incubation at 36.5 °C 48 hr. in 5 % CO_2

Media	The mean increasing times.
CPLM	10.47
Modified CPLM	54.33
Trichosel	11.23

Discussion

We have demonstrated that modified CPLM medium performed the most efficiently for T. vaginalis reproduction. In addition, Candida spp. were slower to appear in these modified formula than in Trichosel. The modified CPLM medium has the advantages of being easily prepared from readily available components and a lower cost than commercial alternatives. This consideration is particularly relevant for laboratories with budgetary restraints, a common situation in developing countries.

Moreover, modified CPLM medium allows more flexibility in providing a supply of culture medium for T. vaginalis and eliminates concerns about expiratory dates. In can be readily prepared according to actual needs. We have also used this modified medium to study the effect of tinidazole and ornidazole on Trichomonas vaginalis by in vitro cultivation. [16]

The combination of culture and wet-mount examination remains the standard approach for detecting T. vaginalis in patient samples. [14] The obvious advantage over culture is that the probe assay takes only 40 minutes to complete.[15] However, modified CPLM medium was found to be more efficient than standard medium for recovering the parasite from clinical specimens. Many investigators have

attempted to harvest the high amount of organisms for drug resistance and immunodiagnosis studies. [17,18] This may provide a readily available and low-cost substitute medium for antigen preparation or in vitro drug sensitivity studies.

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