

## A SIMPLE METHOD OF IN-VITRO CULTURE OF PLASMODIUM FALCIPARUM IN SCREW-CAPPED VIALS\*

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### ABSTRACT

In a preliminary study carried out in a simple field laboratory in Bandar-Abbas, southern Iran, five isolates of P.falciparum collected from the patients was cultured in RPMI 1640 medium with human serum in the tightly closed screw capped vials without any devised system for adjustment of CO<sub>2</sub> and O<sub>2</sub> mixture. The same isolates were also cultured, simultaneously, in petri-dishes using jensen-Trager candle jar method.

Although, the cultures could not be maintained more than 27 days, mostly due to unfavourable conditions existed in the laboratory, the longevity of parasites were more or less the same in both techniques. In this study the multiplication rates of parasites in the screw capped vials were higher than rates obtained in petri dishes in candle Jar method.

### INTRODUCTION

Since the development of a continuous in-vitro culture of P.falciparum by Trager and Jensen in 1976 (7) based

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on slow flow of the RPMI 1640 medium with human serum over a thin settled layer of human erythrocytes under an atmosphere of high CO<sub>2</sub> and low Oxygen, several other methods such as candle Jar method (4), method for large - volume cultivation of malaria parasites (2), application of semi-automated apparatus (5), flow fask apparatus (7), and simple erlenmeyer flask culture system (6) have been applied in the culture of malaria parasites. The technique developed by Haynes et al. in 1976 (3) for the culture of P. falciparum is somewhat different from the above methods.

In our conditions which we wanted to establish continuous culture of malaria parasites in a small laboratory in the Bandar-Abbas Field Research Station located in Southern Iran, even the simplified candle Jar method (2) encountered with some problems. In this present study, for reduction of these problems we used screw capped vial method instead of petri-dishes and candle Jar technique for in-vitro culture of P. falciparum.

## Materials and methods

### Medium

The following two slightly different RPMI 1640 media were used:

a) The liquid fresh medium made from the powder of RPMI Medium 1640 (GIBCO Laboratories) with HEPES Buffer according to the method described by Jensen and Trager 1977(4). After addition of gentamicin sulfate (50mg/liter) the medium was sterilized by filtration through 0.45µ Milipore filter and stored in 100ml screw capped bottle at 4°C. For washing of erythrocytes 100ml of the medium was supplemented with 4.2ml of 5% sterilized solution of sodium bicarbonate. For making cell suspensions for cultures 10% of AB+ type of human serum (received from Iranian National Blood Transfusion Center) was also added to the medium.

b) The liquid TC Medium RPMI 1640 (DIFCO Laboratories) was used without HEPES buffer and antibiotic. For use only 10% AB+ type human serum was added to this commercial liquid medium.

## P.falciparum isolates

Samples of blood were collected in heparinized vacutainer tubes from vein puncture of the out-patients with acute P.falciparum infection in Bandar-Abbas Laboratory of Malaria Eradication Unit.

### Un-infected erythrocytes

A+type un-infected human blood, collected in CPD bag was provided by Iranian National Blood Transfusion Center

### Cultivation procedure

Washing of infected and un-infected erythrocytes and making 12% cell suspensions in the media (a or b) with 10% human serum and setting up the cultures in 4 to 6 small plastic petri-dishes (35mm) were carried out according to the candle Jar method described by Jensen and Trager in 1977 (4). Simultaneously, from the same suspensions 4 to 5ml cultured in each of 1 to 3 sterilized flat-bottomed screw capped vials with about 30ml capacity (85mm height, 28mm outside diameter and 24mm neck opening). The operations of cultivation were done inside a simple local made Perspex hood equipped with an ultraviolet germicidal lamp and a Bunsen gas burner. Before and after pouring the cell suspension into each vials and also in the time of changing the medium, the upper and the neck parts and metal cap of the vials were flamed and finally the cap was tightly screwed and the vials were directly incubated in 37° to 38°C. The depth of the medium above the thin settled layer of erythrocytes in the vials was about 10-12mm.

The changing of medium was carried out everyday. Thin blood smears were prepared every other day, stained with Giemsa and examined microscopically for estimation the number and determination stages of the parasites. To subculture the cultures were diluted two or sometimes three times with fresh washed un-infected A+type erythrocytes every 5 to 7 days.

## RESULTS

Results of the in-vitro culture of P.falciparum in candle Jar and screw capped vial methods in five isolates of P.falciparum are summarized in the following table.

<u>P.falciparum</u> isolates	Number of subcultures		Longevity of parasites in cultures (days)	
	Candle Jar	Screw capped vials	Candle Jar	Screw capped vials
F <sub>1</sub>	1	2	6	16
F <sub>2</sub>	3	2	27	13
F <sub>3</sub>	-	1	5	7
F <sub>4</sub>	2	2	10	19
F <sub>5</sub>	2	1	18	12

Although, the maximum longevity of the parasites (in F<sub>2</sub> isolate) in culture was 27 days in one of the six petridishes in candle Jar method, in general, there is not considerable difference between the two techniques in maintaining parasites alive. The multiplication rates of parasites in the cultures of the vials were relatively higher than those of the petri dishes. The maximum multiplication rate, in the case of F<sub>1</sub> isolate was about 6 times of the original number in the washed erythrocytes of the blood samples taken from the patients. The original number, was 15 infected erythrocytes per 10,000 of red blood cells. The multiplication rate in the case of candle Jar method was about 2 times.

In each of the five P.falciparum isolates the number of parasites started to decrease after 1 to 3 subcultures due to bacterial contamination or the parasites died out without any obvious reason. All stages of asexual form were observed in the stained thin films prepared from the cultures of the vials and petri-dishes. Gametocytes appeared in three isolates of P.falciparum in both methods of cultures after 8 to 15 days.

In the commercial liquid RPMI 1640 medium the rates of the parasite multiplication were rather higher than the rate in the RPMI 1640 medium prepared in our laboratory with HEPES buffer and gentamicin. But the contamination, mostly bacterial forms, were occurred more frequently

in the commercial medium without antibiotic.

## DISCUSSION

In this preliminary study, although, we could not continue the in-vitro culture of the parasites for a considerable long period of time due to unfavourable conditions existed in our field laboratory, nevertheless we found that at least in such conditions, the results of in-vitro cultures of P.falciparum in screw capped vials without any devised equipment for the gas mixture ( $O_2$ ,  $CO_2$  and  $N_2$ ) is more or less the same as the candle Jar method; which it seems to provides the optimum combination of  $CO_2$  and  $O_2$  for growing of malaria parasites.

Perhaps in the case of our applied simple hood (without blower), burning of Bensen gas burner inside the hood, breathing of examiner near the enterance of the hood and flaming the neck and upper parts of the vials during culture operations all have caused the decreasing of  $O_2$  and inctezasing of  $CO_2$  levels.

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