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Research Article

IN VITRO CULTIVATION OF PLASMODIUM FALCIPARUM

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Abstract

Malaria is a human disease which still causes high morbidity and mortality. The present study was undertaken for cultivation of the Plasmodium falciparum in RPMI-1640 medium and observed for their growth. Total 12 malaria positive samples were included in this study out of which 5 were for Plasmodium falciparum and 7 were mixed Plasmodium species. Inoculums were incubated at 37°C for 24 hours. After 48 hours of incubation the culture shows 100% growth of Plasmodium falciparum and 50% growth of mixed Plasmodium species. RPMI-1640 with supplemented with L-glutamine, HEPES buffer, $NaHCO_3$, hypoxanthine, 0.5% Albumax II and 50µg/ml Gentamicin was useful media for cultivation of Plasmodium falciparum.
Keywords: Malaria, Plasmodium falciparum, culture, Roswell Park Memorial Institute-1640 medium, filtration, CO2 incubator

INTRODUCTION

Malaria still poses a threat to the health of residents and travellers in tropical countries. There are currently six species of the genus Plasmodium known to infect humans: Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale curtisi, Plasmodium ovale wallikeri, Plasmodium malariae and Plasmodium knowlesi. Of these, P. falciparum is the species responsible for most of the mortality and morbidity associated with the disease and it is during the asexual intraerythrocytic stages that most of the symptoms of malaria are manifest. Studies into many aspects of human malaria parasite biology was possible by the development of a method to culture asexual blood stages of P. falciparum in vitro in 1976 [1].

The first in vitro development of malaria parasites was reported nearly 80 years ago by Bass and Johns (1912). They obtained defibrinated blood from patients infected with P. falciparum and cultured the sample at 37°C in a glass vial to which a small amount of glucose had been added. Newly developed rings could be observed after one generation time and only occasionally after one to two additional cycles [2].

Number of attempts were made to develop a better culture medium including such methods as short term cultures with Harvard growth medium [3,4] and modified Harvard growth medium [5]. However, only when a new medium, RPMI 1640, developed originally for culture of human leukocytes [6], became available and were proven to be significantly

superior to Harvard medium did it allow for a successful continuous culture of the malaria parasite and which is still being widely used throughout the world [7]. This technique is particularly suitable for small scale cultures, such as those required for drug sensitivity tests, but it is not adequate for large scale cultures where high yield of parasite is needed, for instance, in parasite enzyme isolation and purification and in studies of parasite organelles. Therefore, other techniques were developed for large scale cultures, such as tilting flask [8] and shaken flask [9] methods. Unfortunately, these procedures require wasteful daily changes of medium either by manual or semi-automated methods. A technique for cultivation of P. falciparum without daily medium replacement has been reported which produced high yield of parasites after 3-4 days of cultivation [10].

MATERIALS AND METHODS:

This prospective study was carried out at Department of Microbiology, MGM Medical College and Hospital, Navi Mumbai, India over a period of one year from January 2013 to December 2013.

Ethical clearance: Ethical clearance was obtained from the Institutional Ethical committee of MGM Institute of Health Sciences (Deemed University), Navi Mumbai before starting the project.

Total 12 malaria positive samples were subjected to cultivation using RPMI-1640 medium along with control ATCC strain 3D7 which was procured from Haffkine Institute for Training, Research & Testing, Mumbai, India.

Sample Collection: 5 ml of blood was withdrawn from antecubital vein by venipuncture in EDTA tube taking all sterile precaution and properly labeled the tube with patient's name, date and time. Both thick and thin smears were examined by staining with Giemsa stain (Lot No. 0000167125-HiMedia Laboratories Pvt. Ltd., India) and JSB stain (Batch No. 49677-Bio Lab Diagnostic Pvt. Ltd., India), using standard protocols provided with kit. The blood samples were centrifuged and the plasma was discarded and RPMI – 1640 incomplete medium was taken in test tube and again centrifuged. The procedure was done thrice to remove plasma and buffy coat except red blood cells.

Processing of Samples:

Plasmodium falciparum and mixed *Plasmodium* species along with control strain 3D7 was cultured according to modified method of Jensen and Trager (1980) in 'O' positive human red blood cells at a 5% haematocrit in RPMI-1640 medium, supplemented with L-glutamine (4.2mM), HEPES buffer (25 mM), NaHCO3 (25 mM) hypoxanthine (6.8 M), 0.5% Albumax II (Invitrogen) and 50µg/ml Gentamicin [11]. Culture medium was sterilized by filtration through a membrane filter of 0.22 µm porosity for making stock 500 ml and stored in a sterile screw capped bottle. The working media was again filtered using syringe filter, the filtrate was stored in 125 ml square bottle (Genetix Biotech Asia Pvt. Ltd.), sealed with parafilm (Genetix Biotech Asia Pvt. Ltd.) and stored in refrigerator at 2 - 4°C, which was ready to use. Complete RPMI-1640 medium was taken in a sterile tissue culture flask (Genetix Biotech Asia Pvt. Ltd.) and malarial parasites were cultivated. The inoculum was incubated at 37ºC in 5-10% CO_2 incubator [Figure 1(A-D)]. Smears was prepared, stained and examined each day for growth of malarial parasites [Figure 2(A-D)].

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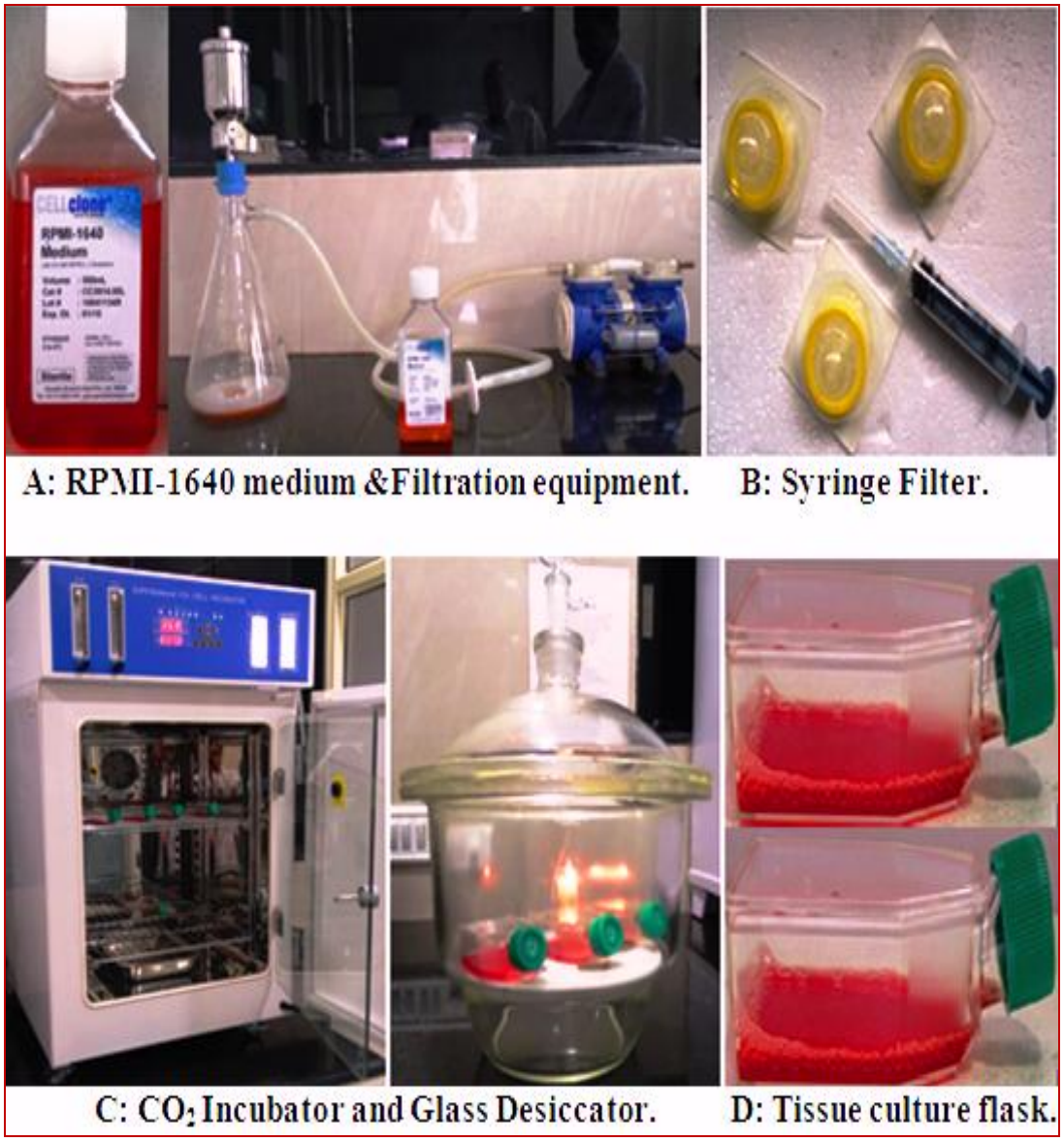


Figure 1(A-D): Showing different equipments and RPMI-1640 medium for cultivation of Plasmodium falciparum.

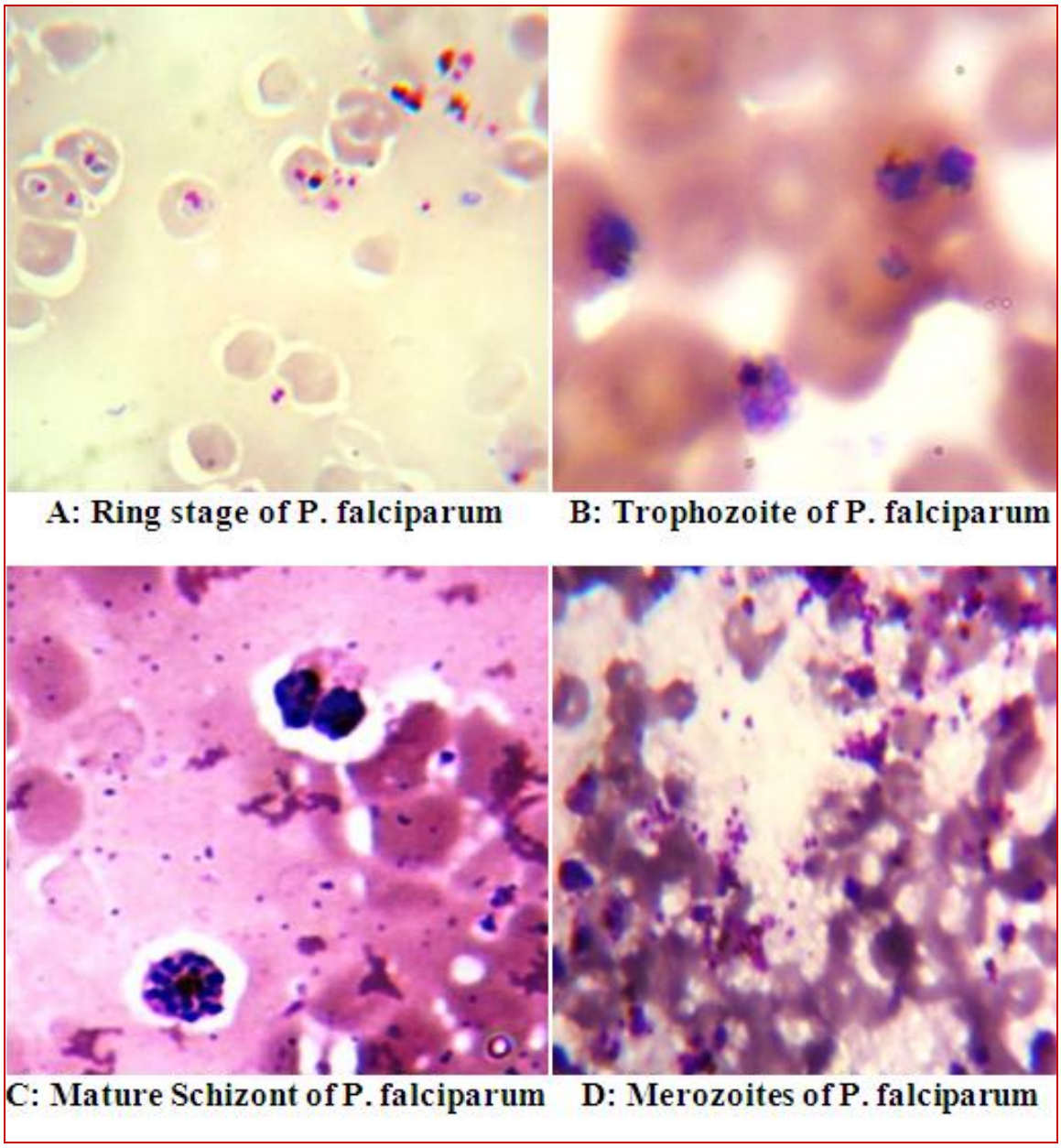
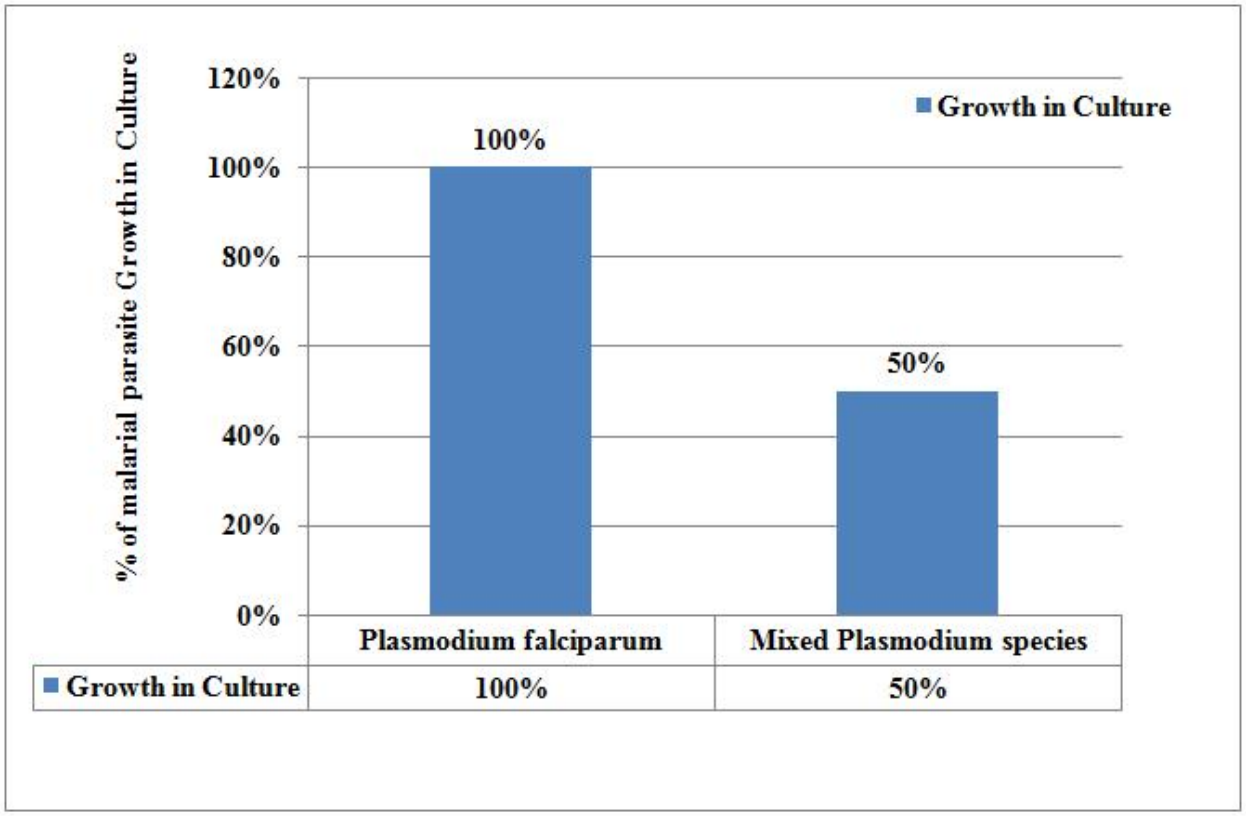


Figure 2 (A-D): Showing different morphology of Plasmodium falciparum after culture on RPMI-1640 medium.

RESULTS:

The present study investigated with 12 malaria positive samples out of which 5 were Plasmodium falciparum and 7 were mixed Plasmodium species. After the 48 hours of incubation the culture shows 100% growth of Plasmodium falciparum and 50% growth of mixed Plasmodium species [Graph 1].



Graph 1: Showing growth of Plasmodium falciparum.

DISCUSSION:

Total 12 blood samples positive for malaria were included in this study and out of which 5 were for Plasmodium falciparum and 7 were mixed Plasmodium species. 12 samples were processed for cultivation. Plasmodium falciparum showed 100% growth whereas mixed Plasmodium species shows 50% growth in culture medium.

P Preechapornkul et al (2010), cultured P. falciparum isolate (TM036) in RPMI, supplemented with 0.5% Albumax II, they reported that the culture could be maintained continuously in the system by daily changes of a small volumes of medium. They observed that cultures attained 8% parasitemia in 40% hematocrit, thereby providing a total parasite biomass of 6.0x109 parasitized erythrocytes. [12]

Srivastava K et al (2007), studied on RPNI, a combination of three commercially available growth media (RPMI-1640, NCTC-135 and IMDM) support long term continuous cultivation of 3D7 strain of Plasmodium falciparum in the presence of 10% bovine calf serum. In their study development of P. falciparum in the presence of horse, goat and rabbit sera as well as various concentrations of Albumax II. RPNI medium supplemented with 10% bovine calf serum (RPNI-BCS) was used as control. They found that the parasitaemia in the presence of Albumax was significantly higher in RPNI than in RPMI-1640 and addition of hypoxanthine in RPMI-1640 caused an increase in parasitaemia whereas no obvious advantage could be observed in RPNI. [13]

A. A. Divo et al. (1982) studied on addition of 50 ml of pooled human serum per litre of RPMI (5% serum) resulted in optimum growth. Batches of RPMI 1640 supplemented with

freshly collected and pooled lots of bovine, porcine, goat, equine, or ovine sera, as well as commercially available fetal-and young-calf sera, were tested and compared with 5% pooled human serum. In their study continuous parasite growth was obtained by transferring parasites directly from 5% human serum into medium plus freshly collected, Neopeptone-supplemented, pooled bovine serum, without any need for an adaptation period. [15]

CONCLUSION:

Plasmodium falciparum was growing well in the RPMI-1640 medium. Parasitaemia start increased after 24 hours of incubation and O positive human red blood cells were added for further sub cultivation. This study support the use of RPMI-1640 medium supplemented with L-glutamine, HEPES buffer, NaHCO3, hypoxanthine, 0.5% Albumax II and 50µg/ml Gentamicin for cultivation of Plasmodium falciparum in the presence of 5-10% CO_2. This culture method can be very useful for diagnosis and drug sensitivity testing of P. falciparum. P. vivax and other species however require different type of culture medium and require reticulocytes for their growth.

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