Chapter 6

Studies with RPNI medium-Growth profile of various laboratory maintained Plasmodium falciparum strains
6.1 Introduction

As evident from the previous chapters RPNI, a combination of three commercially available growth media (RPMI-1640, NCTC-135 and IMDM) supports long term continuous cultivation of *P. falciparum* not only in the presence of FBS but also in the presence of other animal sera as well as ALBUMAX II. Further studies need to be carried out to investigate its potential to cultivate different parasite lines. This chapter contains the results of such studies using three more laboratory maintained strains of *Plasmodium falciparum*.

6.2 Materials and Methods:

During the experiment four chloroquine (CHQ) sensitive *P. falciparum* strains 3D7, NF54, JDP-8 and RKL-9 were used to observe their growth profile in RPNI medium supplemented with 10% FBS, 10% horse serum and 0.5% ALBUMAX II.

Experiments were carried out in 24 well plates as mentioned in chapter 2. In brief, 4 culture suspensions containing 0.5% parasitaemia and 6% Hct were prepared in RPNI medium supplemented with 10% FBS using respective parasite strain. 2ml of each culture suspension was dispensed in duplicate wells and incubated at 37°C in CO₂ incubator. The spent culture medium was replaced once in every 24hrs. Blood smears prepared daily (as mentioned in chapter 1) were examined for parasitaemia and mean of three experiments i.e. of 6 readings was used for analysis.

6.3 Results:

Mean percent parasitaemia as observed using NF-54, JDP-8, 3D7 and RKL-9 strains is depicted in figure 2. It is evident that %parasitaemia increased continuously up to day 7 with minor variations and the respective mean percent parasitaemia was observed to be 14.96, 14.2, 15 and 13.2 for NF-54, 3D7, JDP-8 and RKL-9 strains.
Figure 1: Growth profile of *Plasmodium falciparum* strains NF-54, 3D7, JDP-8 and RKL-9 in RPNI medium supplemented with FBS.

Figure 2a: Growth profile of *Plasmodium falciparum* strain 3D7 in RPNI medium supplemented with FBS, HrSe and ALBUMAX II.
**Figure 2b:** Growth profile of *Plasmodium falciparum* strain NF-54 in RPNI medium supplemented with FBS, HrSe and ALBUMAX II.

**Figure 2c:** Growth profile of *Plasmodium falciparum* strain JDP-8 in RPNI medium supplemented with FBS, HrSe and ALBUMAX II.
**6.4 Discussion:**

The results of studies revealed that all four parasite strains grow well in RPNI-FBS and confirms the utility of modified medium for growth and development of different strains of *P. falciparum*. As has already been reported (Srivastava and Puri, 2004) the RPNI medium is a pool of glucose, amino acids, vitamins, Tween 80 (lipid source), ascorbic acid, glutathione, coenzymes (CoA, NAD, NADP, FAD, cocarboxylase, UTP) and nucleic acid derivatives which are either absent or are present in lower concentrations in RPMI-1640. These findings also revealed that it is the media constituents and not the sera supplements which are crucial for *in vitro* development of malaria parasite. It can thus be concluded that use of RPNI medium is advantageous as it also promotes the development of different strains *P. falciparum* in commercially available FBS and therefore overcomes the risk of exposure to a potentially bio-hazardous blood product.