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Proliferation of Human Lymphocytes in Medium Supplemented with Autologous Plasma – A Novel Technique

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KEY WORDS Leucocyte culture; mitotic index; autologous plasma and culture conditions.

ABSTRACT Several factors contribute to human lymphocyte culture for the procurement of sufficient number of clear well spread metaphase plates for any cytogenetic analysis including culture medium, growth supplements, colchicine treatment, hypotonic treatment, fixation and the technique of slide preparation. Considering all these factors, in the present study, the growth and multiplication of human lymphocytes was observed and studied under different culture conditions and some of the steps were modified so as to give an estimation of the best yield. McCoy's 5a culture medium was supplemented with autologous plasma, which substituted the fetal calf serum. Further, various concentrations and exposure times of colchicine were experimented and standardized so as to obtain good chromosome spreads of both metaphase stage (short chromosomes) and in late prophase stage (long chromosomes). This method can therefore be considered as a less expensive and more suitable procedure, for obtaining the plates for analysing numerical abnormalities employing the shorter chromosomes and high resolution banding studies employing the longer chromosomes for cytogenetic research.

INTRODUCTION

Human mitotic metaphase plates are routinely prepared by employing the modified method of Moorhead et al. (1960). This is further modified in the present study to yield good number of well spread metaphase plates/chromosome spreads for cytogenetic studies at a lesser cost. Mc Coy's 5a synthetic medium was used as its efficiency was tested earlier by Mutchinik et al. (1980). Advantages of using autologous plasma were suggested by Ling and Kay (1975), Auf Der Maur and Berlincourt-Bohni (1976), Bamezai and Singh (1982) and Maria et al. (1990).

MATERIAL AND METHODS

Peripheral blood (5cc) was obtained from equal number of male and female healthy donors in the age group of 20-28 years. These blood samples were coded and placed vertically at 20° for 2 hours, for sedimentation of the blood cells.

Four different culture conditions viz. "A", "B", "C" and "D" were employed for the whole experiment (Table 1). The autologous plasma was added to the vials by bending the syringe needle at an angle of 45° (Fig. 1). From 5ml whole blood, around 2.8 ml of clear plasma could be obtained. Thus 1-2 ml of the plasma was utilized for setting up the cultures in duplicates. The remaining plasma along with the buffy coat was mixed well and from this 0.5 ml of the blood was added to each culture vial. The cultures were incubated at 37° C for a period of 48 and 69 hours.

Experiments were carried out with specific concentrations and exposure timing of colchicine (Table 2) to obtain chromosomes of both metaphase and late prophase stages.

The cultures were then processed according to the standard procedure and slides prepared were coded and analyzed for the growth potential in different culture conditions. One thousand cells were screened at random to know the frequency of the dividing cells by counting the metaphase plates. The growth potential of leucocytes was assessed using mitotic index (MI) as a parameter. The growth of the lymphocytes in culture conditions 'A' and 'C' was compared to that of 'B' and 'D'. The mitotic index of culture conditions with MAP was compared to that with MFCS for both 10% supplement ('10 S') and 20% supplement ('20 S'). All the cultures were terminated at two time intervals viz. 48 hrs and 69 hrs. The mitotic index of MAP and MFCS at both the time intervals was also compared.

RESULTS

Experiments with various concentrations and exposure timings of colchicine gave best results at a final concentration of 10 μg / ml and an exposure time of 15 minutes. This yielded chromosome spreads of both the metaphase stage (short chromosomes) and mid prophase stage (long chromosomes).

The mean MI of the lymphocytes in all the four culture conditions at 48 and 69-hour culture time are shown in Table 3. At culture time of 48 hours the culture condition 'D' showed the

Table 1: Components in *A, *B, *C and *D culture conditions

S. No.	Culture conditions	Components
1.	*A (10%MFCS)	5ml* MPB + 0.5 ml *FCS
2.	*B (10% MAP)	5ml* MPB + 0.5 ml autologous plasma
3.	*C (20% MFCS)	5ml* MPB + 1.0 ml *FCS
4.	*D (20%MAP)	5ml* MPB + 1.0 ml autologous plasma

*MFCS- Medium supplemented with fetal calf serum.
*MAP- Medium supplemented with autologous plasma.
*MPB- 5 ml Medium + 0.2 ml Phytohaemagglutinin + 0.5 ml blood.
*FCS- Fetal calf serum.

Table 2: The various concentration and exposure timings of colchicine treatment

S. No.	Colchicine concentration	Exposure time (in minutes)			
1.	05 µg/m	10	15	20	25
2.	10 µg/m	10	15	20	25
3.	15 µg/m	10	15	20	25
4.	30 µg/m	10	15	20	25

Table 3: Mitotic Indices (MI) obtained after 48 and 69 hours of culture in A, B, C and D culture conditions

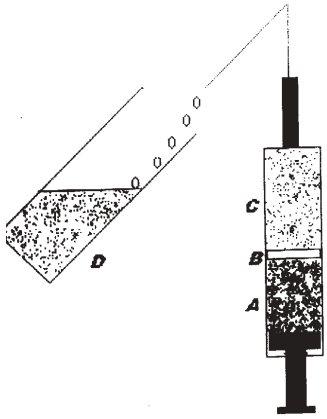
Culture conditions	Mean Mitotic Index (in %)	
	48 Hours	69 Hours
A	12.32 ± 1.001	37.90 ± 0.938
B	17.50 ± 1.366	36.54 ± 4.607
C	14.00 ± 1.272	44.64 ± 2.821
D	17.96 ± 2.469	49.48 ± 2.621

highest %MI of 17.96 ± 2.469 as compared to the other culture conditions. The MI obtained in 'B' was also comparatively higher than that in 'A'. As compared to that of '20 S', the %MI of '10 S' was higher. It was also observed that at both '10 S' and '20 S' of MAP (B and D) the %MI was higher as compared to that of culture condition with MFCS in 'A' and 'C' (Fig. 2).

At culture time of 69 hours, the culture conditions in 'D' showed the highest %MI of 49.48 ± 2.261. The %MI obtained with '20 S' was considerably higher as compared to that with '10 S'. The %MI of culture condition 'A' and 'C' was almost the same. But with 20% supplement the %MI in culture condition 'D' was comparatively higher than that in 'B'.

DISCUSSION

The results of the present study clearly shows



A - Sedimented blood cells
B - Buffy cost of WBCs
C - Autologous plasma
D - Culture medium

Fig. 1. Showing the addition of the autologous plasma to the cultures

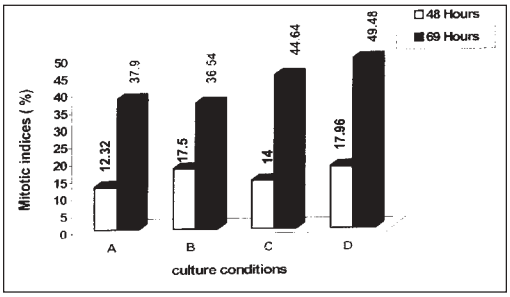


Fig. 2. Mean Mitotic indices (in %) in the four culture conditions A, B, C and D at 48 and 69 hour culture time

the importance in deciding the appropriate exposure period for colchicine and so also the advantages of the use of the autologous plasma over the heterologous serum.

The colchicine concentration of 10µg/ml of medium and exposure time of 15 minutes can be considered as suitable for analyzing the plates for numerical abnormalities on the shorter chromosomes (metaphase stage) and high resolution banding on the longer chromosomes (late prophase stage).

The culture condition 'D' in the present study yielded better results indicating that the autologous plasma is more efficient in providing all the necessary factors and nutrients for adequate proliferation of human lymphocytes in-vitro. Autologous plasma has an added advantage over the heterologous serum as it does not cause any incompatibility related undesirable side effects.

These results agree with the studies of Ling and Kay (1975), Auf Der Maur and Berlincourt-Bohni (1976), Osvaldo et al. (1980) and Bamezai and Singh (1982). Secondly, since the plasma is collected by sedimentation of the blood cells, it eliminates the chances of contamination during centrifugation.

At culture time of 48 hours the %MI in culture conditions 'B' and 'D' was higher as compared to that of 'A' and 'C'. But at 69 hours culture time, the %MI of the culture condition 'C' was considerably lower than that in 'D'. This may be on account of the undesirable side effects of the heterologous sera, which becomes pronounced when the concentration is increased to 20% and exposed for a longer duration of 69 hours.

Thus this method can be considered as a suitable and less expensive procedure for cytogenetic research in diagnostic laboratories, for studying the plates for numerical abnormalities on the shorter chromosomes and high resolution banding on the longer chromosomes.

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