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Various technique of cell culture and slide preparation for human chromosome

Faraj Saad Faraj Mohamed[1]

[1]Department of Laboratory Medicine, Faculty of Health science, Sirte University, Sirte Libya

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Corresponding Author

faraj.saad@su.edu.ly

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A B S T R A C T

The cell culture and slide preparation method of human chromosom is the mechanism that is a corresponding change in DNA content. In this research we used a various techniques of cell culture and slide preparation for human chromosome and examined for 2 days, 3 days and 4 days cell culture because these are average days for approximately for the good results and the treatment will still good also the result has minimum bias.

Research objectives were to determine the appropiate length of cell culture needed for human chromosome with different length and to determine the appropiate method for staining the slide for human chromosome with different length.

Based on the data analysis, the result in this reserach shows that the appropiate length of cell culture needed for human chromosome with different length is 2 days incubation. Although there was no significant differences between 2,3 and 4 days length based on statistical analysis, but still there is little differences between them and the best is 2 days rather than 3 and 4 days based on the graph analysis. The appropiate method for staining the slide for human chromosome with different length is hot plate rather than another technique

1.0 Inroduction

Chromosomes are delicate objects such as rod-shaped straight or bent inside the nucleus because it can absorb the color. Clearly, it can be observed under a microscope. Constituent substance of chromosomes is called chromatin and a tiny threads interwoven in the plasma core. According to Flemming in Maiato (2012), for the first time slicing objects that exist in the core. Flemming use the term to describe the mitotic division strands (chromosomes) in the core into half core and separation to the sub cells. Cell division consists of two sequential process, mainly characterized by DNA replication and chromosome segregation into two cells that replicated separately. During metaphase of the cell cycle, the condensed chromosomes align along the metaphase plate prior to chromatid separation and then move along the spindle fibres to the poles of the spindle. Diagnostic cytogenetic investigations are used routinely within the clinical setting to screen for chromosomal imbalance in a wide range of patients. These methods describe a synchronised suspension culture of cell and an unsynchronised adhesion culture of tissue. According to a Tsafrir et al (2006), cell culture and slide preparation method of human chromosom is the mechanism that is a corresponding change in DNA content. This implies that whereas specific chromosomal abnormalities may arise stochastically, the associated changes in expression of some or all of the affected genes are responsible for selecting cells bearing these abnormalities for clonal expansion. Bone marrow is the most suitable material for cytogenetic diagnostics in hematological malignancies. The most important principle is to aspirate the bone marrow in sterile way to heparin filled probes. Then the procedure of in vitro culture and preparing microscopic slides with metaphase chromosomes and or interphase nuclei is started.

2.0 Materials and Methods

This diagnostic and cross-sectional research analyze the various techniques of cell culture and slide preparation for human chromosome and will examine for 2 days, 3 days and 4 days cell culture in Center for Biomedical Research (CEBIOR), Medical Faculty of Diponegoro University, Semarang, Indonesia. Because these are average days for approximately for the good results and the treatment will still good also the result has minimum bias

(Hamzah, 2011); The best time is 3 days, the reasons to use 4 days because any cycle length of less than 4 days or more than 5 days (e.g. 3 or 7 days) is unusual and should therefore be considered as abnormal in the adult (but not aged) (Goldman, 2007). It is also important to have cells in a logarithmic phase of growth. Splitting of a confluent cell line 2 days before harvesting, and changing the medium 1 day before harvesting, stimulates cell proliferation significant.Metaphase is a stage of mitosis in the eukaryotic cell cycle in which chromosomes are at their second-most condensed and coiled stage. These chromosomes, carrying genetic information, align in the equator of the cell before being separated into each of the two daughter cells. Metaphase accounts for approximately 4% of the cell cycle's duration preceded by events in and followed by anaphase. Microtubules formed in prophase have already found and attached themselves tokinetochores in metaphase. The culture and the staining management in spreading will be analyzed with flame or fire, hot plate and air because according to Xiang and Yang (2014), heating of the sections at a high temperature could prevent the section loss.

Operational Definition

1. Cell culture: the process by which cells are grown under controlled conditions, generally outside of their natural environment.

2. Slide Preparation: slides, cover slips, droppers or pipets and any chemicals or stains that plan to use in microscopic method.

3. Chromosomal analysis or quality of metaphase is the analysis for quality chromosome of methaphase, such as band level, crossing over, broken chromosome.

3.0 Results

From the data, we can see that majority of data samples from band level of all samples in 2 days, 3 days and 4 days the highest is 10 chromosome and the lowest is chromosome 20, the medium one is chromosome 18. This indicated that chromosome 10 is the most chromosome that mostly appear rather than another chromosome.

Table 4.18. The significancy differences between two, three and four days in culture"

ANOVA

Chromosome

	Sum of Squares	df (degree of freedom)	Mean Square	F	Sig.
Between Groups	1.985	2	.993	.674	.518
Within Groups	39.778	27	1.473		
Total	41.763	29			

From the result, the significant or p-value is 0.518 > 0.05 so there are no significant differences between two, three and four days in slide preparation.

Table 4.19. The Significancy differences between the slide preparation method using flame or fire, hot plate and air"

ANOVA

Chromosome

	Sum of Squares	df (degree of freedom)	Mean Square	F	Sig.
Between Groups	8.822	2	4.411	1.125	.329
Within Groups	341.133	87	3.921		
Total	349.956	89			

From the result, the significant or p-value is 0.329 > 0.05 so there are no significant differences between the staining management with flame or fire, hot plate and air drying

4.0 Discussion

Based on result of this research it can be said that the best technique is hot plate rather than another technique, and the best is 2 days incubation. The majority of data samples from band level of all samples in 2 days, 3 days and 4 days the highest is 10 chromosome and the lowest is chromosome 20, the medium one is chromosome 18. This indicated that chromosome 10 is the most chromosome that mostly appear rather than another chromosome.

In a laboratory, when starting any new diagnostic service, had a protocol for training staff and tested equipment so patients are not at risk from inappropriate handling of equipment or slides, etc. One way of doing this is to divide the samples, and send half to an experienced laboratory until the necessary level of competence was achieved. Validation and SOPs of these procedures was required. When molecular genetic techniques were more sensitive than conventional cytogenetics, they should be used once the method has been validated. This could result in onward referral of cases if a laboratory was unable to undertake such analysis. Exclusion of other chromosome abnormalities may still be required in most cases (Barch, M.J, 2015).

Based on result of this research it can be said that the best technique is hot plate rather than another technique, and the best is 2 days incubation. From hypothesis testing it can be say that there are not a lot significant differences between two, three and four days in slide preparation, so hypothesis rejected. Also there are not a lot significant differences between the staining management with flame or fire, hot plate and air drying., but still have differences between three methods. Majority of data samples in 10 patients categorized as moderate (400bphs) that were 50% or 5 patients and good criteria (550 bphs) for 40% or 4 patients, only 1 patients (10%) has Poor category (150 bphs). This indicate that the score 6 majority for respondents and indicated that 4 disctint dark bands and 22 not visible. Moderate indicated that there were 2 disctint dark bands, and poor indicated no fine detail but unequivocal pairing possible

5.0 Conclusion

Based on the analysis data, the conclusion in this research, There is no significant differences between 2,3 and 4 days length of cell culture. The appropiate length of cell culture needed for human chromosome with different length is 2

days incubation. Although there was no significant differences between 2,3 and 4 days length based on statistical analysis, but still there is little differences between them and the best is 2 days rather than 3 and 4 days based on the graph analysis. There is no significant differenceces between staining methods for human chromosome. The appropiate method for staining the slide for human chromosome with different length is hot plate rather than another technique

Recommendations

In the future researchs more samples and other various technique with cytogenetics counseling needed for patient befor and after cytogenetic testing as an informed consent and information finding the of chromosome analysis ,risk of hiring the same condition in the future genetics on other siblings.

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