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Use of Umbilical Cord Serum in Chromosomal Studies

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KEYWORDS Lymphocyte culture; umbilical cord blood serum; AB+ blood serum; comparison; mitotic index; banded preparation; karyotyping

ABSTRACT The precise role of serum in the culture medium is still not fully understood. But, it is evident that without it or an appropriate substitute cells do not thrive in culture. It is clear that certain types of sera perform better than others and that certain lot is more conducive to growth than others. In chromosomal studies, apart from animal sera, human sera were used for a long time. The commercial sera available are costly (FBS, FCS, NBCS). Animal sera (goat, sheep) may not give consistent results. Human blood is preferred for life saving demands. Of the various groups from the human sera, AB+ve gives a good mitotic index. The aim was to find out an alternate, of human origin, cost effective, easily available serum that could be used in cytogenetic laboratories which is better / equivalent to human AB +ve serum. The umbilical cord blood is discarded in almost all the maternity homes. There is a lot of potential to explore the possibilities to use umbilical cord serum in genetic laboratories with the permission from unregistered expected mothers and the authorities. A good quality chromosome preparation is necessary to detect the chromosomal rearrangements for diagnosis purpose. In the present study, adequate number of metaphases and well spread / well - banded chromosomes were obtained with umbilical cord serum. Any literature pertaining to the use of umbilical cord serum for chromosomal studies was not available. Probably, this study may be the first study to find out the use of umbilical cord serum for chromosomal studies as well as to compare with other sera.

INTRODUCTION

Successful chromosome analysis depends not only on the skill of the person performing the work, but also on the media and serum used in the process (Victor et al. 2002). The precise role of serum in the culture medium is still not fully understood. But, it is evident that without it or an appropriate substitute, cells do not thrive in culture. It is clear that certain types and lots are better and more conducive to growth than others. It is a complex mixture that contains 6 to 7.8 g / dl of protein, food substances, metabolites, gases, hormones, plasma proteins and various growth factors. Serum provides a broad spectrum of macromolecules, carrier proteins for lipoid substances, trace elements, low molecular weight nutrients, hormones and growth factors necessary for the growth of cells *in-vitro* (Morgan et al. 1950).

Culture media have been used for the in – vitro growth and maintenance of mammalian cells for more than 100 years. When first developed these media consisted mainly of plasma derived components (serum and clots) and biological fluids (embryonic extracts). Attempts to replace these undefined components led to the development of synthetic media in the 1950s . Synthetic media or basal media consists of mixtures of 60 to 80 components including amino acids, carbohydrates, vitamins and inorganic salts. Most of these basal media (media 199, RPMI 1640, BME) require serum and other supplements such as hormones and growth factors to enhance cell growth.

Serum is a fundamental component and is one of the indispensable items in a cytogenetic laboratory. In this article the application and

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outcome of the umbilical cord serum in the use for chromosomal studies, has been presented.

Aims and Objectives

Once cord blood was considered as discarded human material, but in recent years umbilical cord blood containing a large number of haema-topoietic stem cells have been used successfully for allogenic transplantation to treat a variety of paediatric, genetic, haematologic and oncologic disorders. The umbilical cord blood is rich in nutrients when compared to adult blood. And from experience it has been observed that human AB + ve serum gives better results.

This study has been undertaken to compare the performance of umbilical cord serum and AB + ve serum along with the routinely used newborn calf serum, for chromosomal studies.

MATERIAL AND METHODS

Collection of Umbilical Cord Blood: After obtaining prior permission from the director of the Nursing Home and consent from the mother, informing them that the cord blood will be used only for research purposes, the collection begins after the birth of the baby and the umbilical cord is clamped.The collection site on the cord is surface sterilized with alcohol followed by swabbing in betadine solution. Umbilical cord vein puncture is done with sterile needle.

Blood starts flowing into the sterile container held at a lower level than the placenta so that by gravitational force the blood will flow. As the blood stops flowing, the site is changed by pricking the needle a little above the first prick towards the placenta. Normally the collection will be over within three minutes. After collection, the sterile container having the cord blood is sent to the laboratory for further processing.

Preparation of Serum from Cord Blood: The sterile container is kept slanting at 45°C in the refrigerator for few hours for the cells to settle down. The clear serum above is poured off and centrifuged at 3000 rpm for 30 minutes and filtered using positive pressure pump with 0.45-micron (pore size) cellulose filter (millipore). After filtration the serum is screened for HIV I andII, HBS Ag, HCV and VDRL. It is then inactivated at 56°C for 30 minutes in a water bath, aliquoted in small quantities and frozen.

Sterility test was put up in fluid thioglycolate medium for 7 days and then released for use. The same procedure is followed for human AB + ve serum after collection from the donor.

Lymphocyte cultures were setup by the modified method of Arakaki and Sparkes (1963) using GIBCO Medium and PHA (supplied by Shreyas Solutions, Bangalore). The other chemicals and reagents used were constant. A concentration of 20% serum was used. Two simultaneous cultures were setup for each patients referred for karyotyping to S-DACC using new born calf serum as control and two of the sera (umbilical cord serum and Human AB + ve serum).

RESULTS

Cultures have worked well and culture failure was not observed. The slides were carefully analysed for mitotic index, spreading and banding.It was observed that the morphology of the chromosomes using umbilical cord serum and AB + ve serum were healthy and well banded compared to the newborn calf serum. Between the umbilical cord serum and AB+ve serum, the

Table 1: Mitotic Index (MI) values in %.

	V. Low	*Low*	*Avg*	*Good*	*V Good*	*Grade*
CS (%)	4	20	44	32	-	++ (76%)
AB+ve (%)	-	24	52	24	-	++ (76%)
NBCS (%)	4	68	20	8	-	+ (28%)

Table 2: Quality of metaphase spreading in %

	N Good	*Avg*	*Good*	*V Good*	*Grade*
CS (%)	84	-	48	44	++ (92%)
AB+ve (%)	-	-	76	24	+++ (98%)
NBCS (%)	8	-	84	8	++ (92)

Table 3: G-Banding Quality

	N Good	*Avg*	*Good*	*V Good*	*Grade*
CS (%)	4	64	32	-	+++ (96%)
AB+ve (%)	4	76	24	8	+++ (98%)
NBCS (%)	12	64	20	4	++ (98%)

Abbreviations: CS: Cord Serum, AB+ve: Human AB+ve serum, NBCS: New Born Calf Serum

preparations from AB + ve serum seemed to be better (Tables 1, 2, 3).

DISCUSSION

Collecting, handling, processing and storing should be conducted in a manner so as to protect and maintain the quality of the serum. Serum should not be repeatedly frozen and thawed. Appropriate aliquots should be prepared using sterile techniques and sterile pyrogen free containers. Serum should be stored at - 20°C. A frost–free freezer should not be used to store the serum, since temperature cycling may cause the bottles to crack and may contribute to deterioration. Serum must be added to the medium at concentrations varying from 10 – 30 % depending upon the type of culture media. Compromise of 20 % is probably most beneficial, because excess of serum could be detrimental to the cells and a shortage of serum will not allow maximum growth to be attained. It is the most variable component of the medium and because it is a biological fluid, it may be infected with microorganisms. For these reason commercial suppliers apply stringent quality control measures as well as sterility checks (Barch et al. 1991).

Bottles of sera are usually heat inactivated by incubation at 56° C in a water bath for 30 minutes. Individual bottles of serum can experience different temperature conditions, based on mixing of the contents of each bottle, the number of bottles in a water bath and the temperature uniformity of the bath.

The heat inactivation process is straight forward and subject to several factors, which can lead to variation in product performance.

Some of the potential problems that can arise are:

* Variations in exposure time for heating sera, which can result in either failure to inactivate the desired components or de-naturation of additional proteins.
* Inadequate mixing of sera during heating, which can lead to localized hot spots giving rise to inconsistencies. These problems can seriously compromise the ability of serum to support cell growth (Johnson et al. 2000)
* Heat inactivation of serum is a controversial subject. Heat inactivated serum is commonly used in the preparation of culture media for

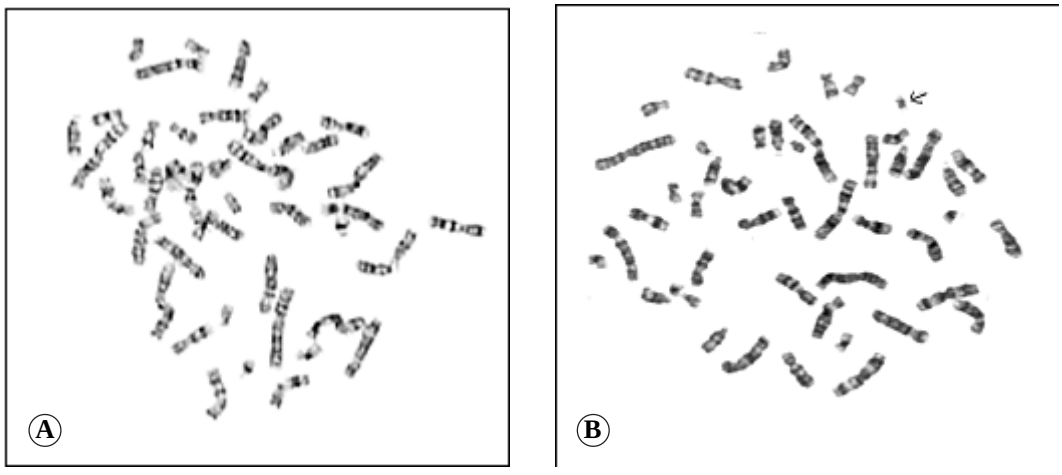

Fig. 1. A: Umbilical cord blood serum B: AB+ blood serum

cytogenetic application. This procedure inactivates heat labile components such as the complement cascade (part of the coagulation system) and other proteins suggested to be cytotoxic. The need for serum inactivation in cytogenetic applications has not been clearly defined (Bogaerde et al. 1998; Leshem et al. 1999).

Many users prefer using heat-inactivated sera since its use has been a historical practice. While the need for heat inactivation of serum has not been demonstrated to be necessary for cytogenetic application, it has also not been shown to be detrimental.

Umbilical cord blood is rich in nutrients. An attempt has been made to find out whether umbilical cord serum can give better results than human AB + ve serum. Of the various groups from the human serum, AB + ve serum gives a good mitotic index. Hence, human AB +ve serum was chosen to compare with umbilical cord serum. The animal sera have been eliminated based on the findings from the earlier study (Amudha et al. 2005). The available groups of A+ ve and B+ve umbilical cord sera were used and it was observed that there was not much difference between the two sera, with regard to the morphology, mitotic index and banding pattern. A comprehensive study of all the groups of umbilical cord serum and the best choice may be taken up later.

The notion was that the umbilical cord serum should give better performance than the human AB + ve serum. But in the present study, it has been observed that human AB + ve serum is better than umbilical cord serum, though cord serum preparations gave healthy and well-banded chromosomes. The availability of human AB + ve serum is hampered by its preferential use for life saving demands. However, small quantities from voluntary donors could be used.

It is possible to explore the utility of umbilical cord serum for chromosomal preparations. The umbilical cord blood is discarded in almost all the maternity homes. With recent awareness for stem cells, many expecting mothers have come forward to register themselves for cryo-preservation of stem cells for future use. There is a lot of potential to explore the possibilities to use umbilical cord serum in genetic laboratories with the permission from unregistered expectant mothers and the authorities. A good chromosome preparation is necessary for the diagnosis of cryptic rearrangements in the chromosomes. In the present study adequate metaphases and well spread out / well-banded chromosomes were obtained with umbilical cord serum.

There is paucity of information in literature on the application of umbilical cord serum for chromosomal studies. Probably this study may be the first to find out the use of umbilical cord serum for chromosomal studies.

CONCLUSION

- Commercially available sera are costly (FBS, FCS, NBCS).
- Umbilical cord serum will be cost effective and easily available.
- Morphology and banding pattern observed in umbilical cord serum seemed to be good.

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