

Use of Porcine Serum in Lymphocyte Culture

R. Victor¹, Pallavi A. Rao¹, Pushpa Vasudev¹, D. Sebastian,¹
Veena A. Rao¹ and Sayee Rajangam²

1. Genetic Laboratory S-Dac Lal Bagh Nursing Home, Jayanagar, Bangalore 560 034, Karnataka, India
2. Division of Human Genetics, Department of Anatomy, St. John's Medical College Bangalore 560 034, Karnataka, India

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ABSTRACT In lymphocyte culture work, apart from human serum, animal serum was in use for a long time. Foetal calf / bovine / goat serum were the common ones used. Porcine serum has not been commonly used though it is said that the constituents of the porcine and human blood are more of the same. Porcine serum in lymphocyte culture for chromosomal studies has been tried and found to be good. It worked better than the goat serum. GTG bands were well seen in the metaphase spreads. Hence, it is suggested that porcine serum could also be used in setting up peripheral lymphocyte culture for karyotyping.

INTRODUCTION

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 are better than others and that certain lots are more conducive to growth than others. Serum acts as a nutrient supplement for the growing cells in culture. There are a number of basic media in common use in cytogenetics laboratory most of them were originally developed for specific purposes and now found wider applications.

Apart from human serum, animal sera were in use for a long time. Goat serum, foetal bovine / calf serum were the common ones used. The most effective one but also the most expensive one is foetal bovine serum. The constituents of porcine blood and human blood are more or less of the same. Hence, in this article the application and outcome of the porcine serum in the lymphocyte culture work has been presented.

Address for Correspondence: Dr. Mrs. Sayee Rajangam, Professor and Head, Division of Human Genetics, Department of Anatomy, St. John's Medical College, Bangalore 560034, Karnataka, India.
Email: sjmcanat@satyam.net.in

MATERIALS AND METHOD

Lymphocyte cultures were set-up by the modified method of Arakaki and Sparkes (1963) using GIBCO medium and PHA (supplied by Shreyas solutions, Bangalore). A concentration of 20% porcine serum was used. Cultures were also set up with fetal bovine serum (Life Technology GIBCO BRL India Limited)

PREPARATION OF PORCINE SERUM

Clotted porcine blood was collected in sterile containers from piggery. The containers were kept slanting at 45 degrees in the refrigerator for a 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). It is then inactivated at 56 degree centigrade for 30 min in a water bath, aliquoted in 20 ml quantities and frozen. Sterility test was put up in fluid thioglycolate medium for 7 days and then released for use.

RESULTS

It has been observed that the chromosome index was good and consistent. From the metaphase spreads it has been observed that the GTG bands were good enough in identifying the chromosomes for the preparation of karyotype. The bands obtained were more or less similar to that of the preparation done with fetal bovine serum.

DISCUSSION

Collecting, handling, processing and storing should be conducted in a manner 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 degree centigrade.

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 to 30 % depending upon the type of culture. 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. At genetic laboratory, Lal Bagh Nursing Home serum is inactivated by heat at 56 degree centigrade for 30 min. This destroys the complement protein and ensures that immunological reaction will not occur against cultured cells. Also at division of human genetics, SJMC, blood is collected from the slaughterhouse, allowed to stand overnight at 4°C, and then the clear serum is poured into centrifuge tubes and centrifuged at 3000 rpm for 30 minutes. The serum is then filtered using pressure pump with 0.45-micron (Pore size)

cellulose filter (Millipore). It is then inactivated at 56 degree centigrade for 30 min in a water bath, aliquot in 20 ml quantities and frozen. Sterility test was put up in fluid thioglycolate medium for 7 days and then released for use.

The sera prepared in the laboratory may not be filtered if it has been collected under aseptic conditions, and the yield is good. It has been observed that porcine serum is better than goat serum. Moreover goat serum doesn't give consistent results. Literature was not available for appropriate discussion (Barch et al. 1991)

CONCLUSION

Porcine serum has not been commonly used in lymphocyte culture work. The use of porcine serum in chromosome studies has been found to be satisfactory and consistent and could be used in lymphocyte culture work.

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