PREPARATION OF LYOPHILISED LIPID RICH QUALITY CONTROL SERUM BY ISOLATING LIPIDS USING LARGE SCALE CHROMATOGRAPHY TECHNOLOGY

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ABSTRACT

The plasma fractionation is a technology to separate therapeutic plasma proteins. The fractionation is carried out either by solvent precipitation (ethanol) or recently by chromatography. During the process of chromatography lipids were found in waste fraction. A new lipid rich lyophilised quality control (Q.C.) sera was prepared inexpensively using this waste plasma fraction. This is probably the first attempt to prepare the Q.C. sera by large scale chromatographic method in India.

KEY WORDS: Quality control, Lipid quality control sera, Cholesterol, Freeze drying, Chromatography.

INTRODUCTION

Modern medicine increasingly relies on the analytical laboratory results for the diagnosis as well as the routine assessment of a patient. Reproducible results can be given by having good standardisation of tests along with regular quality control measures. Control sera are used to assess the reliability of an analytical process, mainly precision and accuracy. In house preparation of control material from human serum was described by Bowers et al (1) and W.H.O. Publication (2). It is essential that control sera should be homogeneous and stable throughout the process. An analytical method involves three types of material whose composition may differ i.e. the calibration standard, the control specimen and the patient's specimen. They differ in chemical or physical characteristics which can affect the validity of results due to matrix effects. Additions may be used to increase stability or to remove or simulate interferences while preparing Q.C. sera. Control serum is prepared from human or animal source. The preparation of control sera for lipid analysis is made by external addition of lipids and poses special problems: a) The sera becomes turbid which interferes with spectrophotometric measurement, b) Unstable in liquid state, c) If lyophilized lipoproteins become insoluble. Various investigators developed the method of preparation of sera for lipid quality control but by using different methods (3,4).

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MATERIAL AND METHODS

National Plasma Fractionation Centre (N.P.F.C.), Mumbai, is involved in the manufacture of therapeutic plasma proteins. The plasma is fractionated by column chromatography for Albumin and Immunoglobulin (5).

Plasma used was tested and found negative for HBsAg, HIV I & II and V.D.R.L. was only processed. The 50 litre of plasma was pooled together in a clean S.S. tank. The lipid rich source was detected in one of the waste fraction (6). Serum was prepared from plasma as described by Buttner et al. (7), and the thrombin required for the removal of fibrin was made in N.P.F.C.

Total lipids*, Cholesterol (Ch), Triglycerides (TG), HDL-Cholesterol (HDL-Ch), were estimated by colorimetric* and enzymatic methods using commercial kits. (E. Merck, Germany* & Ethnor, India). Optical clarity was ascertained at 710nm (8). Moisture content was estimated by using Karl Fischer method (9). Sterility test was also carried out (10).

RESULTS AND DISCUSSION

Preparation of lipid rich quality control sera using chromatography fraction: During the large scale fractionation, after desalting of plasma on sephadex G 25C column, sizable amount of lipids are accompanied along with the immunoglobulin fraction and were separated on DEAE-Sephadex FF column. The procedure is shown in flow diagram (Fig. 1). This lipid rich fraction was

collected at pH: 9.0 on DEAE-Arginine column. The elution of the lipid rich fraction during chromatographic separation is shown in the chromatogram (Fig. 2). This fraction was collected and subjected to alcohol precipitation method with few modifications. The molarity of acetic acid used in our method is 1M instead of 0.1M originally used by Kuchmak et al (11). The yield of total lipids was above 95% (Table 1). We have added sucrose 600 mmol/l as a lyoprotectant during lyophilisation (12). This gave a clear solution on reconstitution. The solubility of lyophilised Q.C. sera was within the prescribed time of 10 mins. No microbial growth was observed and moisture content of the lyophilised sera was within limits (less than 2.0%) indicating process of lyophilisation was satisfactory. The percentage recovery of cholesterol was 71% and triglycerides was 65%. However, recovery of HDL-cholesterol was only 16% (Table 2) and this may be due to different steps in chromatographic process and alcohol precipitation. Different types of commercial quality control sera are available for lipids.

Commercial Q.C. sera was made by only few Indian companies with only cholesterol as a lipid parameter which are discontinued now. Interlaboratory quality control programme is run by Association of Clinical Biochemists of India and Christian Medical College & Hospital, Dept. of Biochemistry, Vellore. They give liquid based noncommercial Q.C. sera.

Beside other common biochemical parameters, cholesterol is the only lipid parameter.

One major problem with routine and commercial lyophilized sera is turbidity and this can lead to inaccuracy where blanks can not be used. This can clog autoanalysers. Only few foreign commercial companies have separate lipid quality control sera. Indigenous lipid quality control sera are not available. Considering the importance, the study of preparation of the stable, economical and indigenous lipid quality control sera was undertaken. This lipid Q.C. sera was tried in various laboratories in the city of Mumbai and some parts of Maharashtra state and results will be reported elsewhere. The method of isolation/concentration of lipids from chromatographic fractionation developed in this study of separation of lipids by chromatography yields the lipids required for the reference material in a sizable amount. This lipid rich fraction may find clinical use as a lipid supplement in parenteral nutrition in future.

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Table 1. Elevation of total lipids by chromatography fraction.

Sr.No.	Serum (g/l)	Serum+ppt.A* (g/l)
1	4.8	10.3
2	4.9	9.8
3	4.8	9.9
4	4.9	10.5
5	5.1	10.6
6	5.2	9.7
7	4.7	9.5
8	5.0	10.6
9	4.9	10.5
10	4.8	9.6
Mean±SD	4.91±0.15	10.1±0.44
% rise		105.7%
		p < .0.001

*ppt.A: Alcohol precipitate from Chromatography Fraction.

Fig. 1 Separation of Lipids by Chromatography

Column : KS 370/15
 Gel : 60% DEAE Seph. FF + 40%
 Arginine Seph.
 Bed height: 15 cm
 Bed volume: 16 lits
 Eluent : NaAc 6.5/0.02, 3.0/0.5
 Sample : Crude IgG from DEAE Seph.,
 pH 6.5 & MS 1.4 adjusted
 Flow rate : (1 lits/min)
 Detector : uv 1, sen. 0.5
 Chart speed: 20 mm/hr.

Fig. 2. Chromatogram : Anion Exchange of IgG & Lipid Rich Fraction.

Table 2. Recovery of Ch, HDL-Ch and TG in serum prepared using alcohol precipitate of chromatography fraction.

Method	Ch (%) [*] n=10	HDL-Ch (%) [*] n=10	TG (%) [*] n=10
Alcohol ppt of Chromatography fraction	71±4.7	16±3.5	65±2.9

^{*}Average percent recovery in serum.

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