The protocol to remove high abundant proteins from HIV-1 infected human plasma

Sushanta Kumar Barik

National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Taj-Ganj, Agra, Uttar-Pradesh, India <https://orcid.org/0000-0002-2911-6117>

Dr Keshar Kunja Mohanty (✉ keshar63@yahoo.com)

National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Taj-Ganj, Agra, Uttar-Pradesh, India <https://orcid.org/0000-0001-7527-4864>

Deepa Bisht

National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Taj-Ganj, Agra, Uttar-Pradesh, India

Partha Sarathi Mohanty

National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Taj-Ganj, Agra, Uttar-Pradesh, India

Shripad Patil

National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Taj-Ganj, Agra, Uttar-Pradesh, India

Rananjaya Singh

National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Taj-Ganj, Agra, Uttar-Pradesh, India

Beenu Joshi

National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Taj-Ganj, Agra, Uttar-Pradesh, India

Srikanth Prasad Tripathy

National Institute for Research in Tuberculosis, Chetpet, Chennai, Tamilnadu, India

Tej Pal Singh

Sarojini Naidu Medical College, Agra, Uttar-Pradesh, India

Rekha Tandon

Sarojini Naidu Medical College, Agra, Uttar-Pradesh, India

Sheetal Tomar

Sarojini Naidu Medical College, Agra, Uttar-Pradesh, India

Srikanta Jena

Ravenshaw University, Cuttack, Odisha, India

Method Article

Keywords: HIV-1, Human plasma, TCA, Acetone, SDSPAGE

Posted Date: July 30th, 2019

DOI: <https://doi.org/10.21203/rs.2.11776/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Removal of high abundance protein from human plasma is an advantage during the analysis of protein in SDS-Poly acrylamide gel electrophoresis. We have adopted a protocol for removal of high abundance protein from human plasma by following several previous protocols. The 50% TCA-Acetone is most suitable method for removal of high abundance proteins like albumin and globulin from HIV-1 infected human plasma samples. We have developed this protocol in our laboratory for carrying out the protein work from HIV-1 infected human plasma samples. This protocol is suitable to run the purified human plasma protein for sodium dodecyl sulfate polyacrylamide gel electrophoresis.

Introduction

The human plasma is a rich source of proteins that leads to biomarker discovery for detection and diagnosis of a disease. The 289 proteins were identified in human plasma by two dimensional and mass spectrometry.¹ The proteins must be up regulated or down regulated in human plasma during the seroconversion period in HIV infected patients or antiretroviral therapy users. These identification of differentially regulated proteins chosen as a suitable marker for detection of opportunistic infection in HIV infected patients has been already published.² Most proteins were involved in HIV infection would be considered as new therapeutic targets against AIDS³. Several plasma protein purification by chemical methods like ammonium sulfate, isopropanol, chloroform:methanol, TCA-Acetone, chromatographic methods and several manufacturers kit were used for depletion of high abundance proteins from human plasma samples. To achieve reliable cost, the TCA-Acetone is a better option to use for depletion of high abundance proteins from human plasma samples.

Reagents

1. TCA (Trichloro acetic acid, Sigma-Aldrich, USA)
2. Acetone(Merck, India)
3. Urea (Sigma-Aldrich, USA)
4. Thio Urea(Sigma-Aldrich, USA)
5. CHAPS(Sigma –Aldrich, USA)
6. ASB-14(Merck ,India)
7. Dithithretol(Sigma-Aldrich, USA)
8. Bromophenol blue (Merck, India, Cat.no:67-64-1).

9. H₂O (HPLC purified ,Merck, India)
10. Acrylamide(Sigma –Aldrich, USA)
11. Bis-acrylamide(Sigma-Aldrich, USA)
12. Tris base(SRL,India)
13. Hydrochloric acid(Merck, India, Cat. No:1.09063.1000)
14. Sodium dodecyl sulfate(Himedia, India)
15. Ammonium per sulfate(Sigma-Aldrich, USA)
16. TEMED(Sigma-Aldrich, USA)
17. Glycerol(Merck,India, Cat No.56-81-5)
18. β-Mercaptoethanol(Sigma-Aldrich, USA)
19. Coomassiae brilliant blue-R250(Merck, India)
20. Acetic acid(Merck, India, Cat No. 64-19-7)
21. Methanol(Merck,India, Cat No.67-56-1)

Equipment

1. -20 °Refrigerator (Krispcold, India)
2. Centrifuge tube(Sigma G 3-18K)

Appropriate biosafety level procedures were followed up for protein extraction from human plasma.

Procedure

Purification of human plasma proteins for SDS Poly-acrylamide gel electrophoresis:

1. Initially 100 µl of plasma was taken in 1.5 ml eppendorf tubes in 5 replicates.

Then 1000 µl each of 10 to 50% TCA-Acetone was added to each of 100 µl plasma samples in eppendorf tubes.

2. The tube was kept at -20°C for overnight.

3. Then the tube was centrifuged at 15000g for 10 minutes.
4. The supernatant was discarded and 90% acetone was added.
5. Then the tube was kept in -20⁰C for 10 minutes.
6. The tube was centrifuged at 15000g for 10 minutes.
7. The supernatant was discarded and kept it for drying.
8. Finally 50 to 500µl rehydration buffer was added in to the dried pellet.
9. After human plasma protein purification, the protein was resolved in a 10% running gel and 5% stacking gel and stained with 0.2% coomassiae brilliant blue R250 solution. Then the gel was clearly visualized with a proper de-staining solution. The gel image was taken by geldoc (Biorad, USA).

Troubleshooting

After centrifugation, the pellet should not be disturbed. Albumins and globulins were removed from the tubes through gentle pipetting. This is the crucial step in purification.

Time Taken

The time taken for the procedure would be 18 hours.

Anticipated Results

SDS-PAGE analysis indicated that 50% TCA-Acetone protocol is suitable for human plasma protein purifications as major unwanted proteins are removed. Ethanol was first used for depletion of high abundance protein from plasma samples before few decades ⁴. Then after few years, ammonium sulfate was used for depletion of albumin and globulin from plasma samples ⁵. Chromatographic methods were adopted for depletion of abundance proteins from plasma samples ⁶. Several manufacturers were developed removal of 7 to 20 high abundance proteins from plasma samples based on the type of disease detection for diagnosis. To reliable the cost effective, TCA-acetone is the best choice for depletion of high abundance proteins from HIV-1 infected plasma samples and is suitable HIV-1 infected for plasma samples analysis in SDS-polyacrylamide gel electrophoresis. This protocol is suitable for clinical applications for electrophoresis of human plasma proteins.

References

1. Anderson, N. L. & Anderson, N. G. The Human Plasma Proteome. *Mol. Cell. Proteomics* **1**, 845–867 (2002).
2. Rasheed, S., Hashim, R. & Yan, J. S. Possible Biomarkers for the Early Detection of HIV-associated Heart Diseases: A Proteomics and Bioinformatics Prediction. *Comput. Struct. Biotechnol. J.* **13**, 145–152 (2015).
3. Arhel, N. & Kirchhoff, F. Host proteins involved in HIV infection: New therapeutic targets. *Biochim. Biophys. Acta - Mol. Basis Dis.* **1802**, 313–321 (2010).
4. Brummelhuis H.G. J. Ethanol fractionation of human plasma: An overview. *Plasma Fractionation and Blood Transfusion*. 47-56 (1985).
5. Bollineni, R.C., Guldvik, I.J., Gronberg, H., Wiklund, M., Mills I.G., Thiede, B. A differentially protein solubility approach for depletion of highly abundant proteins in plasma using ammonium sulfate. *Analyst*, **140**.8109-8117 (2015).
6. Friesen AD. Chromatographic methods of fractionation. *Dev Biol Stand.* **67**:3-13. (1987).

Acknowledgements

Acknowledgement: Indian Council of Medical Research is acknowledged for providing the ICMR-SRFship to Mr Sushnata Kumar Barik.

Figures

Figure: 1 SDS-PAGE of extracted protein from human plasma.

(Lane-1:10% TCA-Acetone, Lane-2:20% TCA-Acetone,Lane-3:30%TCA-Acetone,Lane-4:40% TCA-Acetone, Lane-5-6-7 :50% TCA-Acetone, Lane-8,9:10%TCA-Acetone, Lane-10:Human plasma (Without precipitation))

Figure 1

(Lane-1:10% TCA-Acetone, Lane-2:20% TCA-Acetone,Lane-3:30%TCA-Acetone,Lane-4:40% TCA- Acetone, Lane-5-6-7 :50% TCA-Acetone, Lane-8,9:10%TCA-Acetone, Lane-10:Human plasma (Without precipitation))

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [supplement1.docx](#)